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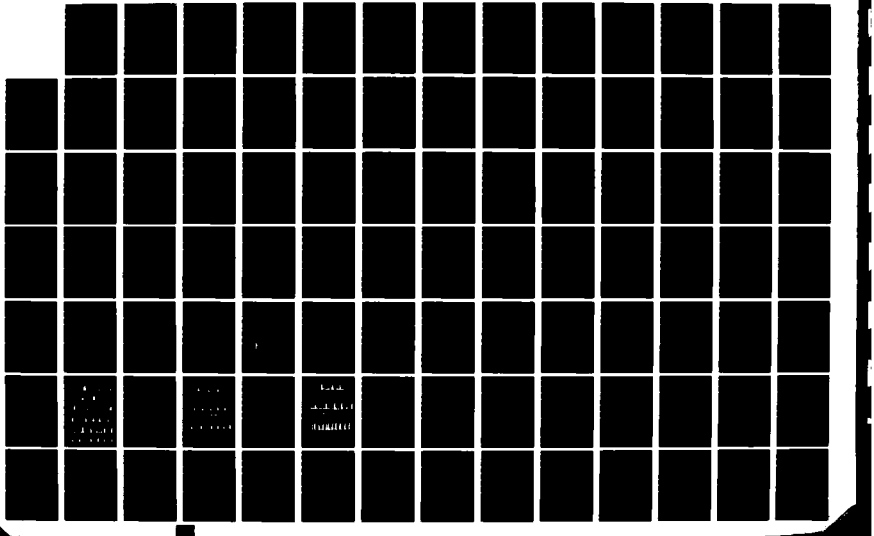

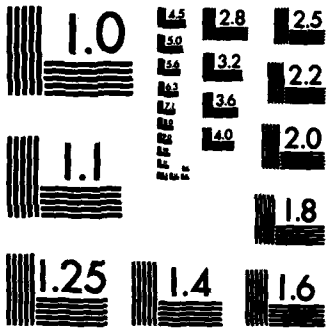

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ABSTRACT

Placental lactogen concentrations in sheep were observed to vary from + 96% to -56.4% in blood samples collected every 15 minutes. These fluctuations did not appear to result from diurnal or behavioral signals and further studies were conducted to elucidate reasons for the large variations and to determine the metabolic role of oPL during gestation.

Fasting, which decreased plasma glucose and increased plasma free fatty acid levels significantly in all ewes, resulted in an increase in mean plasma oPL concentrations in ewes in mid gestation. This effect was due mainly to large increases in two of the five ewes in this group. There was no change or a decrease in plasma oPL concentration with fasting in the other three mid gestation ewes and in another group of ewes in late gestation. The half-life and disappearance rate for glucose following glucose injection did not differ between pregnant and non-pregnant animals. Thus, oPL does not appear to respond consistently to a fast, to changes in plasma glucose or free fatty acids induced by fasting or to have diabetogenic effects.

The intravenous administration of 12.5 or 25 mg of arachidonic acid resulted in a significant increase in plasma oPL concentration approximately 120 minutes after injection. A larger dose of arachidonic acid had no effect or was inhibitory, indicating the possibility of a dose response relationship between arachidonic acid and oPL. Immediately following the arachidonic acid there were increases in the frequency of uterine contractures and in the single animal where uterine blood flow

was measured, it decreased simultaneously with the alterations in contracture frequency. There appeared to be a rapid, transient increase in uterine vein progesterone concentration, approximately 90 minutes before the increase in oPL. The time course of events suggests that the changes in contractures and uterine blood flow could be mediating by prostaglandins but the later increase in oPL is probably via another mechanism.

No significant correlation could be made between changes in uterine electromyographic (EMG) activity and the large fluctuations seen in oPL concentration in sequential samples. EMG activity of the uterus has been associated with changes in intrauterine pressure (IUP) which may alter uterine blood flow. Therefore, the EMG activity seen in this study and associated changes in IUP either do not alter uterine blood flow consistently or a change in uterine blood flow does not effect uterine or jugular vein plasma oPL concentrations. Thus, the physiological basis for the variability in secretion pattern for oPL is still unclear.

82-67

RELATIONSHIPS BETWEEN ARACHIDONIC ACID, UTERINE ACTIVITY AND METABOLIC REGULATION OF PLACENTAL LACTOGEN SECRETION

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment for the Degree of

Master of Science

by

Susan Elizabeth Huyler

August 1982

To my family

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LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Placental lactogen (PL) is a peptide hormone secreted by the placenta during gestation in several species including humans, sheep, goats, and rats (Blank, Chan and Friesan, 1977). Investigations into regulation of PL secretion and its physiological role during gestation have been inconclusive. The secretory pattern for PL shows great variability, plasma concentrations between subsequent samples fluctuate markedly in women (Vigneri et al., 1973), sheep (Taylor et al., 1980) and goats (Hayden et al., 1979).

Such variability in a hormone concentration could be caused by changes in delivery rate from the uterus to the peripheral circulation or the rate of release from the site of production to the uterine circulation. During late gestation in the sheep uterine blood flow is approximately 20% of cardiac output (Carter, 1975). Thus, a change in blood flow through the uterus could easily alter the concentration of a placental hormone in the periphery.

Changes in intrauterine pressure (IUP) in pregnant ewes have been correlated with uterine electromyographic (EMG) activity and changes in basal uterine tone called contractures (Nathanielsz et al., 1980). Since contractures vary with respect to location on the uterus and amount of myometrium involved, they may lead to alterations in uterine blood flow (UBF) (Nathanielsz, unpublished observations). Labor contractions have been demonstrated to decrease UBF in women (Brotanek et al., 1969) and sheep (Assali et al., 1958; Greiss, 1965). Since arachidonic acid has been

used to induce labor in women (MacDonald et al., 1974), it may be involved in a UBF mediated change in release of placental hormones. Arachidonic acid has also been shown to increase production of hPL in vitro (Handwerger et al., 1981), perhaps via a change in membrane permeability or fluidity since it makes up a large percentage of cell membrane phospholipids.

Fasting, which has been demonstrated to decrease UBF in pregnant sheep (Morriss et al., 1980) has also been shown to increase PL concentration in ewes (Brinsmead et al., 1981) and women (Tyson et al., 1971). This effect has been tied to the hypothesized role of hPL as a "growth hormone of pregnancy" (Grumbach et al., 1968). Although receptors for oPL have been identified in sheep liver, adipose tissue, and mammary gland (Chan et al., 1978), indicating perhaps a metabolic function for oPL, there is conflicting data regarding this role and the possibility exists that PL performs different functions during gestation in monogastric and ruminant animals.

The purpose of the following studies, though basically directed towards an understanding of the variability in oPL secretion, was threefold: 1) to study the effect of 84 hours of starvation followed by a glucose or acetate challenge on the plasma concentration of oPL, glucose and FFA of ewes in mid (70-90 days) or late (120-135 days) gestation, 2) to determine in vivo effects of arachidonic acid on oPL production, and 3) to examine possible correlations in late pregnant ewes between fluctuations in oPL and uterine contractures as depicted by EMG activity.

REVIEW OF LITERATURE

Occurence and Characteristics of Placental Lactogen

The appearence of mammary gland development and a transient lactation in the hypohysectomized ewe (Forsyth, 1974) and maintenance of the corpus luteum by the placenta of the hypophysectomized rat (Rothchild et al., 1973) are partial evidence for the production of a compound from the placenta with luteotrophic and lactogenic properties. The search for this compound began in 1938 (Astwood and Greep) but it was not until 1962 that Josimovich and Mac Laren isolated a peptide hormone with growth hormone like properties from the human placenta. Placental lactogen (PL), also known as chorionic somatomammotrophin (CS), has subsequently been identified in monkeys (Grant et al., 1970), sheep (Chan et al., 1974), rats (Robertson and Friesan, 1975), goats (Buttle et al., 1972) and baboons (Josimovich, 1973). Existence of a bovine PL has been reported (Buttle and Forsyth, 1976; Bolander, Fellows and Ulberg, 1976), however, the plasma concentrations found have been very low compared to other ruminants.

Human placental lactogen (hPL) has been the molecule most intensively investigated and has been isolated as a protein hormone of 190 amino acids, molecular weight 21,600 and containing two disulfide bridges (Li et al., 1971). It has 80% homology with the amino acid composition of human growth hormone (hGH). Despite this similarity, hPL has only 13% of the activity of hGH in the rat tibia test (Li, 1972). HPL is also similar to human prolactin (hPrl) and is highly lactogenic in rats and mice (Forsyth and Folley, 1970). However, its role in normal mammary gland

development in women is not known.

Ovine placental lactogen (oPL) has been purified from sheep cotyledons (Handwerger et al., 1975). It has a molecular weight and an amino acid sequence similar to ovine growth hormone (oGH) and hPL. In a prolactin radioreceptor assay, the displacement of I^{125} h Prl from binding sites on mammary gland membranes by increasing quantities of hPL, oPL, and ovine prolactin was parallel (Handwerger et al., 1974). Using a GH radioreceptor assay, oPL, hGH, and hPL inhibited binding of ^{125}I hGH to receptor sites on liver membranes.

Placental lactogens have been measured in plasma and placental extracts by radioimmunoassay (RIA), radioreceptor assay (RRA), and bioassay. Bioassays include the pigeon crop sac stimulating assay, rat tibia test, in vivo rabbit intraductal assay, and an NB2 node rat lymphoma cell suspension culture assay (Tanaka et al., 1980).

The isolation and purification of hPL and oPL have led to the development of RIAs. These involve the production of anti-sera to a specific hormone and are based on the principle of competitive inhibition between radioactively labelled and unlabelled hormone. Successful RIA's for oPL and hPL have been developed using a variety of techniques (Handwerger et al., 1977; Chan et al., 1978b).

The development of radioreceptor assays have made it possible to identify placental lactogens from many additional species. These assays measure binding of hormone to a membrane receptor, and as such the hormone need not be isolated or purified, nor is the assay species specific. Competition for binding sites is established between a radioactively

labelled GH or Prl standard and the unlabelled hormone in question. Bound and free hormone are separated and the radioactivity in the bound phase counted and compared with standards. The receptor-hormone interaction is thought to involve the biologically active site of the hormone molecule and thus gives an indication of biological potency.

Synthesis of Placental Lactogen

Messenger RNA and polyribosomes which synthesize hPL *in vitro* can be extracted from full term human placentae (Boime *et al.*, 1976). Translation of hPL in a cell free system of polysomes results in an amino acid sequence 20 residues longer at the N terminal end than hPL which is secreted (Birken *et al.*, 1977). This extra sequence is thought to serve as a signal peptide, (Blobel and Dobberstein, 1975) directing the nascent peptide to the endoplasmic reticulum where it is removed before release of the hormone.

Secretion of Placental Lactogen

Little is known regarding the mechanisms of PL secretion. The secretion rate of hPL varies with stage of gestation and method of analysis and has been determined to be 300-1000 mg per day in late gestation (Grumbach *et al.*, 1968; Solomon and Friesan, 1972). The half life of hPL has been shown to be 12-30 minutes by different methods (Pavlou *et al.*, 1972; Grumbach *et al.*, 1968). OPL has a half-life established to be approximately 20 minutes (Kelly *et al.*, 1974) or 29 minutes by Handwerger *et al.* (1977).

Using immunoflourescent techniques Reddy and Watkins (1978) showed that the site of PL secretion in the ewe appears to be the binucleate cells of the chorion. These cells are evident by day 16 of pregnancy in the ewe, the same time as the first appearance of oPL as measured by RRA (Martal and Dijiane, 1977). The migrate from the chorion into the uterine epithelium which then under goes a syncytical transformation. It has also been demonstrated that hPL is secreted by the syncytiotrophoblast of the human placenta (Boime et al., 1982). This migration of binucleate cells may represent a release mechanism. One hypothesis of migratory control (Steven et al., 1978) is that the fetal pituitary is responsible for secreting, under inhibitory control of the hypothalamus, a binucleate cell mobilizing factor. The authors have demonstrated that migration of the cells is accelerated by fetal pituitary stalk secretion. As pregnancy progresses, PL is secreted mainly into the maternal circulation, the ratio of fetal to maternal oPL falling from 1.2 $\pm$ .4 in pregnancies less than 100 days of gestation to .14 $\pm$ .04 at term (Gluckman et al., 1979).

Metabolic Regulation of Placental Lactogen Secretion

The secretion of hPL is thought to be controlled by the plasma concentrations of the metabolic substrates; carbohydrate, fat or protein. Plasma free fatty acid (FFA) concentrations have been altered experimentally in attempts to vary PL levels. Infusion of nicotinic acid (NA) is known to decrease FFA by decreasing cAMP activity, to a low point at 40-50 minutes

post infusion, followed by a rise above baseline (Nye and Buchanan, 1969). Plasma FFA can be increased by an infusion of triglyceride mixture plus heparin which activates lipoprotein lipase (Blackard, Hall and Lopez, 1971). Infusions of NA into women in late pregnancy depressed FFA by 50% (Gaspard et al., 1977) while triglyceride infusion plus heparin increased FFA from 468 to 2478 ueq/l. Neither treatment altered the level of hPL (Gaspard et al., 1975). Morris et al. (1974) treated women near the end of pregnancy with NA, triglyceride with or without heparin, and found no alteration in plasma hPL concentration. Infusion of various doses of NA into pregnant ewes decreased FFA from 846 to 94 ueq/l by 90 minutes, followed by an increase to 1942 ueq/l, but did not cause significant effects on plasma oPL (Handwerger, personal communications).

Conflicting data has been reported regarding the effect of carbohydrate on PL secretion. Burt et al. (1970) reported that a 25 gram dose of glucose given intravenously (IV) as a 50% solution resulted in decreasing hPL corresponding with the initial hyperglycemia. After one hour hPL increased above pre-test fasting levels in association with increased insulin levels. There was no secondary decrease in hPL after a second 25 gram glucose load. In contrast, Samaan et al. (1966) reported no significant change in hPL during or following induced hyperglycemia (glucose infusion) or hypoglycemia (insulin injection) in late pregnant women. Sustained hyperglycemia followed by a bolus injection of a glucose solution had no consistent effect on hPL concentrations in women in late gestation (Ajabor and Yen, 1971). Lowering blood glucose in ewes by infusion or injection of insulin, raising blood glucose by

infusing or injecting glucagon, glucose or dextrose, had no significant effect on oPL levels (Handwerger, personal communication).

A more physiologic means of lowering blood glucose and raising FFA is by fasting. Starvation and pregnancy lead to similar metabolic adaptations. If nutrient intake is inadequate during gestation, an accelerated starvation of pregnancy may develop. Glucose tolerance is impaired in human pregnancy despite hyperinsulinemia following glucose administration (Hager et al., 1972). Also, insulin sensitivity is decreased and FFA levels increased (Schalch and Kipnis, 1965). The number of insulin receptors on adipocytes has been shown to increase during gestation in rats (Flint et al., 1979). This may be an adaptation to counter the anti-insulin effects of PL and allow lipid deposition to occur in early gestation.

Knopp (1973) has described two metabolic phases of pregnancy: 1) the anabolic phase- The first two-thirds of gestation when there is maternal hyperphagia and hyperinsulinemia leading to increased maternal fat storage. The conversion to glucose of adipose tissue triglycerides increases 200% and release of FFA is decreased as placental and maternal fuel needs are met. 2) the catabolic phase- The effect of insulin on adipose tissue is opposed as fatty acid formation from glucose decreases. The mobilization of maternal fat stores as FFA is synchronized with growth of the feto-placental unit. Any ingested glucose is diverted from maternal tissues to the fetus, stored lipids being used as the maternal energy source. Since maternal hyperinsulinemia persists through the second half of gestation, an insulin antagonist seems necessary to

establish a state of insulin resistance in maternal adipose tissue. If this were not true, lipogenesis would continue, leaving the fetus at a disadvantage in competing with the mother for circulating glucose. Knopp suggests that phase 2 may be under control of PL since this hormone has been shown to produce insulin antagonism and fat mobilization. Malaisse et al. (1969) report that changes in the functional activity of pancreatic beta cells with gestation are secondary to development of the placenta and its hormonal function.

A study by Felig and Lynch (1970) demonstrated that an 84 hour fast in the second trimester of human pregnancy resulted in decreased glucose and insulin in plasma, increased urinary ketone bodies and NH_3 excretion, all of which were exaggerated when compared with non-pregnant women. Tyson et al. (1971a; 1976) have noted that hypoglycemia and hypoinsulinemia are accelerated during fasting of pregnant women. In these studies, hPL concentrations increased by 32 to 56% during a 72 hour fast in one study and 33.2% in another. The FFA increase paralleled plasma hPL increase during the first 48 hours. Changes in response to a fast differ depending on the stage of gestation of the woman being investigated. The greatest increases are seen in women between the tenth and fourteenth week of gestation while little response is seen after week 30. The authors feel their data support a role for the feto-placental unit in the mobilization of maternal lipid and protein stores during starvation as a means of preserving maternal glucose for fetal use. These results are equivalent to those obtained with a hypopituitary dwarf, indicating that the effect was independent of growth hormone. Women at 16-22 weeks of gestation fasted

for 84-90 hours showed a 30-40% increase in plasma hPL (Kim and Felig, 1971).

A similar study has been conducted in sheep by Brinsmead et al. (1981). Fetal and maternal blood glucose were lowered via insulin injection or raised by a glucose infusion. Neither treatment significantly altered oPL concentrations in the plasma. However, maternal fasting for 72 hours, which decreased maternal glucose from 2.6 to 1.38 mmol/l and fetal glucose from .72 to .30 mmol/l, resulted in a rise in maternal oPL concentration by 24 hours to a peak at 72 hours of 195% of control levels. The increase in fetal oPL concentration was significant only at 72 hours.

In another study in fasting sheep uptake of glucose and essential amino acids by the uterus of pregnant animals decreased by 63% during the first three to four days of fasting. There was no subsequent decrease during days 5-7 of the fast, in fact, amino acid uptake increased from 2.3 $\pm$ 1.7 to 7.8 $\pm$ 3.8 μM/kg/min indicating, perhaps, a metabolic adaptation to starvation (Morriss et al., 1980).

The normal range of blood glucose in fed sheep is 25-50 mg/dl, approximately half the normal human value. The ruminant has adapted to a diet low in carbohydrate, but problems with glucose conservation and gluconeogenesis still exist. Ingested carbohydrate is rapidly fermented to the volatile fatty acids (VFA): acetate (97% of total plasma VFA values), butyrate, and propionate. The role of glucose in omnivores is fulfilled by acetate in herbivores. Acetate is oxidized by muscles, used for lipogenesis by adipose tissue and mammary gland, and is the major precursor of adipose tissue triglycerides in ruminants. Acetate is lower in a fasted than a fed animal due to decreased rumen fermentation. During a

fast, the largest part of energy for metabolism comes from fatty acid carbons which must flow through butyryl and acetyl CoA and condense with oxaloacetate to enter the citric acid cycle. Oxaloacetate may be in short supply due to lack of carbohydrate precursors, if so, the pool of ketone bodies is increased, another indication of starvation.

In ruminant animals the response of blood glucose levels to a fast is similar to that of non-ruminants, only delayed (Reid, 1950). There was little change in blood glucose when non-pregnant sheep in good condition were fasted for up to 46 hours. However, a 24 hour fast in ewes in poor condition during the last two months of gestation led to blood glucose levels as low as 8.6 mg/dl. Thus, although ruminants have low blood glucose and rely on gluconeogenesis and VFA formation as energy sources, they are still susceptible to lowering of glucose by fasting, especially if they are pregnant or in poor condition.

The synthesis of placental proteins has been shown to change with nutritional status. Wunderlich et al. (1979) found that rat PL synthesis on a 5% protein diet was only 53% of that found in rats on a normal 18% protein diet. Rats on an 18% protein plus 20% alcohol diet synthesized only 52% as much rPL as controls. Studies in primates involving administration of an isocaloric protein deficient diet to ten rhesus monkeys had a negative effect on fetal birth weight and maternal weight gain during pregnancy but no effect on placental lactogen levels or placental weight (Novy et al., 1981). Following ingestion of a meal consisting of 64 grams protein plus 100 grams glucose in 200 ml water, no significant change in hPL concentrations in women in late gestation was observed

(Tyson et al., 1971b).

Protein or amino acid concentrations have also been investigated as possible controlling mechanisms for oPL secretion. Handwerger et al. (1978) infused arginine in doses of 25 and 50 grams into pregnant ewes. Only one ewe showed an increase in oPL concentration after the 25 gm dose, but two or three hours after infusion of the 50 gram dose oPL levels had increased 79-115% in seven ewes, 454% in one ewe, and 1142% in two ewes. In a similar experiment (Handwerger et al., 1981) infusion of 25 gm orinithine stimulated oPL secretion by 124 ± 25% in all eight pregnant ewes within one hour post infusion. In the same study administration of citrulline had no effect on oPL. These amino acids are compounds of the urea cycle, indicating a possible conversion of arginine to ornithine for induction of PL synthesis.

Spontaneous Variations in Plasma Placental Lactogen

It has been noted that the hPL levels for individual women in late pregnancy may vary within a day by ±15% (Zlatnik et al., 1979). Maternal blood samples taken hourly from pregnant ewes for a 24 hour period showed spontaneous fluctuations in plasma oPL of up to two fold between samples. No evidence was found for a circadian variation. Plasma oPL from ewes sampled every 10 minutes for 140 minutes were more stable, the maximum change being 35% from one sample to the next with no evidence for pulsatile release (Taylor et al., 1980). In research involving goats (Hayden et al., 1980), plasma levels of caprine placental lactogen (cPL) in adjacent samples showed marked fluctuations of up to two to three-fold

which were not related to changes in plasma glucose or FFA. Lindberg and Nilsson (1973) also noted significant variations in hPL in samples taken every four hours during a 24 hour period with no evidence of circadian variation and no relationship to sleep, meals, or light exercise. The results of this work are supported by that of Pavlou, Chard and Letchworth (1972) where sampling occurred every 15 minutes for 24 hours from women in late gestation.

Vigneri et al. (1973) used a continuous blood sampling technique in third trimester women for 10-15 hours plus samples were taken at 10 minute intervals for 60-90 minutes. The fluctuations in hPL varied from +26% to -49% between samples from both methods. There was no correlation with meals, stress, age, or number of pregnancies and no periodicity or ultradian rhythm. These fluctuations cause a wide overlap between hPL values of a normal pregnancy and those where there is possible placental failure. This, according to some authors, reduces the value of a single sample as a diagnostic tool (Josimovich et al., 1970; Singer et al., 1970). Other authors have found hPL concentrations to be of value in diagnosis of intrauterine growth retardation and placental insufficiency (Granat et al., 1977).

In general, hormone levels in plasma are dependent on the distribution space in addition to secretion rate and metabolic clearance rate. These spontaneous fluctuations may therefore be related to changes in placental circulation caused by uterine muscle activity or vasoactive influences on the vessels (Vigneri et al., 1973).

Lippert et al. (1973) investigated changes in utero-placental blood

volume using a radioactive isotope that is retained in the bloodstream. The activity detected is directly proportional to the blood volume surveyed. The activity of the uterus was reflected in blood volume changes seen in the tracings from the detector, indicating increases in blood volume approximately every five minutes. These volume changes are contributed to by uterine muscles activity as well as vasoconstrictor and dilator influences on the vessels.

The uterine vasculature has been demonstrated to be sensitive to the vasoconstrictor effects of epinephrine. Endometrial blood flow decreases by 58.7% and cotyledonary blood flow by 34.5% during epinephrine infusion to 85-140 day pregnant ewes (Rosenfeld, Barton and Meschia, 1976). Fasting also has a significant effect on blood flow in sheep (Morriss et al., 1980). After a five day fast, 12 late gestation ewes demonstrated a 25% decrease in total blood flow with a 20% decrease in placental blood flow at the same time that hepatic blood flow is increasing. Since synthesis of hPL is dependent on adequate placental blood flow (Tyson et al., 1972) any decrease in uterine blood flow may influence plasma hPL concentrations.

If intrauterine pressure readings (IUP) are taken simultaneously with blood flow they may give an indication of the role of uterine muscle activity in regulation of placental blood volume changes. Investigations into IUP by Scheffs et al. (1971) demonstrated that blood flow increases during the relaxation phase and decreases as the IUP increases indicating a contraction. They conclude that normal uterine contractions are associated with changes of impedence in the myometrial vascular bed. Nathanielsz et al. (1980) has correlated changes in IUP in pregnant ewes with uterine

electromyographic (EMG) activity and with changes in the basal uterine tone called contractures. A contracture is defined as a change of at least 3.5 mm Hg above the previous existing IUP baseline lasting for a minimum of five minutes. The contractures were present in all pregnant ewes beyond 100 days gestation, occured with an average frequency of .96/hour and were closely related to EMG activity of the uterus. This is evidence that they were not caused by other muscular activity such as rumen movements or changes in posture. The contractures vary as to location and amount of myometrium involved. They may alter cotyledonary blood flow, which could lead to changes in amount of placental hormones in the peripheral circulation.

Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) is known to alter uterine activity via its effect on smooth muscle (Weeks, 1972). HPL concentrations in women where abortion was induced by either 56 mg $PGF_2\alpha$ injected between the uterine wall and membranes or 20 mg $PGF_2\alpha$ injected transabdominally, were significantly reduced in the two hours post injection (Ylikorkala and Pennanen, 1973). In patients injected with saline extraamniotically no change in plasma hPL was seen. The authors conclude that injection of $PGF_2\alpha$ must decrease hPL levels by reducing synthesis or secretion by the placenta rather than by mechanical disturbance of the placenta since those factors were present in the controls also.

An *in vitro* study conducted by Genbacev *et al.* (1977) obtained similar results. They found that addition of $PGF_2\alpha$ to the incubation medium of human placental decidua resulted in decreased incorporation of ^{14}C leucine into protein in a dose dependent manner that was correlated

with the age of the placenta. There was a significant decrease in hPL synthesis by 24-26 week gestation placentas. Also a 25% decrease in hPL production was seen by incubation of placentas obtained after abortion was induced by intraamniotic injection of $PGF_2\alpha$. Many members of the prostaglandin family exert their effects via changes in cAMP. However, incubation of human placental decidua with theophylline or dibutyryl cAMP had no significant effect on the synthesis of hPL (Handwerger et al., 1973).

In an attempt to correlate the prostaglandin levels seen in human pregnancy with hPL concentrations, Spellacy (1972) infused $PGF_2\alpha$, PGE_2, and, to control for the effect of uterine contractions, oxytocin, into the antecubital vein of term pregnant women. Saline only was infused during the first 60 minutes of the five hour infusion. No significant change in hPL was evident in response to any treatment.

Several studies have been conducted examining the effect of labor on hPL levels. Pavlou et al. (1972) found that the variation of hPL during labor was no greater than the daily fluctuations of late pregnant patients. Eleven out of fifteen women did have a significant rise in hPL at the time of delivery. During spontaneous labor, 22% of women showed significant variation between samples with a maximum difference of 1.2 µg/ml. When labor was induced with oxytocin 33% had variations of up to 1.8 µg/ml. When samples were taken during periods of known uterine activity, no significant correlation between hPL and activity were seen.

Variations in plasma concentration of placental lactogen may also be regulated by changes in its rate of release from the site of synthesis, a process which can be affected by membrane permeability. Arachidonic

acid, twenty carbon essential fatty acid comprising a large proportion of membrane phospholipids, has been implicated in hPL release in vitro (Handwerger et al., 1981a). Arachidonic acid is metabolized via several different pathways (Nutrition Reviews, 1978; Ramwell et al., 1977). It is first cleaved from the plasma membrane by phospholipase A_2 and then can follow the cyclo-oxygenase pathway to prostaglandins, thromboxanes, or prostacyclins; or the lipoxygenase pathway. The concentration of arachidonic acid is independent of the total FFA level, is not altered by starvation, and is thought to be the limiting factor in prostglandin synthesis. As mentioned previously, prostaglandin families have a variety of effects on many different organs, depending on which family is involved (Weeks, 1972; Hinman, 1972).

In the study by Handwerger et al., concentrations of arachidonic acid from 20-500 μM stimulated hPL release from placenta descidua in a dose dependent manner. Incubation with phospholipase A_2 also resulted in hPL release as did the arachidonic acid precursors linoleic and linolenic acids. Inhibition of the cyclo-oxygenase pathway by indomethacin or flufenamic acid had no effect on hPL secretion, nor did the lipoxygenase inhibitors EYTA and BM755C. This indicates that the effect of arachidonic is independent of these pathways. Arachidonic acid has also been found to stimulate secretion of luteinizing hormone from a two day culture of pituitary cells (Naor and Catt, 1981). The necessity for calcium in arachidonic acid action has been suggested by an experiment involving rabbit peritoneal neutrophils (Volpi et al., 1980). The addition of arachidonic acid to the incubation medium caused a significant increase in calcium flux

through the membrane. This effect was sensitive to the inhibitors of the lipoxygenase pathway.

Conflicting data has been obtained regarding the effect of calcium on hPL secretion. Handwerger et al. (1981b) and Choy and Watkins (1976) reported a stimulatory effect of low calcium or the calcium flux inhibitor methoxyverapimil in the incubation medium on hPL release. Welsch (1979) demonstrated an increase in hPL with a high calcium concentration, indicating perhaps a bell-shaped dose response curve to calcium levels (Horrobin et al., 1977). Calcium is known to be involved in the secretion of many different hormones (Rasmussen and Goodman, 1977).

Effect of Placental Mass on Placental Lactogen Secretion

The relationship between placental-fetal mass and plasma PL has been extensively investigated. Spellacy (1972) has found a significant relationship between placental weight and hPL and between infant birth weight and hPL. HPL in women carrying twins is approximately twice that seen in single gestations (Grumbach et al., 1968). This relationship holds true in sheep for oPL (Handwerger et al., 1975) where concentrations of oPL are related directly to litter size regardless of breed (Butler et al., 1981). In Finnsheep ewes with singles the oPL concentration was 1197 $\pm$ 599 ng/ml; twins 1584 $\pm$ 186 ng/ml; and triplets 2997 $\pm$ 289 ng/ml. In crossbred ewes mean oPL concentrations during late pregnancy in the maternal circulation were significantly correlated to combined fetal weight at birth. In another study, concentrations of oPL in maternal plasma for ewes with a single fetus averaged 718 $\pm$ 227, twins 1387 $\pm$ 160, and triplets 1510 $\pm$ 459 ng/ml (Taylor et al., 1980).

Functions of Placental Lactogen

Receptor sites for oPL have been identified in sheep adipose tissue, mammary gland, and liver (Chan et al., 1978a), indicating that the hormone may exert some control over metabolic events. Placental lactogens can be detected early in gestation in maternal plasma. The concentration increases throughout gestation, peaks, plateaus, and then declines, either before parturition as in the ewe or immediately following parturition as in women.

The initial increases in PL concentration occurs at the same time as the homeorhetic adaptations in the animal which are necessary to support pregnancy. Homeorhesis is defined by Bauman and Currie (1980) as the "orchestrated or coordinated control in metabolism of body tissues necessary to support a physiological state such as pregnancy." Nutrients are used by body tissues for maintenance and growth of an animal. During pregnancy the fetus imposes an additional demand for nutrients on the system and metabolism must be altered to supply these needs. The authors hypothesize an endocrine control mechanism arising from the conceptus (fetus and fetal membranes) which regulates nutrient partitioning to support pregnancy. If these adjustments are not made, metabolic disorders may result. Both the flow of nutrients across the placenta and into the mammary gland would be under this hormonal control.

Many studies have been conducted in an attempt to discover if PL has control over intermediary metabolic events. Bleicher et al. (1964) using a rat epididymal fat pad bioassay, found that a lipid mobilizing substance was present in the serum and placental extracts of pregnant women. In a

subsequent study, a preparation of purified hPL at concentrations from 5 to 100 µg/ml incubated with the same type cells showed lipolytic action which increased in a dose dependent manner. The release of FFA was not correlated with that of glycerol indicating a possible enhanced rate of reesterification (Genazzani et al., 1969). The authors hypothesize that hPL must be a substance which diminishes maternal glucose consumption through elevation of FFA or by direct inhibition of glucose uptake.

Turtle and Kipnis (1967) found that rat epididymal fat pads incubated with 500 µg/ml hPL for one hour did not show stimulation of lipolysis as measured by glycerol or FFA release. However, after four hours there was a 200% increase in both parameters which was inhibited by the addition of Actinomycin D or puromycin at the beginning of the incubation, but not if the inhibitors were added after the first hour. The authors concluded that hPL activated lipolysis is dependent upon new protein synthesis. In the same paper, 20 µg/ml hPL increased glycerol release by 200% with no effect on FFA levels.

Injection of human purified placental protein (PPP), which is the same as hPL, into fasted rabbits and monkeys resulted in increased plasma FFA concentration (Riggi et al., 1966). When PPP was administered for 25 days, hyperglycemia, hypertriglyceridemia and increased FFA synthesis resulted. Felber et al. (1972) found that hPL stimulated lipogenesis when the incubation medium contained cells from fed rats plus 2 mg/ml glucose and stimulated lipolysis when using cells from 18 hour fasted rats and only .5 mg/ml glucose.

In an in vivo study (Grumbach et al., 1966) injection of 100 to 400 mg hPL intramuscularly (IM) into four children with hypopituitary dwarfism resulted in a sharp rise in plasma FFA within four hours. The study was undertaken to determine hPL effects in GH deficient individuals using

a dose of hPL which would maintain concentrations near those found in the last trimester of pregnancy. Berle et al. (1974) gave a single dose of four mg hPL to non-pregnant women and saw no effect on FFA or glycerol levels in the plasma. No significant change in FFA concentrations was seen after infusion of oPL into pregnant and non-pregnant sheep (Butler, personal communication). PL may have various effects depending on basal FFA levels. If FFA levels are raised above physiologic concentrations both endogenous lipolysis and lipolytic actions of PL are impaired. If FFA levels are lower, PL may be an effective lipolytic agent.

The effect of PL on glucose metabolism has also been studied. Addition of 100 µg/ml hPL to an incubation of rat epididymal fat cells containing 2 mg/ml glucose stimulated metabolism as measured by CO_2 production, synthesis, glucose uptake and glycogen synthesis (Felber et al., 1972). In studies conducted by Grumbach (1968), hypopituitary male dwarfs were injected with 100 mg hPL every six hours for ten days. Their fasting blood glucose was not significantly different from control periods. However, there was a large decrease in the rate of glucose disposal as shown by decreasing k values. The concentration of immunoreactive insulin and insulin response to a glucose load were increasing during this time. The authors conclude that hPL has two effects on glucose metabolism: 1) a diabetogenic (anti-insulin) effect and 2) enhancement of insulin secretion. These characteristics are also observed during pregnancy (Baird, 1969).

HPL (1 µg/ml) has been found to augment insulin secretion and DNA production by rat pancreatic islets in vitro (Neilson, 1982). Using islets from hypophysectomized rats, treated with hPL for four days before

sacrifice, this effect was demonstrated to be independent of growth hormone (Martin and Friesan, 1969). Thus, as previously discussed, the increased insulin response to glucose during gestation may be a direct effect of PL on the beta cell (Tyson et al., 1970). Leake and Burt (1969) discovered that basal glucose uptake and glucose response to insulin was greater in adipose tissue from pregnant rats than non-pregnant rats. When adipose tissue from non-pregnant rats treated with 5 mg hPL for six days, was tested in the same manner, it responded as did tissue from pregnant rats.

Handwerger et al. (1976) infused 50 mg oPL into pregnant and non-pregnant sheep, causing significant changes in plasma FFA, insulin, glucose, and amino nitrogen. FFA decreased by 65% at one hour and returned to baseline only after six hours; insulin fell 68% at one hour and reached 150% above baseline by four hours; both glucose and amino nitrogen decreased after two hours. There was no difference in response between pregnant and non-pregnant ewes. The pregnant ewes were injected with 1, 5, 10, 25, and 50 mg oPL to establish a dose response relationship. There was a significant negative correlation between the log of the oPL dose and concentration of FFA, glucose, and amino nitrogen.

Mammogenic and Lactogenic Function of Placental Lactogen

A mammogenic function of PL has been hypothesized since it was observed that mammary growth and a transient lactation occurred in hypophysectomized, ovariectomized pregnant rats treated with estrogen and progesterone (Lyons, 1944). Fetectomy also did not limit mammary growth in the rat (Selye, 1934). Hayden et al. (1979) demonstrated that the increase in cPL secretion in goats from 10 to 16 weeks of gestation occurs at the same time as rapid lobulo-

alveolar development of the mammary gland. This development of lobulo-alveolar tissue, largely complete by parturition, determines the ability of the gland to synthesize and secrete milk (Richardson, 1973). This tissue weight is positively correlated with placental mass and fetal number as is PL secretion. In hand milked goats, milk yield is related to the number of kids. Goats bearing twins or triplets had 27% and 47% higher milk yields respectively than those bearing singles. Milk yields in these animals was also correlated with cPL titers from weekl 1 to term, giving evidence that PL has a role in control of development and function of the mammary gland in goats.

Buttle et al. (1979) investigated mammary gland growth in hypophysecomized and bromocryptine, which inhibits prolactin secretion, pregnant goats. Development of lobulo-alveolar tissue in both groups was similar, approximately 50% of control levels. This indicates that a placental factor may be acting in a manner similar to prolactin.

Milk production has been found to increase by 150% in ewes bearing and nursing twins when compared with single gestations (Alexander, 1959). The production was not increased by greater suckling intensity, indicating again that this greater milk yield was determined prior to parturition. Multiple offspring have a direct effect on mammary growth during late pregnancy in ewes (Rattaray, 1974).

There is evidence that hPL injections stimulate lactogenesis in the rat (Forsyth, 1970) and that breast development and a brief lactation occur in women and rhesus monkeys after hypophysectomy during gestation (Agate, 1952; Kaplan, 1961). HPL has a lactogenic potency of 20 IU/mg

as measured in the rabbit intraductal mammary gland assay (Forsyth, 1967). Thus, hormones produced by the feto-placental unit may influence the development of the mammary gland before delivery, and affect milk yield (food supply) after birth (Gaines, 1915).

Luteotrophic Influence of Placental Lactogen

The corpus luteum in the rat is maintained after hypophysectomy and fetectomy, but if the placenta is also removed the corpora lutea degenerate (Deanesley et al., 1941). Luteotrophic activity has been discovered in vitro in the day 12 rat placenta (Averill et al., 1950). In the sheep, the corpus luteum is necessary during the first 50 days of gestation for the maintenance of pregnancy. Hypophysectomy before day 60 results in regression of the CL. From day 30 to 60 the conceptus gradually acquires luteotrophic properties and also begins to secrete progesterone. After day 60 neither pituitary or ovaries are necessary for pregnancy maintenance (Denamur et al., 1973). Thus, the ability to maintain a pregnancy in sheep and rats depends on placental functioning, either by secretion of progesterone itself or maintenance of progesterone production by the CL via a placental luteotrophin, a role which may be fulfilled by PL.

Influence of Placental Lactogen on Utero-Placental Vascularization

Many fascinating observations have been made on the development of uterine vasculature during gestation. In 1933 Sir Joseph Barcroft noted that in rabbits and sheep there is extensive development of the vascular system in early gestation, before the large increase in fetal weight.

In late gestation the fetal weight increases more rapidly than the corresponding vascularization. This led to the formation of theories regarding the physiological mechanisms establishing the relationship between fetal weight and uterine circulatory development. Is the growth of the fetus limited by the ability of the uterine blood flow to supply metabolites or does the fetus provoke changes in circulation at a rate proportional to its own growth? Many studies have been done using sheep as a model since 1) circulatory changes are altered with the stage of gestation and 2) fetal growth occurs after development of the circulation (Huckabee, 1972).

It has been demonstrated that uterine blood flow in the sheep is 650 ml/kg (combined weight of fetus, placenta, and uterus)/minute up to 60 days of gestation while the flow averages less than 250 ml/kg/min from 90 days to term (Huckabee, 1972). Total uterine blood flow may be 1000 ml/min., approximately 20% of cardiac output. Changes in blood flow occur at the same time as changes in the endocrine relationship between fetus and mother. Estrogen is known to increase blood flow in sows (Dickson, 1969) while progesterone, given after estrogen administration, decreases blood flow. PL may be involved by acting as a capacitance hormone, enhancing the secretion rate or potency of several different hormones which orchestrate maternal adaptations to pregnancy (Solomon and Friesan, 1968). The fetus would therefore have control over uterine circulation, ensuring its supply of nutrients via production of hormones by the placenta.

Pope et al. (1979) have also suggested that a substance released by the conceptus may directly or indirectly influence both blood flow and

maintenance of the corpus luteum. It has been demonstrated that the ovine conceptus is capable of altering uterine vasoconstrictor responses to $PGF_{2\alpha}$ and sympathetic nerve stimulation (Ford et al., 1976). Information is needed on metabolism of hormones versus fetal growth and limits of hormonal control over uterine growth, before conclusions can be made.

MATERIALS AND METHODS

I. Fasting Study.

Pregnant and non-pregnant Dorset ewes weighing 50 to 55 kg were selected from the Cornell sheep flock: Group 1 ewes were 120-135 days of gestation n=4, Group 2 ewes 70-90 days of gestation n=5; and Group 3 ewes were non-pregnant n=8. (Intentions were to have equal size groups, however, 4 ewes in Group 1 and 3 ewes in Group 2 were diagnosed as nonpregnant by plasma oPL concentrations). Animals were acclimated to the sampling barn for one week before study and were fed hay, water and a pelleted dairy ration. On the afternoon before the study, a teflon catheter (16 ga. -6", Jelco Laboratories) was inserted into a jugular vein of each ewe. The animals were kept in elevated pens, each holding two animals separated by a partition, and large enough to allow standing or reclining.

The ewes in each group were randomly chosen to be fasted or fed. Three ml blood samples were taken every 15 minutes for four hours once each day of the 84 hour study period. Fed animals received hay and pellets once a day after the blood sampling period, fasted animals were given only water after the start of the fast. Following sampling on the fourth day, 100 ml of a 50% glucose solution was injected into the cannulae of all animals and blood samples were taken for an additional three hours. Animals were fasted before each challenge to increase the metabolic sensitivity of the system and hence its ability to respond. Samples were centrifuged, plasma decanted and stored in

vials containing 38% sodium citrate at -20°C until assay.

After completion of sampling on day four, catheters were removed, ewes were returned to a group pen and fed. Following a repletion time of 5 days for Group I and 10 days for Groups 2 and 3, the treatments were reversed (ewes previously fed were fasted). Animals were handled and blood samples taken as before, and following this four day period ewes were injected via the catheter with sodium acetate (50 millimoles per animals dissolved in 10 ml distilled water). Three additional hours of blood samples were taken and ewes were returned to the barn.

Samples from pregnant animals taken on days 2 and 4 were assayed for ovine placental lactogen (PL)(Handwerger et al., 1977). Glucose and FFA determinations were made using a Technicon Autoanalyzer II (Dalton and Kowalski, 1967) and samples assayed for acetate using the gas chromotographic methods of Chase et al. (1977).

Changes in oPL concentration were analyzed by two-way ANOVA. Regression analysis was performed between glucose, FFA, and acetate and oPL levels. The half-life ($T_{\frac{1}{2}}$)(time taken for glucose to decrease from a selected value to half that value) and the rate of uptake by tissues (K) in % min^{-1} for glucose were computed for all animals after the glucose challenge.

II. Arachidonic Acid Study

The pilot study involved one 65 kg Morlam ewe and one 55 kg Finnsheep ewe, both approximately at 90 days of gestation. They were housed in individual pens and fed hay, water and pelleted dairy ration. They were not fasted prior to the study.

Teflon catheters were inserted into a jugular vein of each ewe the morning of the experiment. Four control blood samples were taken at 10 minute intervals followed by an injection of 25 mg. arachidonic acid through the catheter at time zero. Three ml blood samples were taken into syringes containing heparinized saline for a total of five hours. The following day bovine serum albumin (BSA) in saline was injected as a vehicle control and samples taken as previously described.

Based on the preliminary data obtained an additional study was conducted involving a group of six Dorset ewes, all approximately 55 kg and 130 days of gestation.... Animals were housed in pens as described in part I and randomly divided into three groups of 2 sheep each.

Teflon catheters were inserted into a jugular vein of each ewe, animals were placed in pens and fed the day prior to the study. The study was designed as a 3x3 Latin Square with the treatments of palmitic acid, saline, and arachidonic acid given to each of the three groups in a random order on three days with a day between each treatment. Blood samples were taken as in the pilot study with additional samples at 240 and 300 minutes after treatment. Plasma was frozen at -20^{o}C until it was assayed for oPL (Handwerger et al., 1977).

Arachidonic acid solution for both studies were prepared by complexing either 25 or 50 mg arachidonic acid as the sodium salt (Calbiochem Lot 010046) to essentially fatty acid free BSA (Sigma A-6003) in a ratio of 17.4 μmole arachidonic acid to 10 μmole BSA (Laurell, 1957). The volume used to dissolve the BSA was reduced from 200 ml in the pilot study to 20 ml of .97% saline in the main study.

Palmitic acid solutions were prepared by adding 7.8 ml .02M NaOH to 25 mg palmitic acid (ICN Pharmaceuticals) plus 31.2 ml distilled deionized water. The solution was warmed until the palmitic acid dissolved. This solution was complexed to BSA in the same molar ratio as above and pH adjusted to 7.4 (Bauman, personal communication).

An ANOVA of split plot in time was performed on the combined data from both studies (Steele and Torie, 1960). A regression analysis was done on the oPL concentrations following injection of saline, palmitic acid and 50 mg arachidonic acid.

III. Uterine Activity Study.

Ten Rambouliet-Columbia ewes between 90 and 128 days of gestation were used for this study. They were put in elevated metal cages, similar to those described in study I, at least five days prior to surgery. Ewes were starved the day preceding surgery and were anaesthetized with 800 mg Ketamine (Vetalar-Parke Davis) intramucularly (IM), and 1.25 mg Atropine IM. After securing the animal to the operating table, a jugular catheter was inserted through which Ketamine was infused during surgery. The abdomen was scrubbed with Betadine and then alcohol and a mid-ventral incision was made to expose the uterus. Amniotic catheters and flow probe cables were exteriorized from the abdominal cavity through the maternal flank via a metal trocar and cannula. Vascular catheters and EMG wires were passed in the opposite direction using a second trocar and cannula.

The fetal head and neck were located in the uterus and the uterus was incised in layers. The choriallantois, choriamnion, and maternal layers were held together with hemostats to minimize loss of fluid. According to Nathanielsz et al. (1978) an incision (1.5 cm) was made in the fetal skin below the larynx and the thyroid, and .5 cm from the midline. The jugular vein and carotid artery on the side of the incision were located and dissected free of surrounding tissue. The vessels were ligated, and catheterized using .40"x.070" Tygon tubing (Scientific Products). The fetal skin incision was closed and a balloon was inserted through the uterine incision into the amniotic cavity to record intra-amniotic pressure, (the amniotic catheter was made of .050 x .90" Tygon tubing) before the fetal membranes and uterus are closed.

Branches of the uterine veins to both the pregnant and nonpregnant uterine horns were located in the broad ligament. Vessels were dissected free of surrounding tissue and catheterized, the tip of the catheter being threaded centrally. For insertion of the electromagnetic flow probes (Cand C Instruments) a 4-5 cm. segment of uterine artery was freed from surrounding tissue. The vessel was slipped through the gap in the magnet into the flow probe channel. The flow meter cable (Micron Instruments Inc.) was anchored to surrounding tissue and the lead was pushed subcutaneously to the location of the original trocar and cannula insertion where it was exteriorized.

To record electromyographic (EMG) activity of the uterus vinyl insulated multistrand stainless steel wires were sewn into the muscle so that a bare portion of the wire remained within the muscle (Nathanielsz et al., 1980). The pairs of EWG electrodes were sewn 5 to 8 mm apart into the myometrium

of various parts of both uterine horns. An indifferent electrode was placed at least 2 cm from any of the other electrodes.

The abdomen was closed, catheters, EMG wires and flow probe cables were attached to the back of the animal. The animal was returned to the cage and allowed to recover from surgery. Fetal blood samples were taken frequently for the measurement of pO_2, pCO_2, pH and hemoglobin on a blood gas analyzer (Acid Base Lab. 2, Radiometer-Compenhagen) to monitor fetal status. Chloramphenicol (Chloromycetin Sodium Succinate, Parke Davis), 800 mg to the mother and 200 mg to the fetus, and Ampicillin (Polycillin, Bristol Labs) 250 mg to the mother were administered IV immediately following surgery and twice a day for three days post surgery.

All vascular catheters were attached to a pump for continuous infusion of heparinized saline (10 IU/ml)(.25 ml/hour). All animals were allowed to recover the five days before any studies were conducted.

Experiment 1 - Uterine EMG activity was monitored on either a Beckman R711 or Grass polygraph, for a period of 24 hours before blood samples were taken. After a contracture pattern was established (a contracture was defined as a burst of EMG activity lasting 5-7 minutes) a series of baseline samples were taken. Two samples from the maternal jugular vein and the uterine vein was taken during a contracture and one sample was taken between contractures for a series of 10 contractures.

Experiment 2 - To determine if arachidonic acid injection had an effect on uterine EMG activity and maternal oPL concentrations, 12.5 mg arachidonic acid (prepared as described in part II) was injected into the

jugular catheter following three hours of recording and blood sampling. Blood samples were taken as described in part II.

Experiment 3 - This was conducted according to the protocol for experiment 2 except that 25 mg arachidonic acid was injected.

Not all animals were used for each experiment. In two cases the fetus died and the ewe was sacrificed, in two cases the ewe died before the treatment, and in some other cases the catheters necessary became inoperable. Electromagnetic blood flow measurements were obtained in one animal. In all cases, two ml samples were taken and put into heparinized tubes, centrifuged, plasma decanted into two equal aliquots and stored at -20^{o}C until assay. Samples were assayed for oPL (Handwerger et al., 1977) and progesterone (Gengenbach, 1978).

Contracture intervals were measured and the average for each animal before and after arachidonic acid compared using the student t-test.

RESULTS

I. Fasting Study.

Fasting resulted in an increase in plasma oPL concentrations in mid-gestation animals at both 36 and 84 hours of the fast as compared with oPL levels during the fed state (Table 1). The change is due mainly to the large increase seen in animals #84 and #151. In contrast, in animal #212 the fasting oPL levels are much lower than those seen in the fed state. In animal #62 and #215, oPL values show little change between the two metabolic states. The time course concentrations for oPL in each animal are shown in Figures 1 and 2. In contrast to mid-gestation, ewes in late gestation showed a decrease in plasma oPL concentrations at 36 hours of fast when compared to levels in the fed state (Table 1). Fluctuations in plasma concentrations are shown in Figures 3 and 4.

No evidence of a circadian variation in plasma oPL concentration was seen in the samples taken every 15 minutes during the fed and fasted condition, however, there were fluctuations in plasma oPL concentrations of +96.2% to -56.4% between successive samples (Fig. 1-4).

Fasting decreased plasma acetate in all animals and acetate injection raised plasma concentrations above physiologic levels for approximately 120 minutes (Table 2). Basal free fatty acid (FFA) levels were increased and plasma glucose level decreased in all three groups during the fast (Table 3). The only significant correlation was a negative relationship between plasma glucose and oPL in mid gestation animals (Table 4).

The half-life ($T_{1/2}$) of plasma glucose and disappearance rates (k) following the glucose infusion are listed in Table 5. No differences were seen among means for pregnant and non-pregnant ewes.

Table 1. Effect of fasting on mean (±SEM) plasma oPL concentrations (ng/ml)

Sheep no.	Treatment		
	Fed[1]	36 hour fast[2]	84 hour fast[2]
	MID-GESTATION		
62	100.0±14.2	61.3±6.2	75.1±17.3
84	248.3+11.3	991.4±31.3	1027.8±51.5
151	78.2±8.0	334.4±31.8	592.4±99.3
212	746.3±57.0	175.4±6.2	217.3±9.5
215	77.6±32.8	118.5±16.4	149.6±9.6
$\bar{x}$	$250.1^{a,3}$	$336.2^{b,3}$	$412.4^{b,3}$
	LATE GESTATION		
30	1028±71	1338±50.5	1080+21
56	774±38	635±83	476±57.6
70	3231±141	2487±322	3687±128.5
141	2235±135	1839±129.5	1605±23.5
$\bar{x}$	$1817^{a,3}$	$1575^{b,3}$	$1712^{a,b,3}$

[1]Mean of samples taken over the four day fed period.

[2]Mean of samples taken during a four hour period on that day.

[3]Pooled standard deviation was 74.4 for mid gestation animals and 253 for late gestation animals.

[a,b]Different superscripts indicate significant differences between means as tested by the student's t-test ($p < .01$).

Fig. 1. Plasma concentrations of oPL in samples taken every 15 minutes during a four hour period on day 2 and day 4 in ewes in mid gestation. Closed circles indicate the fed state and open circles the fasted state.

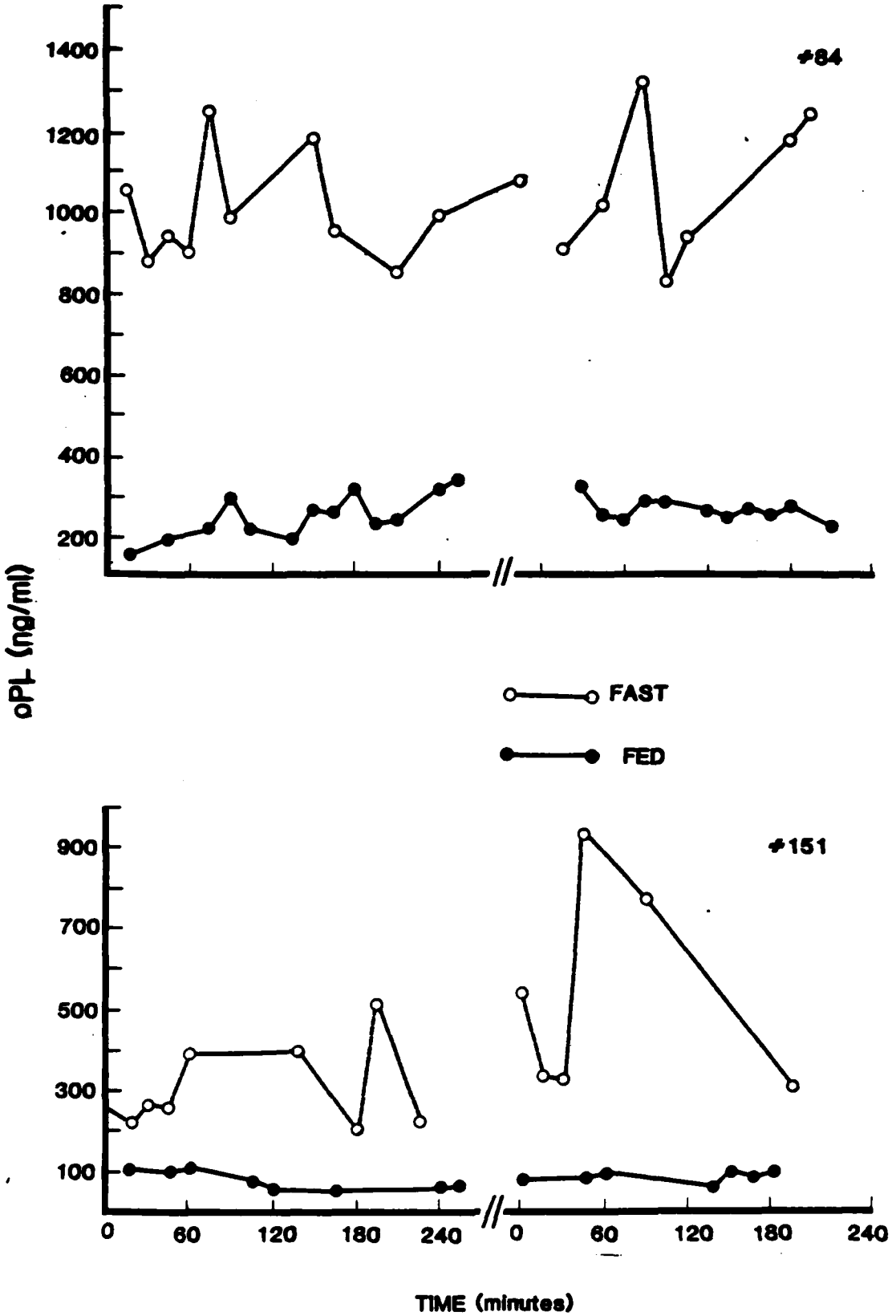

#84
1400
1200
1000
800
600
400
200
oPL (ng/ml)
FAST
FED
#151
900
700
500
300
100
0
60
120
180
240
TIME (minutes)

Fig. 2. Plasma concentrations of oPL in samples taken every 15 minutes during a four hour period on day 2 and day 4 in ewes in mid gestation. Closed circles indicate the fed state and open circles the fasted state.

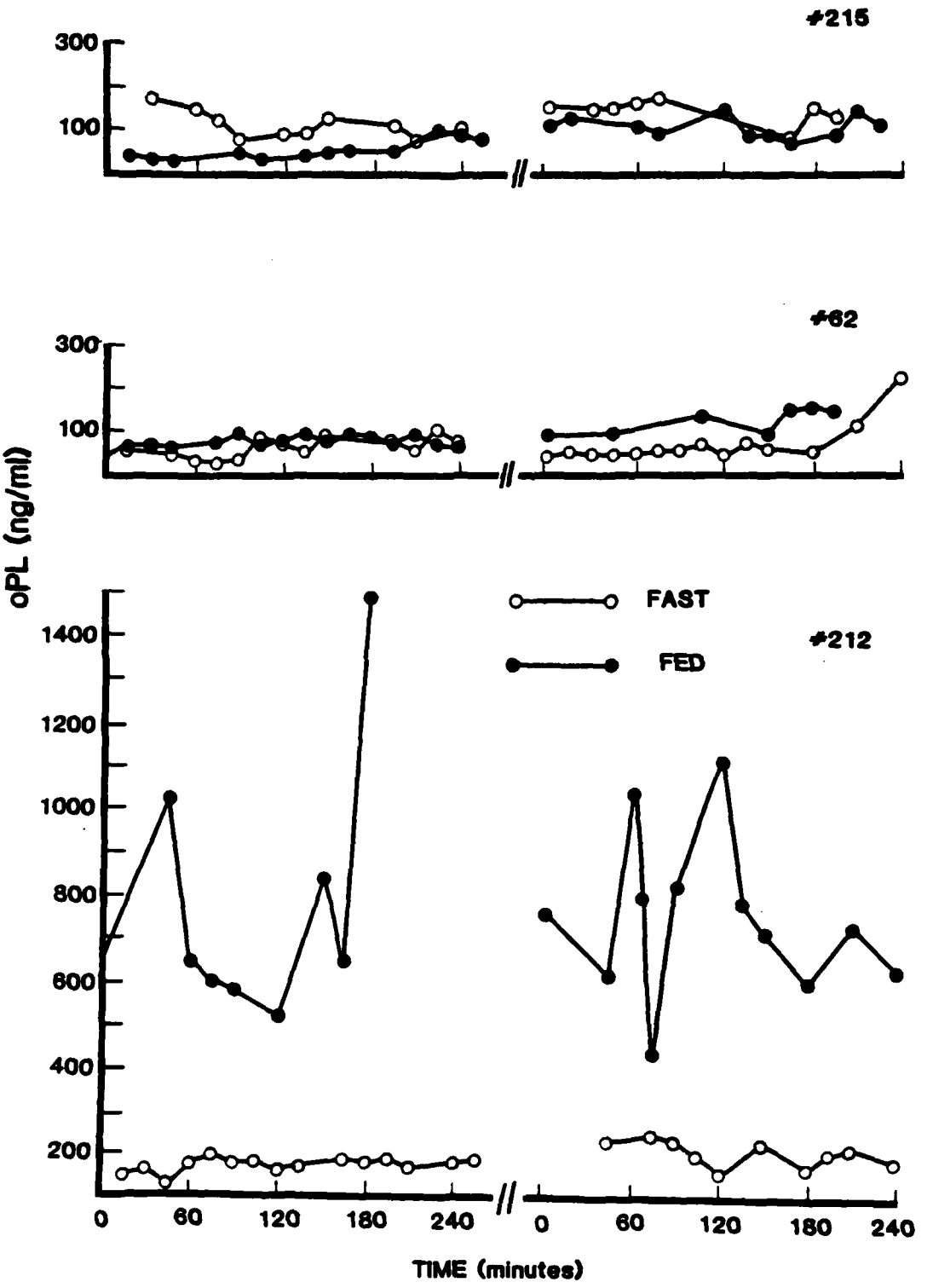

#215
300
100
#62
300
100
oPL (ng/ml)
FAST
FED
#212
1400
1200
1000
800
600
400
200
0
60
120
180
240
0
60
120
180
240
TIME (minutes)

Fig. 3. Plasma concentrations of oPL taken every 15 minutes during a four hour period on day 2 and day 4 in ewes in late gestation. Closed circles indicate the fed state and open circles the fasted state.

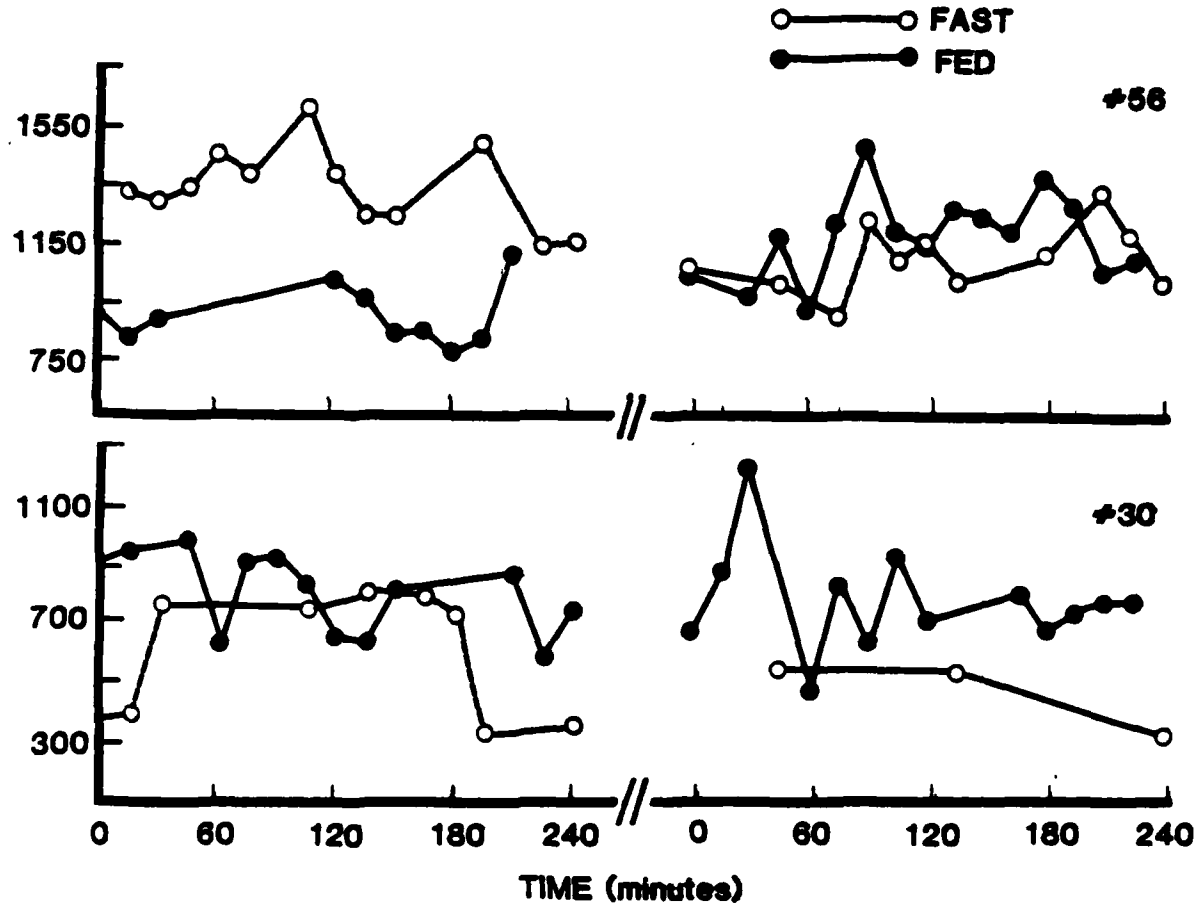

FAST
FED
#56
#30
1550
1150
750
1100
700
300
0
60
120
180
240
0
60
120
180
240
TIME (minutes)

Fig. 4. Plasma concentrations of oPL taken every 15 minutes during a four hour period on day 2 and day 4 in ewes in late gestation. Closed circles indicate the fed state and open circles the fasted state.

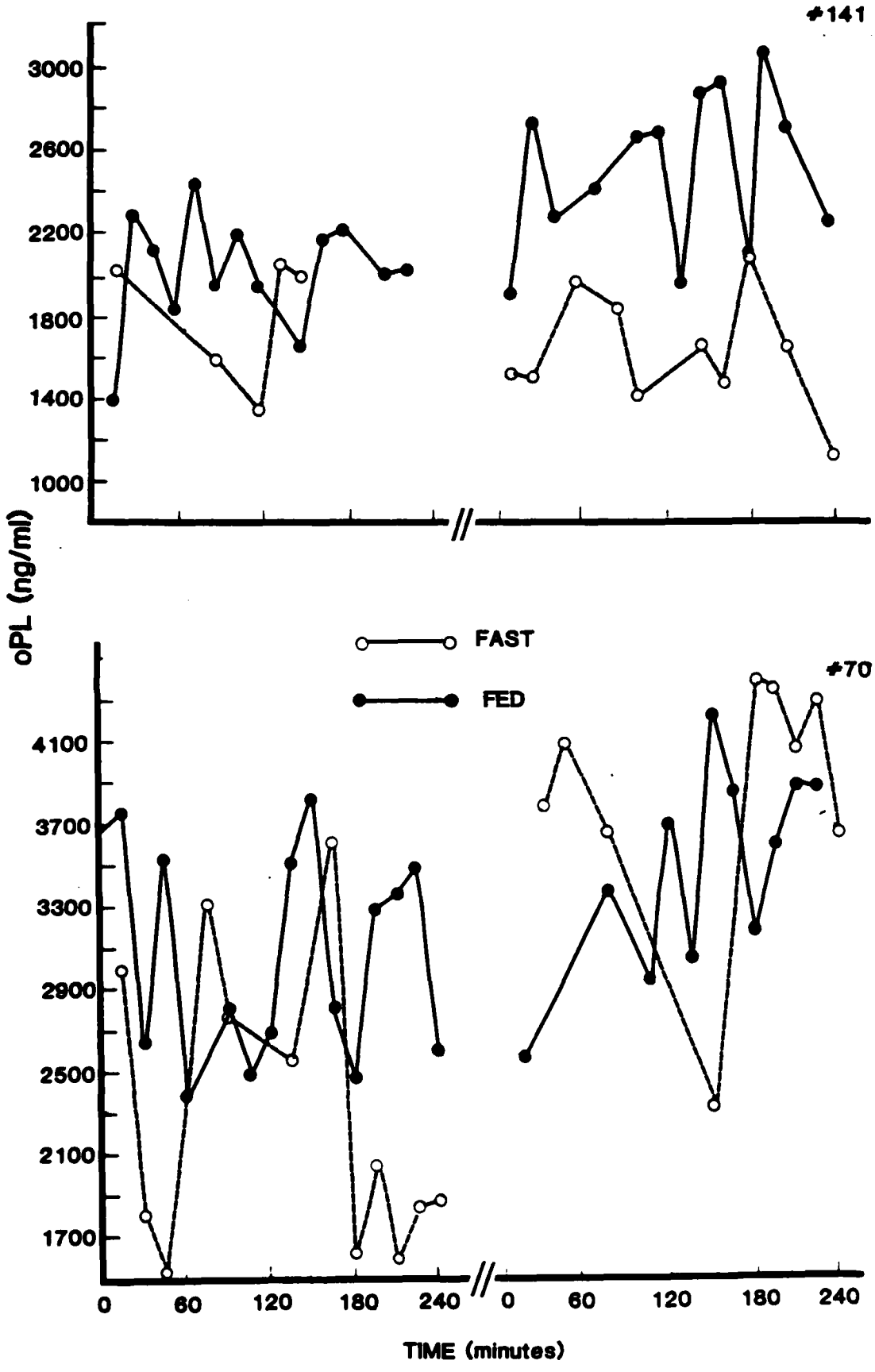

#141
3000
2600
2200
1800
1400
1000
oPL (ng/ml)
FAST
FED
#70
4100
3700
3300
2900
2500
2100
1700
0
60
120
180
240
0
60
120
180
240
TIME (minutes)

Table 2. Effect of fasting and acetate injection on mean (±SEM) plasma acetate concentrations (mg %) in pregnant ewes.

Time [1]	Treatment	
	Fed (5)[2]	Fast (4)
12 hours	5.19±.58	6.97±.9
36 hours	4.97±.49	4.59±.56
60 hours	5.86±.79	4.38±.67
84 hours	4.68±.8	5.44±.77
Acetate injection		
+5 minutes	17.39±1.1	15.39±.85
+15 minutes	12.5±1.6	12.77±1.4
+90 minutes	6.58±1.38	6.03±.91
+135 minutes	5.18±.48	6.24±1.15

[1] From the start of the fast.

[2] Number of animals is in parentheses.

Table 3. Effect of fasting and stage of gestation on mean (±S.D.) plasma FFA concentrations (μeq/l) and glucose concentrations (mg %) in pregnant and non-pregnant ewes.

Time[1] (hours)	Non-pregnant (8)[2]		Mid-gestation (5)		Late gestation (4)	
	Fast	Fed	Fast	Fed	Fast	Fed
			FFA			
12	210.0±25	288.5±3.5	233.8±37.8	169.8±21.1	394.0±51.4	409.8±50.5
36	454.5±3.5	255.5±2.1	496.0±97.7	516.0±29.3	1159.0±154.8	586.0±98.8
60	702.5±53	194.0±29.7	871.8±165	280.5±18.9	1515.8±63	578.8±49
84	787.5±64[a]*	222.0±15.6	903.0±131[a†]	211.1±27.5	1526.5±108.3[b†]	472.5±40.8
			Glucose			
12	44.5±.7	49.0±0	42.5±.6	43.5±4.5	40.5±4.9	42.0±4.5
36	40.5±.7	43.0±1.4	34.0±1.4	44.5±.82	27.3±1.3	39.0±.82
60	37.0±0	46.0±0	27.0±1.15	45.3±1.5	20.8±.96	38.0±1.4
84	39.5±[c]**	48.0±0	30.3±2.3[d]***	49.0±1.73	21.5±.58[e†]	35.5±1.3

[1]Measured from start of fast.

[2]Number of animals is in parenthesis.

*The mean is significantly different from mean in fed state, $p < .01$.

**Significant difference at $p < .025$.

***Significant difference at $p < .005$.

†Significant difference at $p < .001$.

Different superscripts indicate different means as tested by the student t-test, $p < .005$.

Table 4. Correlations between plasma oPL concentrations and plasma FFA, glucose, and acetate.

Treatment	Mid-gestation (5)[1]			Late gestation (4)		
	FFA	Glucose	Acetate	FFA	Glucose	Acetate
Fast 36 hours	.55	-.98*	-	-.74	.57	-
84 hours	.48	-.82	-	-.29	.09	-
Fed	.11	-.04	-	-.68	.01	-
Acetate injection	-	-	.18	-	-	.04

*Significant at $p < .05$.

[1]Number of animals is in parentheses.

Table 5. Effect of fasting and stage of gestation on mean (+SEM) half-life ($T_{\frac{1}{2}}$) and disappearance rate (k) of glucose following glucose infusion.

	$T_{\frac{1}{2}}$ (minutes)			k (% min^{-1})		
	Non-pregnant	Mid-gestation	Late gestation	Non-pregnant	Mid-gestation	Late gestation
Fast	138.0±8.7	123	115.7±19.1	.51±.03*	.56	.56±.08
Fed	91.4±12.1	77±13.3	81	.87±.15	.96±.2	.86

*Significantly different from the mean in the fed state, $p < .005$.

II. Arachidonic Acid Study

The effects of 25 and 50 mg doses of arachidonic acid on plasma oPL concentrations are depicted in Figure 5. The ANOVA performed on the data up to the 180 minute sample showed a trend ($p < .1$) toward differences in treatment effects which may have become significant with a greater number of animals. Due to unequal group sizes at the 240 and 300 minute samples, these values could not be included in the ANOVA. Therefore, student t-test were performed which showed a significant difference in mean oPL concentration in response to 25 mg (n=2) and 50 mg (n=4) arachidonic acid at 240 minutes ($p < .05$) and 300 minutes ($p < .025$) after injection. Plasma oPL concentration decreased after palmitic acid and 50 mg arachidonic acid as evidenced by a significant negative regression coefficient (Table 6). No changes were seen after saline.

III. Uterine Activity Study

Plasma oPL concentrations in samples (from either the uterine or jugular vein) taken before a period of contracture activity (representing a baseline) showed no consistent changes when compared to oPL concentrations in samples taken within 20-25 minutes ($T_{\frac{1}{2}}$ for oPL in plasma) after cessation of activity (which would reflect changes in oPL production during that period)(Table 7).

The 12.5 mg dose of arachidonic acid caused an immediate increase in frequency of uterine contractures (Table 8) and an increase in jugular vein plasma oPL concentration after approximately 120 minutes in two out of three animals. The third animal showed a transient increase followed by a decrease (Fig. 6). OPL concentration in the uterine vein increased

Table 6. % Change in plasma oPL concentrations (mean ± SEM) following injection of palmitic acid, saline and arachidonic acid (50 mg).

Time[1] (min.)	Treatment		
	Palmitic acid (6)[2]*	Saline (5)	50 mg arachidonic acid (4)**
0	100	100	100
5	101.7±5.6	117.9±12.3	119.6±10.1
10	99.0±5.18	113.6±11.4	112.8±14.7
15	100.3±6.1	108.6±11.2	114.9±17.7
20	93.6±6.0	103.4±14.6	104.9±15.7
30	88.0±5.55	106.8±11.2	98.3±5.3
45	98.6±27.8	88.8±2.3	110.4±5.9
60	100.8±8.8	117.5±10.5	92.1±7.3
75	84.3±4.4	103.8±12.5	104.6±6.9
90	97.0±6.7	119.4±14.3	102.3±2.6
105	92.0±2.4	100.8±19.2	90.4±7.6
120	95.0±5.0	113.8±5.1	92.5±8.2
135	99.0±6.08	104.0±11.2	93.1±9.1
150	89.4±8.45	110.6±9.9	85.1±4.4
180	99.8±12.9	111.0±13.1	82.1±9.7
240	87.3±12.4	113.2±12.1	72.8±9.8
300	79.8±6.4	109.5±12.5	79.3±10.0

[1]Following treatment injection.

[2]Number of animals is in parentheses.

*Slope(b)≠0; significant at $p < .025$.

**Slope(b)≠0; significant at $p < .001$.

Fig. 5. % of baseline plasma oPL concentration in samples taken following injection of 25 mg (open circles) and 50 mg (closed circles) arachidonic acid. Each point is a mean + SEM. N=4 except for 25 mg at 240 and 300 minutes n=2.

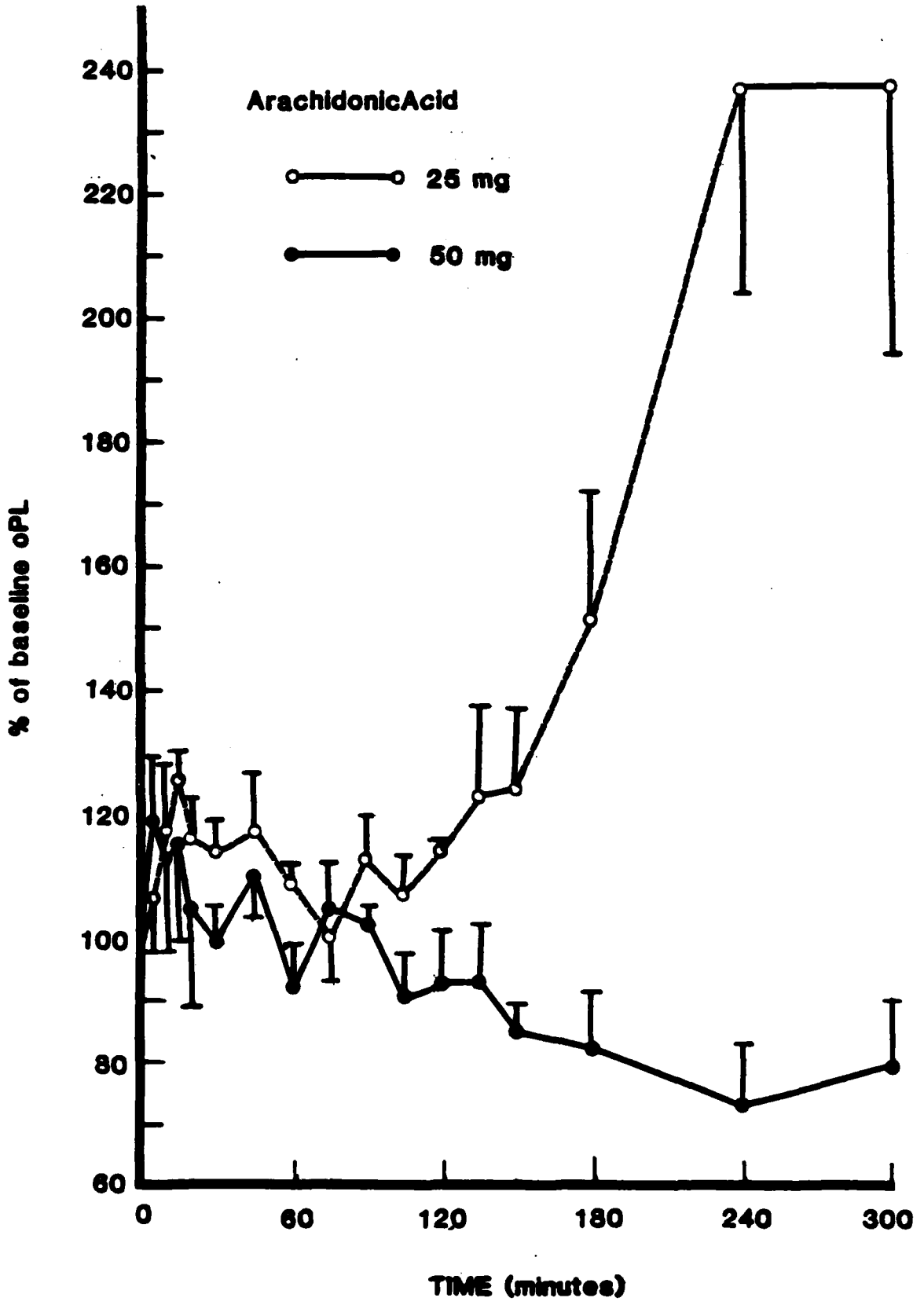

ArachidonicAcid
25 mg
50 mg
% of baseline oPL
240
220
200
180
160
140
120
100
80
60
0
60
120
180
240
300
TIME (minutes)

Table 7. Changes (%) in plasma oPL concentrations during contractures.

Change in oPL[2]	Jugular vein Sample during contracture[1]			Uterine vein Sample during contracture		
	1	2	3	1	2	3
+	36.8[3]	38.9	32.4	16.0	57.7	20.0
0	47.4	41.7	51.4	56.0	42.3	64.0
-	15.8	19.4	16.2	28.0	0.0	16.0
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1]Sample # refers to: 1) first sample during a contracture, 2) second sample during a contracture, and 3) post contracture sample.

[2] + = >10% increase
0 = ±10%
- = ≥10% decrease
Changes are relative to the plasma oPL concentration in the sample preceding the contracture.

[3]% of total observations in the category.

Table 8. Effect of arachidonic acid injection on the mean (±SD) time interval (minutes) between contractures.

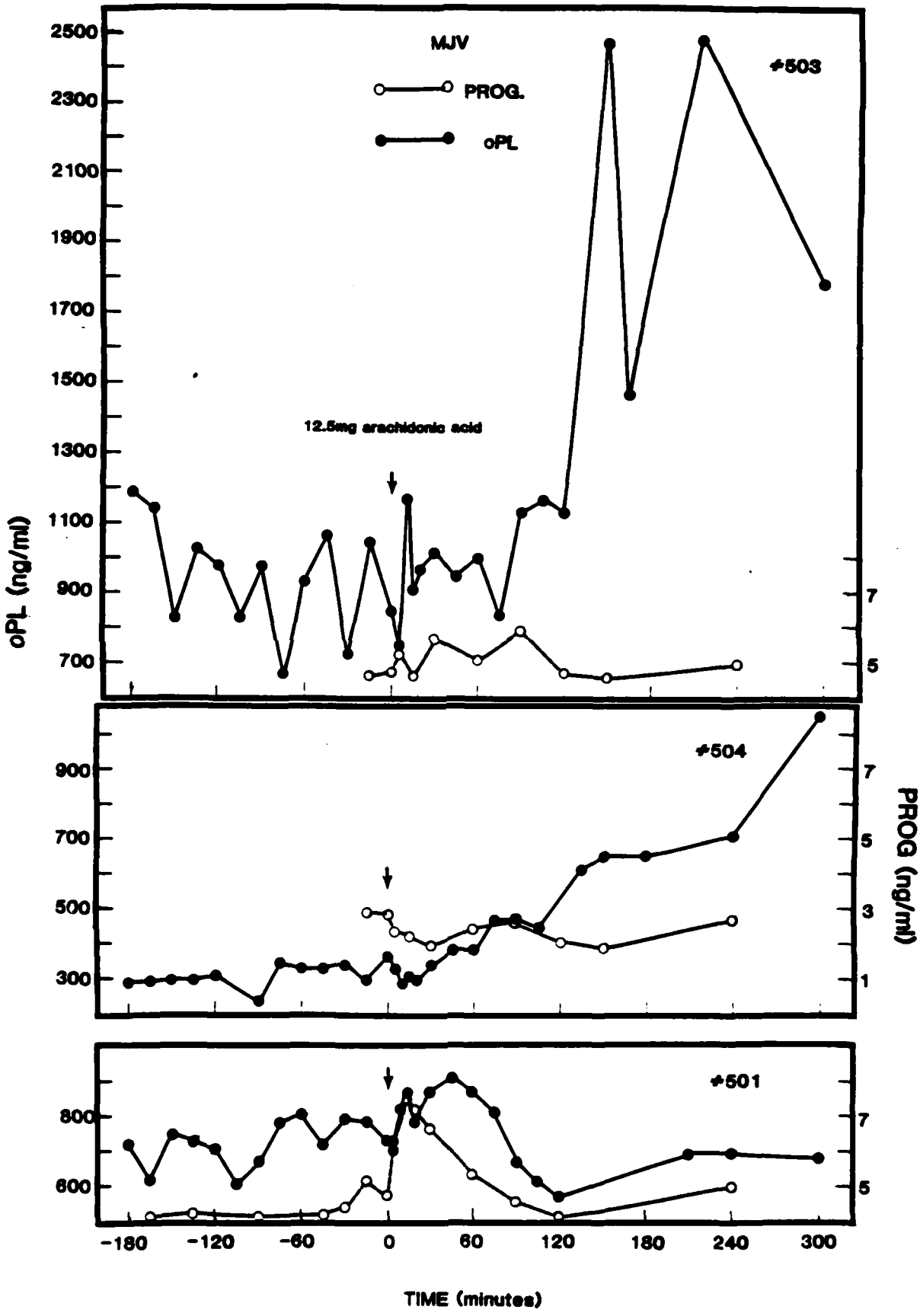
	Arachidonic acid (mg)				
	0	12.5		25.0	
Sheep no.	Control period	Before injection	After injection	Before injection	After injection
501	36.2±4.8	36.2±4.8	35.5±8.7	35.5±8.7	19.7±5.6**
503	41.8±9.5	65.2±17.8	44.4±17.9	93.1±37.2	98.1±22.4
504	23.6±3.4	32.5±6.5	18.1±4.8*	33.0±6.6	
506	53.2±16.5				
507	28.7±7.8				
508	33.5±6.2	33.5±6.2	3.5±2.9***		

*Mean is significantly different from the before injection mean at $p < .025$.

**Significant at $p < .005$.

***Significant at $p < .001$.

Fig. 6. Maternal jugular vein (MJV) plasma oPL (closed circles) and progesterone (open circles) concentrations in three ewes before and following injection of 12.5 mg arachidonic acid.


MJV
PROG.
oPL
#503
12.5mg arachidonic acid
#504
#501
oPL (ng/ml)
PROG (ng/ml)
TIME (minutes)
2500
2300
2100
1900
1700
1500
1300
1100
900
700
900
700
500
300
800
600
7
5
3
1
-180
-120
-60
0
60
120
180
240
300

in the single animal where it was measured.

In contrast, jugular vein progesterone did not change in two out of three animals following arachidonic acid while the third animal showed a transient increase followed by a decrease (Fig. 6). Uterine vein progesterone appeared to increase dramatically within 15 minutes following 12.5 mg arachidonic acid in all three animals in which it was measured (Fig. 7). Uterine blood flow measurement in a single animal indicated that blood flow in the uterine artery decreased following injection of 12.5 mg arachidonic acid (Fig. 10).

OPL concentrations in jugular vein plasma following a larger dose (25 mg) of arachidonic acid increased in only one of the three animals tested (Fig. 8). In the animal that responded there was also a significant increase in frequency of contractures (Table 8), a decrease in uterine arterial blood flow (Fig. 11) and a delayed increase in jugular vein progesterone (Fig. 8).

Uterine vein progesterone and oPL concentrations did not change following 25 mg arachidonic acid in the single ewe where it was measured. In general, the concentration of uterine vein progesterone was approximately 4-5 fold greater than jugular vein progesterone concentration in samples taken concurrently. In contrast, the ratio of uterine to jugular vein oPL concentration was near unity.

Due to the small number of animals in each category further statistical analyses were not performed on the data.

Fig. 7. Plasma oPL (closed circles) and progesterone (open circles) in the uterine vein of the pregnant uterine horn (UVPH) in three ewes following injection of 12.5 mg arachidonic acid.

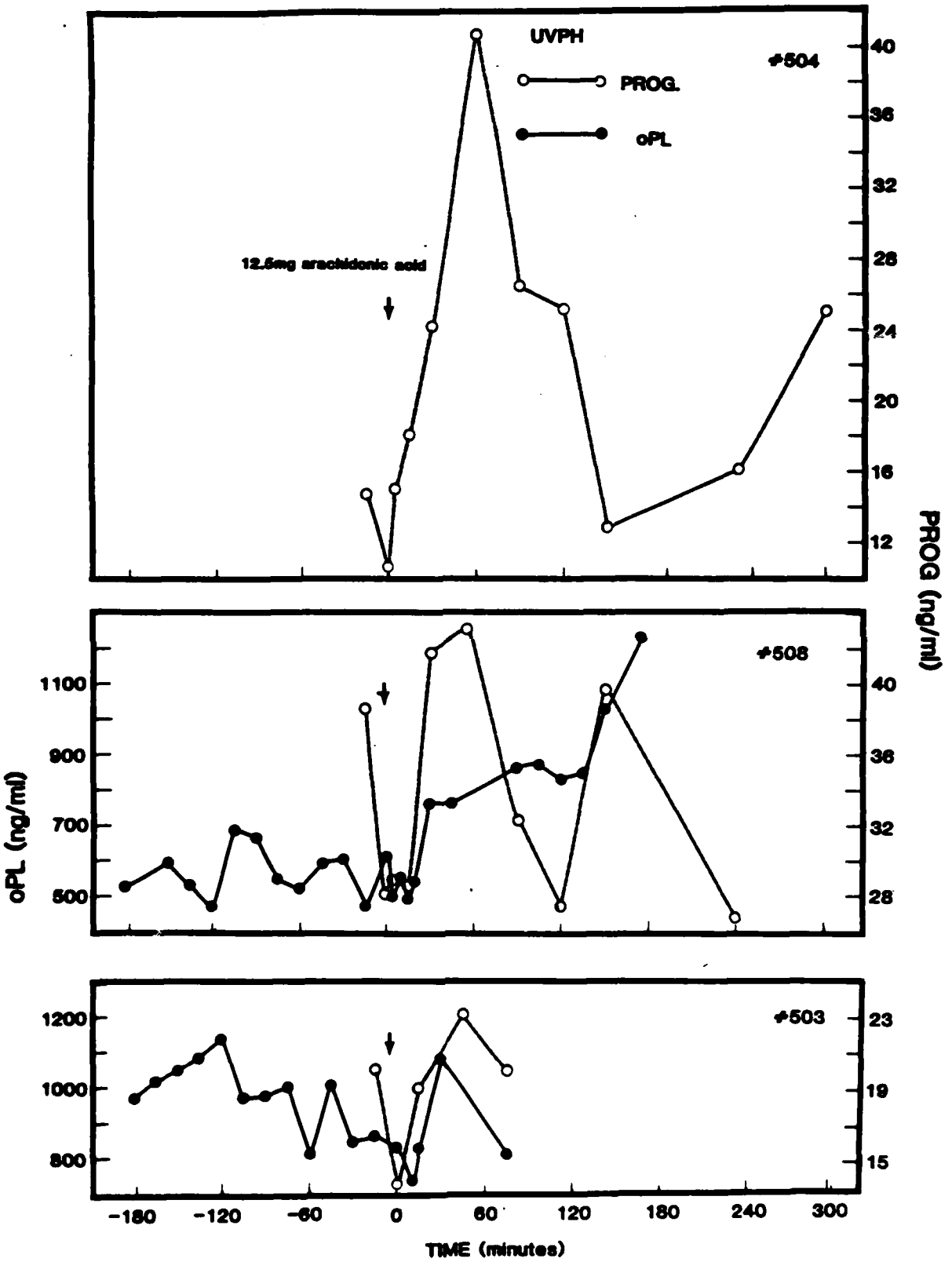

UVPH
#504
PROG.
oPL
12.5mg arachidonic acid
40
36
32
28
24
20
16
12
PROG (ng/ml)
#508
1100
900
700
500
oPL (ng/ml)
40
36
32
28
#503
1200
1000
800
23
19
15
-180
-120
-60
0
60
120
180
240
300
TIME (minutes)

Fig. 8. Maternal jugular vein (MJV) plasma oPL (closed circles) and progesterone (open circles) in three ewes following injection of 25.0 mg arachidonic acid.

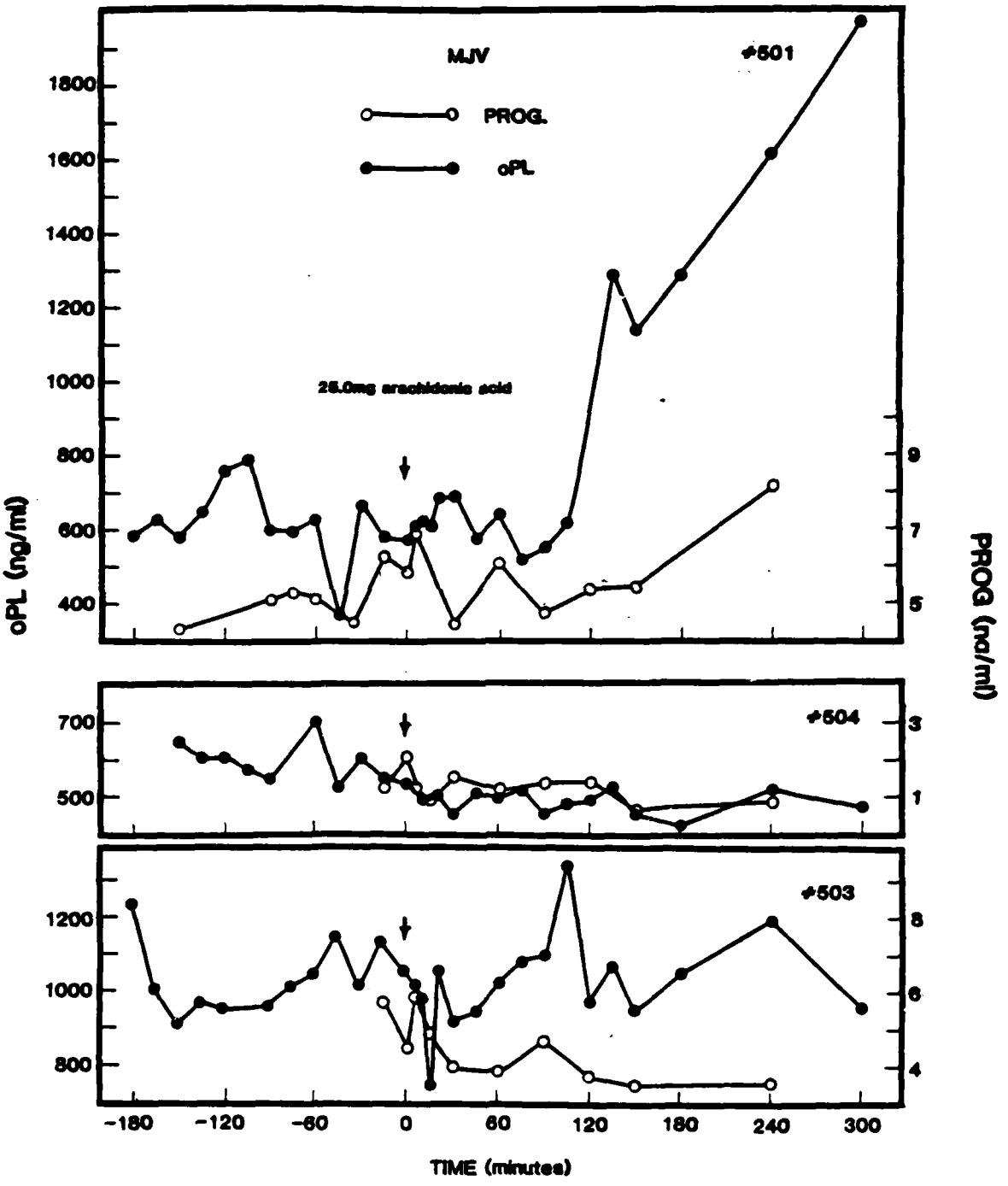

MJV
#501
PROG.
oPL
25.0mg arachidonic acid
#504
#503
oPL (ng/ml)
PROG (ng/ml)
TIME (minutes)
1800
1600
1400
1200
1000
800
600
400
700
500
1200
1000
800
9
7
5
3
1
8
6
4
-180
-120
-60
0
60
120
180
240
300

Fig. 9. Uterine electromyographic (EMG) activity in two pregnant ewes during control periods.

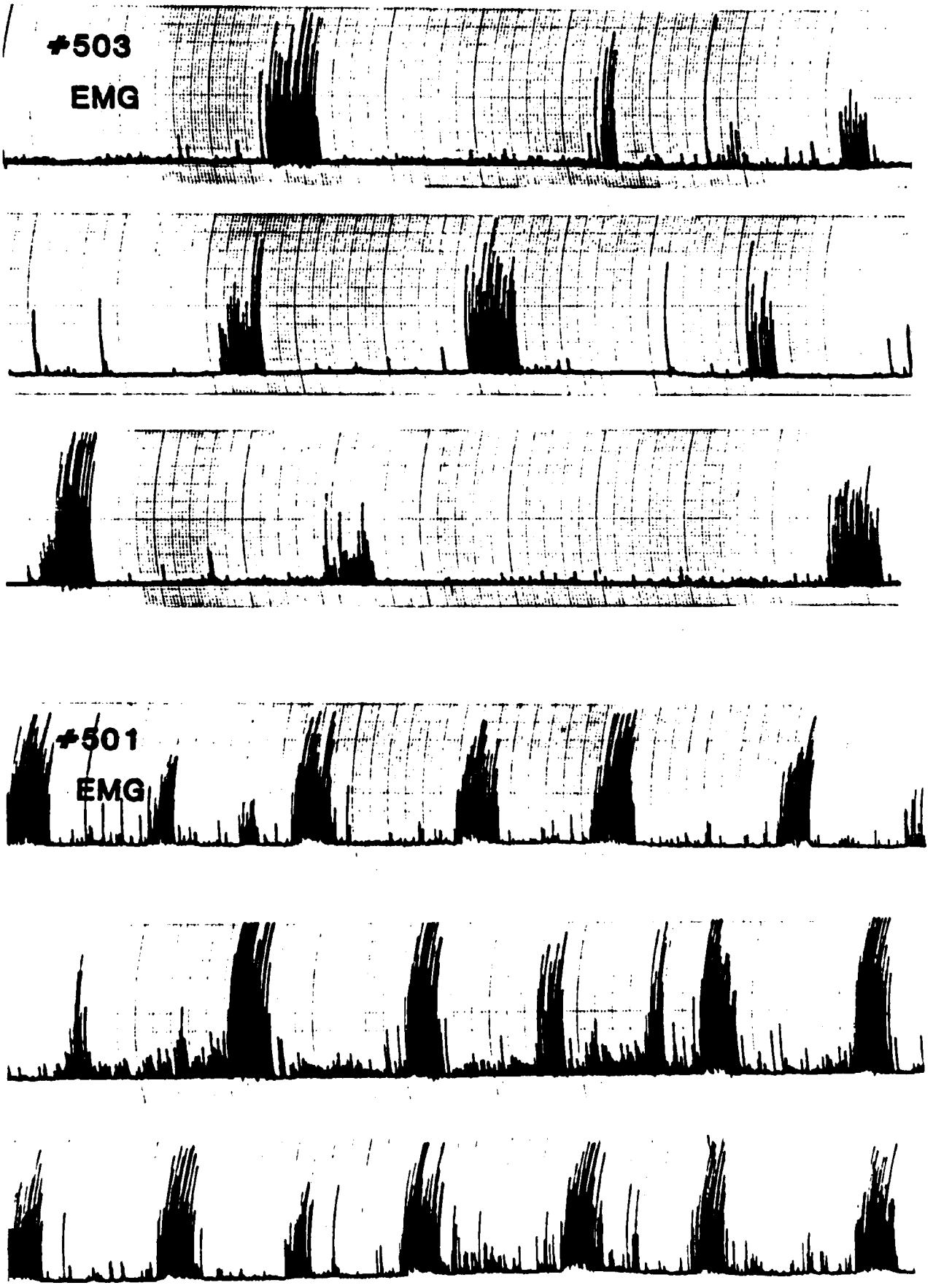
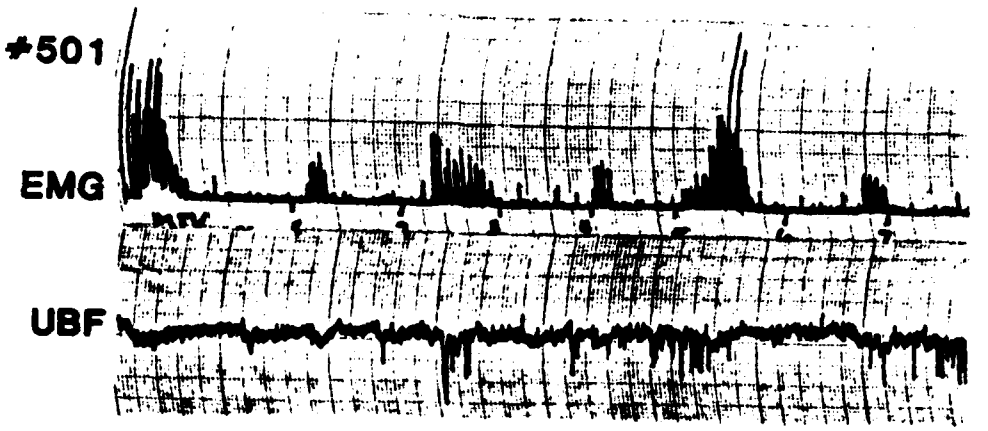
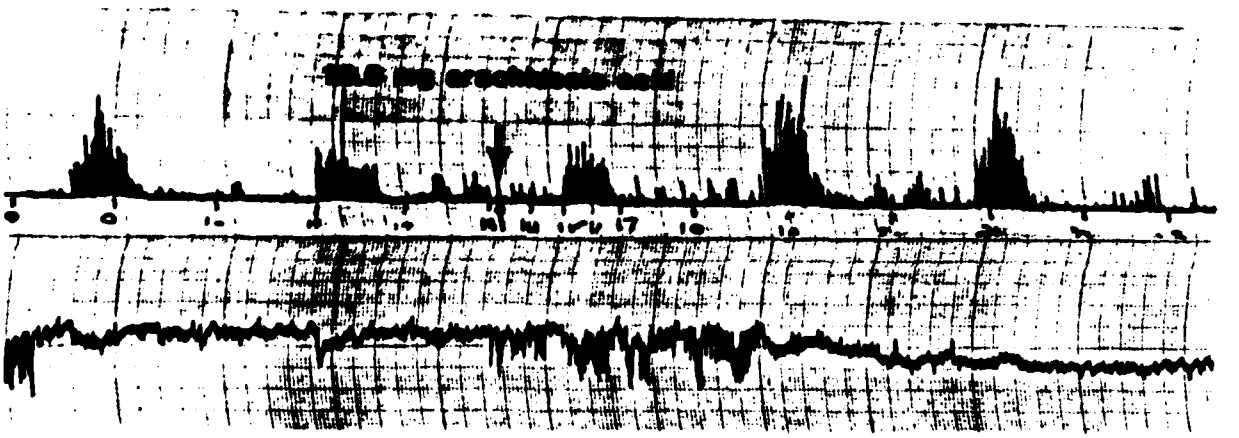

#503
EMG
#501
EMG

Fig. 10. Uterine EMG activity and uterine blood flow (UBF) in one pregnant ewe before and after injection of 12.5 mg arachidonic acid.


#501
EMG
UBF



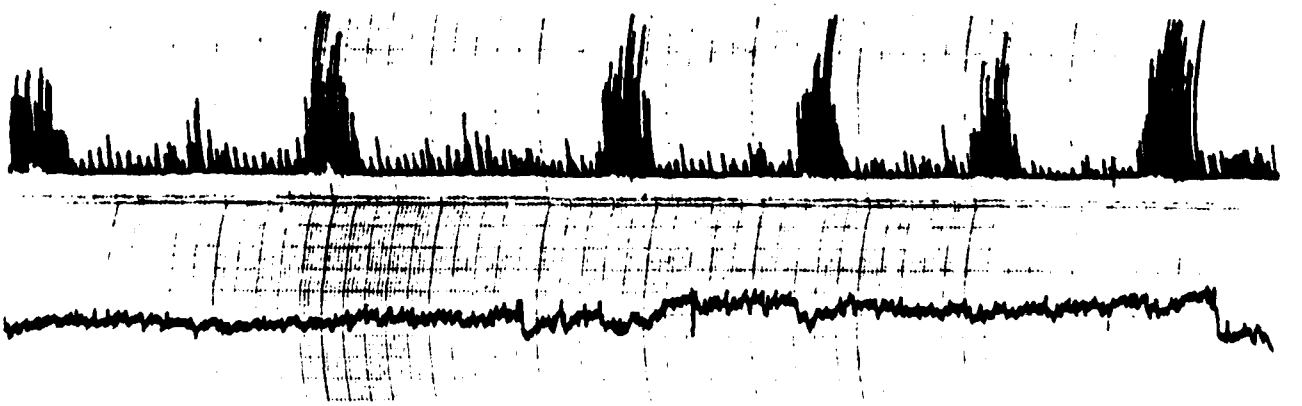
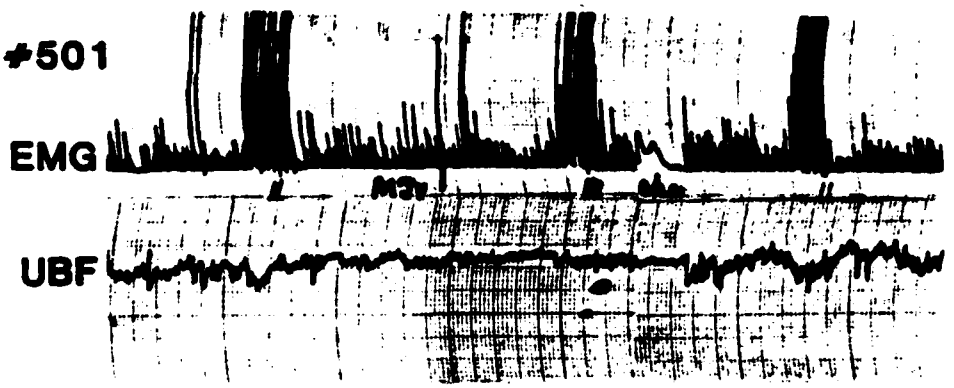
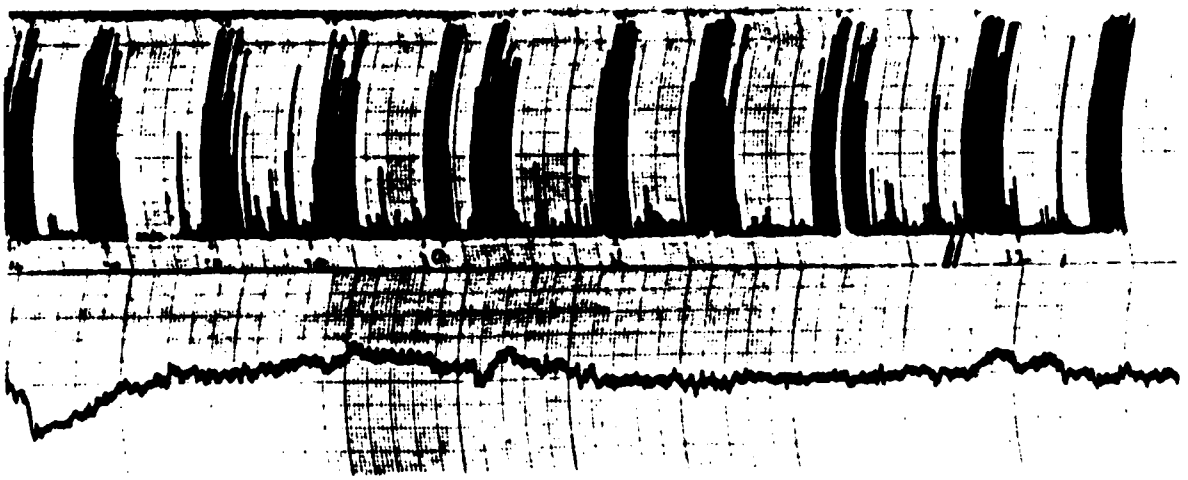


Fig. 11. Uterine EMG activity and UBF is one pregnant ewe before and after injection of 25.0 mg arachidonic acid.


#501
EMG
UBF



DISCUSSION

The existence of great variability in the secretory pattern of oPL was evident from samples taken during the fasting study confirming results of others in sheep (Taylor et al., 1980) and women (Vigneri et al., 1973). As a result, investigations were conducted to determine possible causes for this variability.

Blood samples were taken before and after periods of uterine electromyographic (EMG) activity in an attempt to determine if the fluctuations seen in oPL concentrations in sequential blood samples were related to uterine activity. Depending on the amount of myometrium involved and the intrauterine pressure (IUP) generated, contraction of the uterus could either increase uterine blood flow by forcing blood out of the uterus, decrease uterine blood flow (UBF) by compression of vessels, or not alter flow.

Since no consistent patterns of change in oPL levels were evident form precontracture samples to samples taken during a contracture and within 20 minutes ($T_{\frac{1}{2}}$ for oPL) after a contracture, uterine blood flow changes at these stages in gestation either have no effect on PL release into the circulation or occur in a random fashion independent of uterine EMG activity. The latter condition has been demonstrated in humans (Brotanek et al., 1969). In contrast, during spontaneous or induced labor in sheep (Assali et al., 1958; Greiss, 1965) and women (Brotanek et al., 1969) UBF decreased proportionally to the intensity of the contraction and increased during the relaxation phase. When IUP exceeds 60-70 mm Hg in sheep, UBF practically ceases (Assali et al., 1958)

Since IUP increases by only approximately 3.5 mmHg in these pre-labor contractures (Nathanielsz et al., 1980) it is also possible that the UBF is not altered or that more frequent blood samples need to be taken and assayed for oPL during a contracture.

Possible effects of arachidonic acid on PL variability were investigated in two studies. Arachidonic acid could work directly or through conversion to $PGF_2\alpha$, a vasoconstrictor which also increases myometrial activity; prostacyclin (PGI_2), a vasodilator; PGE_2; thromboxanes; or products of the lipoxygenase pathway. The active substance might possibly increase oPL by influencing membrane permeability, vascular activity, myometrial activity, or synthesis directly. By influencing membrane permeability, arachidonic acid or a metabolite could alter the membrane flux of an ion or metabolite that effects oPL production. Arachidonic acid has been shown to influence calcium flux in neutrophils (Volpi et al., 1980) but the effect of calcium or other ions on PL production has not been conclusively defined (Handwerger et al., 1981; Choy and Watkins, 1976; and Welsch, 1979).

The results of the first arachidonic acid study indicate a stimulatory effect of injection of 25 mg arachidonic acid on plasma oPL concentration while a higher dose had no effect or was inhibitory. This data confirms the findings in vitro of increased hPL release from placental decidua following incubation with arachidonic acid (Handwerger et al., 1981).

In the Rambouillet ewes used in the uterine activity study, the 12.5 mg dose of arachidonic acid stimulated production of oPL and the 25 mg dose was ineffective in three out of four animals. In the fourth animal the 25 mg dose had the same stimulatory effect on oPL concentrations

as was seen in the first study, but oPL levels did not increase in response to the 12.5 mg dose. Thus, a threshold dose, which may vary due to breed or surgery effects, of arachidonic acid appears to lead to an increase in oPL production. At a sub-threshold or supra-threshold dose there may be inhibition of oPL response. The time lag between arachidonic acid injection and the increase in PL concentration, approximately 90 minutes, is adequate for the induction of cellular machinery necessary for new protein synthesis. Palmitic acid inhibited PL production in the same manner as the large dose of arachidonic acid. Since it is not metabolized to and is not a metabolic product of arachidonic acid, another mechanism must be in effect. The inhibition was not seen with the BSA-saline, eliminating a non-specific vehicle effect.

Before the alterations in oPL concentration there were immediate increases in the frequency of contractures, indicating that there are two mechanisms of action, one effecting contractures and the other oPL. In the single animal where uterine blood flow was measured, it decreased simultaneously with the alterations in contracture frequency. The decrease in UBF seen following the 12.5 mg dose of arachidonic acid could lead to the rapid, transient increase in uterine vein progesterone. However, if the increase in progesterone was UBF related, oPL concentration would be expected to change concurrently since they are both produced by the placenta in the ewe. The increase in plasma oPL is seen 75-90 minutes after the increase in progesterone indicating that two distinct stimulatory mechanisms are again in effect.

Alternatively, the arachidonic acid may directly effect production of placental or ovarian progesterone. It has been demonstrated that

arachidonic acid decreases peripheral progesterone and increases ovarian $PGF_2\alpha$ (Shemesh and Hansel, 1975) and PGI_2 increases ovarian progesterone production (Milvae and Hansel, 1980) when injected directly into the bovine corpus luteum. Thus, both arachidonic acid or its metabolite may alter the uterine vein progesterone production, which is approximately 35% of the total progesterone produced during late gestation in the sheep (Bedford et al., 1973).

In addition to possible direct effects of arachidonic acid on placental hormone variability, or conversion of arachidonic acid the ovary, the uterus and feto-placental unit may also metabolize arachidonic acid. Early in gestation the ovine endometrium and embryo will preferentially synthesize PGI_2 from exogenous arachidonic acid (Marcus, 1981). In late human gestation injection of arachidonic acid into the amniotic cavity induces labor and subsequent abortion which may be blocked by aspirin ingestion, indicating the effect is via a product of the cyclo-oxygenase pathway (MacDonald et al., 1974). Thus, by MacDonald et al., propose that release of arachidonic acid from the cell membrane by phospholipase A_2 is the limiting factor in $PGF_2\alpha$ synthesis by the uterus, injection of exogenous arachidonic acid allows the limiting step to be by-passed and early labor is induced.

$PGF_2\alpha$ has been demonstrated to decrease hPL production both in vitro (Genbacev et al., 1977) and in vivo (Ylikorkala and Pennanen, 1973). Handwerger et al. (1981) found that addition of $PGF_2\alpha$ to incubation medium containing human placental descidua had no effect on arachidonic acid stimulated hPL release. These results support a hypothesis that a mechanism other than conversion to $PGF_2\alpha$ is responsible for the increases

seen in oPL production after arachidonic acid injection. However, the decrease in UBF and increase in frequency of uterine contractures seen in this study could possibly be initiated subsequent to conversion of arachidonic acid to $PGF_{2}\alpha$.

There are other conditions which may cause variability in plasma PL concentrations. Morriss (1980) demonstrated that UBF decreases during a fast of ewes in late gestation. Fasting also results in an increase in plasma PL concentration in sheep (Brinsmead et al., 1981) and women (Tyson et al., 1971). It may be that PL production is sensitive to the alterations in blood flow or plasma metabolite concentrations which occur during a fast.

HPL has been demonstrated to have both lipolytic and diabetogenic properties (Knopp, 1973) and has been associated with the appearance of these characteristics during human gestation (Baird, 1969). Investigations into regulation of oPL have been used as a model for control of hPL production and action. However, there is little evidence that oPL and hPL are regulated by the same factors or perform the same function.

Despite the occurrence of maternal hypoglycemia and hyperlipidemia in all fasted animals, there was great variation between animals in response of plasma oPL concentrations to the fast. This is in contrast to responsiveness of hPL levels in women in equivalent stages of gestation (Tyson et al., 1972).

In this study there was an increase in plasma FFA concentration during the fasting period in all three groups of ewes. Although the pre-fast FFA level was highest in the ewes in late gestation, the percent increase in FFA levels over the four day fast was nearly identical in all cases; 387%,

386%, and 375%, in late gestation, mid gestation, and nonpregnant ewes respectively. This indicates that oPL may be enhancing lipolysis during pregnancy in sheep, but is probably not responsible for the accelerated lipolysis of starvation. Plasma oPL concentrations were not correlated with FFA levels in fed or fasted sheep.

Maternal plasma glucose concentrations were depressed most severely in ewes during a fast in late gestation, probably due to removal of 1.2 grams glucose/hour per fetus from the maternal pool at this stage in gestation. Glucose turnover rate, the combination of production and use, increases during gestation in sheep as fetal energy needs increase. In a twin pregnancy, glucose turnover is increased by approximately two-thirds, emphasizing the extent of the carbohydrate drain of pregnancy on an animal which is already dependent on gluconeogenesis (Bergman, 1963).

There is a significant negative correlation between plasma oPL and glucose concentrations during the fast in mid gestation animals. This implies that as glucose levels decrease, oPL concentration increases, perhaps in an attempt to prevent a metabolic crisis in the mother or fetus. Mid gestation is the time when the homeorhetic adaptations of pregnancy are taking place (Bauman and Currie, 1980) and metabolism is altered to supply fetal need. OPL may coordinate the changes which occur to ensure use of FFA as the primary maternal energy source, so glucose is spared for almost exclusive use by the fetus.

The $T_{\frac{1}{2}}$ and k values obtained in this study indicate that ruminants have a much lower tolerance to a glucose load than do non-ruminants. Average values in the normal human are $T_{\frac{1}{2}}$ = 30-60 minutes, k = 1.2-2.2%

min^{-1} and in diabetic individuals, $T_{\frac{1}{2}}$ = 80 minutes and k = .9% min^{-1}. There are no differences seen in these values in the three groups. Thus in contrast to hPL action, it appears that oPL is not exerting a diabetogenic effect in sheep.

Fasting also resulted in decreased rumen fermentation as reflected by decrease in acetate production. Acetate is the most important lipogenic precursor for both adipose tissue and mammary gland in ruminants, making it the primary energy source. The lack of correlating changes in oPL concentration after acetate injection indicates that these concentrations are not influenced by acute changes in energy levels as expressed by plasma acetate concentrations. This is in contrast to human studies where levels of glucose, the primary energy source in ommnivores, have been correlated with decreased hPL concentrations (Burt et al., 1970).

Changes seen in plasma oPL concentrations during the fasting study may be confounded by the shifts that take place during the normal course of gestation; oPL levels are increasing from 60-90 days, plateau near 100-110 days, and begin to decrease 5-10 days before parturition.

Perhaps there is another regulatory factor involved in PL production. Knopp and Ruder (1970) propose that hormones produced by the placenta limit glucose use by extra-uterine tissues but are not subject to feed back regulation by maternal nutritional status. Thus, placental hormone production might respond to a UBF mediated decrease in delivery of nutrients to the placenta, which would not necessarily be reflected in decreased nutrient concentration in the peripheral plasma. The variations seen in PL may be the result of a physiologic response to fluctuations in placental perfusion.

CONCLUSIONS

The purpose of these studies was to investigate reasons for the great variability in plasma oPL concentration seen between sequential blood samples and to clarify the possible metabolic role of oPL during gestation. Although the effect of fasting in mid gestation ewes was statistically significant, it may not be biologically significant since the response was not consistent in all ewes and was confounded by the increase in plasma oPL concentration seen normally in gestation. Pregnancy had a lipolytic effect which was enhanced by starvation, but the diabetogenic effect of fasting was not altered by pregnancy. This indicates that in contrast to results seen with hPL, oPL does not appear to have diabetogenic effects.

A threshold dose of arachidonic acid, which differed between two trials, was associated with an immediate increase in the frequency of uterine contractures and a decrease in uterine blood flow and elicited a delayed increase in plasma oPL concentration. The possibility exists that there is a dose-response relationship between arachidonic acid and oPL. Since animals were only treated at two doses, the exact nature of the relationship between oPL and arachidonic acid is difficult to evaluate. Additional studies using doses of arachidonic acid over a wide range in individual animals would elucidate this question. Progesterone, which is also produced by the placenta in pregnant ewes, showed only a rapid, transient increase in concentration, indicating that it is not affected by the same mechanism which stimulated oPL production. The

time course of events suggests that the immediate alterations in uterine contracture and uterine blood flow may be mediated by a prostaglandin, while the delayed changes seen in oPL are controlled by another mechanism of action. To understand changes which may occur in placental hormone production, the uterus should be viewed as an organ capable of synthesis, perfused by blood from the uterine artery and drained by the uterine veins. Placental hormones, such as oPL, are released at a constant rate by cells in the placenta into the uterine circulation. If the production rate stays constant, a decrease in blood flow through the uterus should result in a decrease in delivery of oPL to the periphery and a decrease in jugular vein plasma oPL concentration, an increase in blood flow through the uterus would have opposite effects. In this study, perturbation of the system by arachidonic acid resulted in a decrease in uterine arterial blood flow. Since plasma oPL concentrations increased rather than decreased following the injection, the effect must be mediated via a non blood flow related route. If the bolus of arachidonic acid is able to alter the rate of membrane phospholipid turnover, a change in membrane fluidity might increase the rate of oPL release from the site of synthesis. Alternately, since the increase seen in plasma oPL concentration lags the arachidonic acid injection by 90-120 minutes which would allow time for new protein synthesis, the synthesis rate for oPL may be increased. The trigger for the increase in production could be either the direct effect of arachidonic acid or its metabolites, or the simultaneous decrease in uterine blood flow (UBF). If PL has a metabolic role during gestation to ensure a constant supply of nutrients to the

fetus, then a decrease in UBF and/or the accompanying decrease in nutrient supply to the fetus, might activate PL production.

Further investigations into the mode of action of arachidonic acid need to be accomplished, perhaps initially by administering arachidonic acid, both *in vivo* and *in vitro*, blocking its metabolism and examining changes in cell membrane composition and oPL concentration. Also, the possibility that oPL production responds to a decrease in uterine blood flow, either directly or via the corresponding decrease in placental perfusion of metabolites, needs to be elucidated by closely monitoring these parameters during the fed state and examining changes during the fasted state. Direct examination into oPL-UBF-arachidonic acid relationships could be accomplished by administering epinephrine (known to decrease UBF) and measuring oPL, or injecting oPL into the uterine circulation, monitoring UBF and possible alterations in phospholipid composition of cell membranes.

These studies would attempt to answer the questions raised regarding the physiological role of oPL during gestation and the function of arachidonic acid in mediating that role.

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APPENDIX

AD-A125 367 RELATIONSHIPS BETWEEN ARACHIDONIC ACID UTERINE ACTIVITY AND METABOLIC REG.. (U) AIR FORCE INST OF TECH WRIGHT-PATTERSON AFB OH S E HUYLER AUG 82 2/2

UNCLASSIFIED AFIT/CI/NR-82-67T F/G 6/1 NL

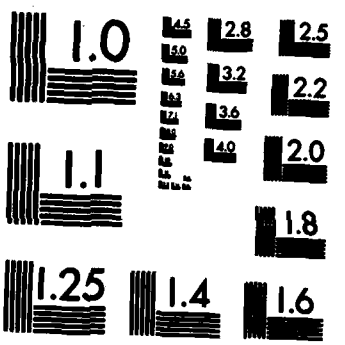

Table 1. Analysis of variance for plasma oPL concentrations in the fed and fasted state during gestation.

Source of variation	d.f.	Mean square	F value
	MID GESTATION		
Sheep	4	879167	158.8***
Treatments	2	131965	23.8***
Sheep x treatments	8	329587	59.5***
Residual	36	5534	
	LATE GESTATION		
Sheep	3	14188469	220.81***
Treatments	2	236452	3.68*
Sheep x treatments	6	612630	9.5**
Residual	36	64249	

*Significant at $p < .05$.

**Significant at $p < .01$.

***Significant at $p < .001$.

Table 2. Split plot in time analysis of variance for plasma oPL concentrations following treatments of saline, palmitic acid, 25 mg arachidonic acid and 50 mg arachidonic acid.

Source of variation	d.f.	Mean square	F value
Sheep	3	803	.35
Treatments	2	8066.6	3.497
Sheep x treatments	6	2306.6	7.94*
Time	13	347.9	1.198
Time x treatments	39	244.9	1.33
Residual	78	290.4	

*Significant at $p < .01$.