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PREECLAMPSIA: AN IMBALANCE IN PLACENTAL PROSTACYCLIN, THROMBOXANE AND PROGESTERONE PRODUCTION

By

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"The correlations found between changes of weather (particularly sudden changes of pressure) and the occurrence of toxaemia were neither constant nor absolute. In all probability, these weather-factors act merely as stress agents, and influence only those pregnant women who have an unstable hormonal and neurovegetative equilibrium."(Azzarone, 1959)

With the etiology of preeclampsia still in question it is comforting to know that one of the most unpredictable phenomenons in the world has been eliminated as causative agent in preeclampsia.

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ABSTRACT

PREECLAMPSIA: AN IMBALANCE IN PLACENTAL PROSTACYCLIN, THROMBOXANE AND PROGESTERONE PRODUCTION

By

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The production of the vasoactive substances, prostacyclin (PGI) and thromboxane (TXA), and the steroids, progesterone and estradiol, were investigated in normal and preeclamptic placentas. Tissues (350 mg) were incubated for 4 h (steroid studies) and 48 h (eicosanoid studies) at 37° C with 95% O2, 5% CO2 in a metabolic shaker. PGI production was decreased ($P<0.001$) in preeclamptic vs. normal tissue, whereas the production of TXA and progesterone were increased ($P<0.0001$ and $P<0.01$, respectively). The production of estradiol was not significantly different ($P>0.50$) in normal vs. preeclamptic tissues. Addition of progesterone or progesterone and estradiol (100 uM) decreased PGI production ($P<0.01$) but not TXA production ($P>0.50$). Estradiol alone (100 uM) had no effect. Addition of a thromboxane synthase inhibitor selectively inhibited TXA production. These data indicate preeclampsia is characterized by an imbalance in placental PGI and TXA production. A local increase in progesterone could contribute to the ↑TXA/↓PGI imbalance of preeclamptic placentas. Thromboxane synthase inhibitors may be useful in treating preeclampsia.

I. INTRODUCTION

The hypertensive disorders of pregnancy account for about a fifth of the maternal deaths that occur each year (Chesley, 1978). In addition, these disorders pose a serious threat to the fetus and to the newborn infant who is often delivered prematurely, either following spontaneous labor or by therapeutic termination of pregnancy (Chesley, 1978). This thesis is concerned with the etiology of one of the hypertensive disorders of pregnancy, preeclampsia. According to a survey of eleven major hospitals in the late 1970's preeclampsia accounted for 48-92 percent of the cases diagnosed as a hypertensive disease of pregnancy (Chesley, 1978). The clinical symptoms of preeclampsia include; high blood pressure, edema, proteinurea, central nervous system hyperactivity and disseminated intravascular coagulation (Dennis et al, 1982). To date, the etiology of preeclampsia has been extensively investigated but not elucidated.

For many years the prostaglandins and the thromboxanes have been implicated in the etiology of preeclampsia. Because of their antagonistic vasoactive properties it is increasingly likely that prostacyclin (PGI2) and thromboxane A2 (TXA2) may be involved in preeclampsia. Prostacyclin is a potent vasodilator and an inhibitor of platelet aggregation

(Moncada and Vane, 1979). Whereas, TXA2 is a potent vasoconstrictor and a stimulator of platelet aggregation (Moncada and Vane, 1979). Thus an imbalance in PGI2 and TXA2 could contribute to the clinical manifestations of preeclampsia. The following study was undertaken to simultaneously determine the TXA2 and PGI2 production rates by normal and preeclamptic placentas. In addition, experiments were performed to determine if the placental production of steroids had any effect on the placental production of PGI2 and TXA2.

II. LITERATURE REVEIW

A. Preeclampsia

Preeclampsia ranks as one of the leading causes of maternal death occurring in approximately five percent of all pregnancies (Chesley,1978; Dennis et al., 1982). Two major complications that may occur during preeclampsia are fetal growth retardation and premature delivery. In both cases the infant is usually of low birth weight and therefore is in greater jeopardy during the first few months after delivery. Exact statistics on the incidence of these two complications in association with preeclampsia are not available. However, several studies have documented fetal growth retardation secondary to preeclampsia (Willocks, 1962; Willocks et al., 1964; Hellman et al., 1967). Premature delivery may be due to either premature labor or therapeutic termination of pregnancy (physician induced labor or caesarean section) [Chesley, 1978]. Therapeutic termination of pregnancy is a common occurance because the symptoms of preeclampsia rapidly dissipate after parturition (Dennis et al., 1982). Therefore it is likely that an organ unique to pregnancy, such as the placenta, may be involved in preeclampsia.

The principle pathophysiological changes in preeclampsia are increased vasoconstriction, increased platelet aggregation and reduced uteroplacental blood flow (Dennis et al., 1982; Pritchard et al., 1976). These

abnormalities manifest themselves clinically as high blood pressure, proteinurea, edema, central nervous system hyperactivity and disseminated intravascular coagulation (Dennis et al., 1982). The clinical criteria for the diagnosis of preeclampsia are presented in Table 1 (Chesley, 1978; Dennis et al., 1982). It is important to note that changes at the vascular level could account for, or contribute to, all of these symptoms.

B. Eicosanoids in Preeclampsia

1. Hypersensitivity of the vasculature to angiotensin II

The plasma concentrations of renin, aldosterone (Pedersen et al., 1983) and angiotensin II (A-II)[Chesley, 1978] are significantly lower in the preeclamptic patient than in the normotensive patient. In spite of this, Gant et al. (1973) demonstrated that preeclamptic patients have an enhanced sensitivity to the pressor effects of A-II. In a study of 192 primigravid women, Gant et al. (1973) found that the amount of A-II required to increase the diastolic blood pressure by 20 mm Hg was significantly less in preeclamptic women than in normotensive women. In fact the different responsiveness became apparent as early as 18 weeks before hypertension developed in the preeclamptic patient (Gant et al., 1973). A subsequent study (Everett et al., 1978) revealed that the dose of A-II required to increase the diastolic pressure 20 mm Hg was

TABLE 1

Clinical Criteria for Preeclampsia

SYMPTOM	Necessary Criterion for a Preeclamptic Diagnosis
hypertension	maternal blood pressure during the third trimester greater than 140/90 mm Hg
proteinurea	urinary concentration of protein in a 24 h collection is more than 300 mg/liter
edema	the important factor is the rate at which edema occurs, a weight gain of more than 2 kg/week is abnormal
central nervous system hyperactivity	the maternal reflexes become more sensitive to stimulation with progression of the disease
coagulation disorders	some level of disseminated intravascular coagulation is likely to accompany the disease

significantly less in normotensive patients treated with prostaglandin synthetase inhibitors than in the untreated normal pregnant women. Everett et al. (1978) concluded that the vascular sensitivity to A-II seen in preeclampsia might be the result of defective prostaglandin synthesis. In addition, Glance et al. (1984) found a dose-related attenuation of the vasoconstrictive actions of A-II by prostacyclin when both were infused into human placental cotyledons in vitro. Therefore, these studies support the hypothesis that there is a vasodepressor prostaglandin deficiency in preeclampsia (Gant et al., 1983).

2. Prostaglandins

The initial interest on prostaglandins during preeclampsia centered on prostaglandin E (PGE) and prostaglandin F (PGF). However, after a decade of studies (1973-1983) no roles for PGE and PGF in the etiology of preeclampsia were convincingly demonstrated. During this time two other vasoactive eicosanoids, prostacyclin (PGI2) and thromboxane A2 (TXA2) were identified. Their antagonistic vasoactive properties have brought them to the forefront of consideration in the etiology of preeclampsia.

3. Prostacyclin

Prostacyclin is a potent vasodilator, an inhibitor of platelet aggregation (Moncada and Vane, 1979) and it decreases uterine activity (Lye and Challis, 1982; Omini et al., 1979; Wilhelmsson et al., 1981; Zahradnik et al., 1983). The major site of production of prostacyclin in the normal adult is the vascular endothelium (Demers, 1980). Another site of production and release of prostacyclin are the tissues in the lung (Gryglewski et al., 1978; Moncada et al., 1978; Hensby et al., 1979). In addition, Dusting et al. (1978) determined that prostacyclin produced by tissues anterior to the pulmonary circulation is not inactivated as it passes through the lungs (unlike other prostaglandins). Therefore prostacyclin may act locally and as a circulating hormone. Because preeclampsia is characterized by increased vasoconstriction frequently associated with increased platelet aggregation and reduced uteroplacental blood flow (Pritchard et al., 1978; Dennis et al., 1982) a decrease in prostacyclin production may play a causative role in preeclampsia.

Prostacyclin increases during normal pregnancy. This has been demonstrated by an increase in urinary levels (Goodman et al., 1982; Brash et al., 1983) and an increase in plasma levels (Lewis et al., 1980) of the stable metabolite of prostacyclin, 6-keto-prostaglandin F1-alpha (6-keto PGF1). The increase in prostacyclin has not been attributed to any specific source. However, many

pregnancy-associated tissues produce prostacyclin in vitro, including uterine tissues (Liggins et al., 1980; Ylikorkala et al., 1983), the placenta (Mitchell et al., 1978; Behr, 1984), the fetal membranes (Mitchell et al., 1978) and the umbilical vessels (Remuzzi et al., 1979). The large mass of the placenta with its highly vascular decidua make it a likely source for the significant production of eicosanoids.

The importance of prostacyclin in pregnancy is exhibited by the regulatory functions in which prostacyclin has been implicated. Tuvemo (1980), utilizing strips of umbilical arteries, determined an integral role for prostacyclin in the regulation of umbilical blood flow. Coceani and Olley (1980) elucidated a contributory role for prostacyclin in maintaining the patency of the ductus arteriosus in the fetus. This has been corroborated by several other investigators (Cassin, 1980; Terragno, 1980; Makila et al., 1983; Ylikorkala et al., 1984). Remuzzi et al. (1979) found that prostacyclin activity from placental and umbilical vessels was greater than that by vessels from normal adults. These investigators concluded that high prostacyclin generation could contribute to maintaining low peripheral resistance in the fetus despite the high cardiac output. Another study by Resnik and Brink (1980) proved that prostacyclin was a vasodilatory agent in the uterine circulation (as well as the peripheral

vessels) of the sheep. Thus, alterations in the production of prostacyclin by the placenta (or other pregnancy-associated tissues) during pregnancy could have grave consequences for the mother and fetus.

Several authors have described depressed levels of prostacyclin in preeclamptic patients. Early investigators determined protacyclin activity utilizing a bioassay to determine platelet aggregation inhibitory activity. Remuzzi et al. (1980) found that prostacyclin activity was significantly lower in umbilical and placental vessels from preeclamptic patients than from normal pregnant women. Bussolino et al. (1980) confirmed these findings in placental veins, subcutaneous vessels and uterine vessels. Prostacyclin-like activity was decreased in amniotic fluid (Bodzenta et al., 1980) as was the concentration of 6-keto PGF1α (Ylikorkala et al., 1981). The levels of urinary metabolites of prostacyclin are decreased in preeclampsia (Goodman et al., 1982; Brash et al., 1983; Fitzgerald et al., 1984). In fact, this change has been shown to occur as early as 20 weeks gestation in the patient destined to become preeclamptic (Fitzgerald et al., 1984). Also, plasma concentrations of prostacyclin have been reported to be decreased (Lewis et al., 1981; Yamaguchi and Mori, 1985) or unchanged during preeclampsia (Ylikorkala et al., 1981).

Studies investigating the production rates of prostacyclin have resulted in similar conclusions. The

production of prostacyclin from preeclamptic umbilical vessels, either rings of vessel or cells in culture, is significantly less than the production from normotensive umbilical vessels (Carreras et al., 1981; Stuart et al., 1981; Makila et al., 1984; Ylikorkala et al., 1984). Also, the production rate of prostacyclin from preeclamptic placental tissues is significantly lower than that for normal placental tissues (Walsh et al., 1985). Not only is less prostacyclin produced in preeclampsia but the responsiveness of platelets to the prostacyclin present is diminished (Lewis et al., 1981; Wallenburg and Rotmans, 1982; Briel et al., 1983). Thus, changes in the levels of prostacyclin during preeclampsia are well documented. However, it is unlikely that deficient production of one substance would mediate all the symptoms/complications of preeclampsia. Several authors have hypothesized that normal vascular function is maintained by a balance in the production of prostacyclin and thromboxane. Furthermore, the vascular changes which occur during preeclampsia are due to a disruption of this homeostasis.

4. Thromboxane

Thromboxane is a potent vasoconstrictor, a platelet proaggregatory agent (Moncada and Vane, 1979; Granstrom et al., 1982), it has a strong contractile effect on airway smooth muscle (Svensson et al., 1977) and it increases

uterine activity (Wilhelmsson et al., 1981). Essentially the actions of thromboxane are in opposition to those of prostacyclin. The circulating platelets are the major source of thromboxane production in the normal adult (Demers, 1980). A balance between prostacyclin formed in the vessel wall and thromboxane formed in platelets may be the regulatory control mechanism of thrombus formation in blood vessels (Gorman, 1979; Moncada et al., 1977; Moncada and Amezcua, 1979; Moncada and Vane, 1979; Needleman et al., 1979). Therefore, the decrease in prostacyclin production during preeclampsia coupled with consistent (or increased) thromboxane levels could contribute to the clinical manifestations of preeclampsia (Pritchard et al., 1976; Chesley, 1978; Dennis et al., 1982).

Similarly to prostacyclin the production of thromboxane is increased during normal pregnancy because maternal plasma concentrations of its stable metabolite, thromboxane B2 (TXB2), are higher during late pregnancy than during mid pregnancy or the nonpregnant state (Ylikorkala and Viinikka, 1980). Again, as with prostacyclin no specific source has been identified for the increased thromboxane during pregnancy. But there are many tissues in addition to platelets that produce thromboxane. Ingerman-Wojenski et al. (1981) determined that bovine endothelial cells produce thromboxane as well as prostacyclin. Another study by Neri Serneri et al. (1983) obtained human arterial and venous segments from

patients undergoing vascular surgery and found that these tissues produce prostacyclin and thromboxane. Liggins et al. (1980) perfused human endometrium over a seven hour period and determined it was capable of producing thromboxane. In addition, placental tissues produce thromboxane (Mitchell et al., 1978). Two groups (Mitchell et al., 1978; Martensson and Wallenburg, 1984) determined that concentrations of thromboxane in the umbilical vein were higher than the concentrations in the peripheral plasma. This suggests that the elevated umbilical vein levels are due to synthetic or metabolic activity in the fetoplacental unit. Therefore the placenta becomes a likely candidate as a source for the production of thromboxane during pregnancy.

During preeclampsia, levels of thromboxane in the amniotic fluid are unchanged (Ylikorkala et al., 1981). Peripheral plasma levels of thromboxane have been reported to be either increased (Koullapis et al., 1982; Martensson and Wallenburg, 1984) or unchanged (Mitchell et al., 1978; Ylikorkala et al., 1984; Yamaguchi and Mori, 1985). Perhaps of greatest interest was the study by Martensson and Wallenburg (1984), in which the concentrations of prostacyclin and thromboxane were concomitantly measured in plasma from the umbilical vein, the uterine vein and a peripheral vein. Their results revealed a significant shift in the production of these eicosanoids in favor of

thromboxane during preeclampsia. The possible contribution of eicosanoids of placental origin to this imbalance has not been documented. Thus there is a need to simultaneously determine the thromboxane and prostacyclin production rates by normal and preeclamptic placentas. In addition, several authors have reported alterations in the production of prostacyclin and thromboxane from cells incubated in media containing steroids. Because the placenta produces steroids during pregnancy it is possible that local steroid production may effect local eicosanoid production.

C. Steroids in Preeclampsia

During pregnancy the placenta functions in concert with maternal and fetal tissues to produce the estrogens. Plasma levels of the estrogens increase throughout pregnancy. During preeclampsia urine levels of estriol are decreased, but those of estrone and estradiol are unchanged (Wurterle, 1962). Several investigators (Allen and Lachelin, 1978; Chew and Ratnam, 1976; Mustchler et al., 1963; Townsley et al., 1973) have reported no difference in the plasma levels of the estrogens between normal and preeclamptic patients when no fetal complications were present in either group. However, two groups (Rahman et al., 1975; Ranta et al., 1980) determined that preeclamptic patients with associated fetal

distress did have plasma estradiol levels significantly lower than normotensive patients. A similar pattern was true for estriol (Allen and Lachelin, 1978; Easterling and Talbert, 1970; Mather et al., 1973; Ostergard et al., 1971; Reid et al., 1968). Because of this, plasma estrogen levels (mainly estriol) have been used in conjunction with other clinical tests as an indicator of fetal well-being.

The placenta has long been known to be the site of increased secretion of progesterone during pregnancy (Diczfalusy, 1952; Pearlman and Cerceo, 1952; Salhanick et al., 1952). This is evidenced by the fact that plasma progesterone levels in the umbilical vessels are higher than those in the maternal plasma (Llauro et al., 1968; Tulchinsky and Okada, 1975; Farquharson and Klopper, 1984).

During preeclampsia plasma levels of progesterone have been reported to be either the same as (Simmer and Simmer, 1959; Allen and Lachelin, 1978) or slightly higher than normal (Lindberg et al., 1974). However the urinary excretion of pregnanediol, a metabolite formed by the reduction of progesterone, is significantly less in pre-eclampsia than in normal pregnancy (Louros et al., 1960; Kankaanrinta and Forssell, 1961). Also, the activity of 20-alpha-hydroxysteroid dehydrogenase (20α HSDH), which degrades progesterone to 20-alpha-dihydroprogesterone (20αDHP), is decreased in preeclampsia (Diaz-Zagoya and

Arias, 1981). For this reason, the plasma levels of progesterone in the preeclamptic patient remain higher after delivery than in the normal patient (Diaz-Zagoya and Arias, 1981). In addition, Everett et al. (1978) determined that the increased responsiveness to angiotensin II (A-II) seen in preeclamptic patients can be attenuated by IV infusion of another enzymatic metaboite of progesterone, 5-alpha-dihydroprogesterone (5 DHP). However, IV infusion of progesterone does not alter the increased responsiveness to A-II (Everett et al., 1978). Interestingly, IM injection of progesterone does reinstate the A-II refractoriness (Everett et al., 1978). These investigators concluded that a progesterone metabolite may be important in the maintainance of normal blood pressure during pregnancy. In another study, rabbits treated with progesterone developed hypertension (Mati et al., 1977). This hypertension was alleviated by IM injection of prolactin (Mati et al., 1977). Resnik et al. (1977) increased uterine blood flow by infusing estradiol. Subsequent infusion of progesterone significantly decreased this estradiol-induced flow increase (Resnik et al., 1977).

The steroids have also been shown to effect the production of the eicosanoids from pregnancy-associated tissues. Liggins et al. (1980) determined that progesterone decreases while estradiol increases the

production of prostacyclin and thromboxane from cultured endometrium cells. Progesterone also had a slight inhibitory effect on prostacyclin production from term placental cells in vitro (Myatt et al., 1983). Estradiol slightly increased the production of prostacyclin in this preparation (Myatt et al., 1983). In contrast, progesterone had no effect on the production of prostacyclin or thromboxane from umbilical cord endothelial cells in vitro (Witter and DiBlasi, 1984).

The preceeding studies implicate estradiol and progesterone (or one of its metabolites) in the direct modulation of blood flow or in the mediation of vasoactive substances (i.e. prostacyclin and thromboxane). Because the steroids and the eicosanoids are produced in the placenta, it is possible that any alterations in the production of the steroids could have profound effects on eicosanoid production. In turn, this situation could contribute to the clinical manifestations of preeclampsia; the vasoconstriction, increased platelet aggregation and decreased uteroplacental blood flow.

D. Objectives

The specific research goals of this thesis included:

1. To simultaneously determine the production rates of prostacyclin and thromboxane by normal and preeclamptic placentas.

2. Inhibit thromboxane synthesis to validate the in vitro production of thromboxane from placental tissues.

3. Incubate placental tissues with excess substrate to determine if the function of the thromboxane synthase enzyme in either preeclamptic or normal placentas is limited due to precursor availability.

4. Test the effect of a low oxygen environment on the production of thromboxane by normal and preeclamptic placentas.

5. To determine if selective inhibition of thromboxane will affect prostacyclin production, thereby evaluating this as a treatment for preeclampsia.

6. Measure the production of progesterone and estradiol by normal and preeclamptic placentas.

7. Incubate placental tissues with excess substrate for the steroids to determine their impact on the production of progesterone and estradiol, respectively.

8. Determine if supplementing the incubation media with progesterone and estradiol will alter the production of prostacyclin and thromboxane by normal and preeclamptic placentas, respectively.

III. MATERIALS AND METHODS

A. Patients

Full term placentas from twenty-three normotensive and fifteen preeclamptic women were utilized. All tissues were obtained within fifteen minutes of delivery from E.W. Sparrow Hospital (Lansing, Michigan). The following criteria were used for normal pregnancies: a)maternal blood pressure less than 110/70 mm Hg (systolic/diastolic); b) no proteinuria; c) gestational length between 36-43 weeks; d) labor duration less than 18 hours for primigravida and less than 12 hours for multigravida; e) no overt medical complications, such as: chronic infection, metabolic, endocrine, nutritional or hematologic complications; f) no neonatal abnormalities, such as developmental defects, abnormal apgar scores (1 and 5 min tests), or birth weights less than 2.5 kg or greater than 4.5 kg; g) no third trimester bleeding; h) no gross placental abnormalities; i) no positive findings in high risk antenatal assessment; j) no oligo- or polyhydramnios; k) minimal or no smoking or alcohol consumption during pregnancy. Preeclamptic pregnancies were evaluated with the same criteria except: a) maternal blood pressure greater than 140/90 mm Hg (systolic/diastolic) ; b) proteinuria: urinary protein greater than 0.3 g /24 hour urine collection; c) one preeclamptic patient had gestational diabetes.

B. Experimental Protocols

Immediately after delivery, placental tissues were placed in a sterile bag, put on ice and transported to the laboratory. Processing of the tissues began within thirty minutes of delivery with all experiments conducted under sterile conditions. To insure maximum viability, fragments of placenta (approximately 1cm x 1cm) were separated and placed in 50 mls of Dulbecco's Modified Eagles Media (DMEM)(Gibco,Grand Island,NY). The DMEM was sterilized by microfiltration through a prefilter, a 1.2 uM filter and a 0.22 uM bell filter (Millipore Corp., Bedford, MA). This suspension was perfused with 95% oxygen and 5% carbon dioxide. The fragments of placenta were removed from the oxygenated buffer and prepared for incubation as needed. Excess buffer was removed with sterile gauze.

Tissues utilized in experiments were processed in duplicate. Approximately 350 mg of each tissue was incubated in 7 ml of DMEM for 48 hours at 37^{o} C in a Dubnoff metabolic shaker (Lab-line, Melrose Park, IL). In most experiments tissues were oxygenated with 95% oxygen and 5% carbon dioxide, with some experiments carried out in 20% oxygen with 5% carbon dioxide and 75% nitrogen. Partial pressures and hydrogen ion concentration in the incubation media were consistent at approximately PO2 = 250 mm Hg, PCO2 = 20 mm Hg, and pH = 7.50 (Behr, 1984). Tissue viability after 48 hours was assessed by trypan

blue exclusion staining of trypsinized dispersed cells (Behr, 1984). Samples of media (200 ul) for the eicosanoid studies were collected at 0, 8, 20, 32 and 48 hours of incubation. For the steroid studies samples of media (200 ul) were collected at 0, 30, 60, 120, 180 and 240 minutes of incubation. In some cases the incubation media was enriched with one of the following: indomethacin, arachidonic acid, progesterone, estradiol, pregnenolone, pregnenolone sulfate, dehydroepiandrosterone sulfate, aromatase inhibitor (all from Sigma, St. Louis, MO) or thromboxane synthase inhibitor (U63557A, Upjohn, Kalamazoo,MI).

The production of prostacyclin and thromboxane A2 in the incubation media were estimated by concentration of their stable metabolites, 6-keto PGF1α and thromboxane B2, respectively. The concentrations of progesterone and estradiol in the incubation media were assessed directly. In all cases highly specific radioimmunoassays (RIA) were utilized.

C. Radioimmunoassays

A brief description of each RIA will be given here while a more detailed description and the validation of each assay is provided in the Appendices.

1. Prostacyclin, Thromboxane and Progesterone Assays

These three radioimmunoassays were very similar, therefore a general explanation will be provided with specific points listed in Table 2. Standards, with known amounts of eicosanoid or steroid (Table 2), were used in each assay to generate a standard curve from which unknown quantities in samples could be calculated. Each assay consisted of competitive binding of radioactive and natural eicosanoid or steroid to a highly specific antibody (Table 1). Dextran-coated charcoal, pre-cooled to 0-4^{o}C, was utilized to separate the bound from the free fraction. All tubes were vortexed and allowed to incubate 12-15 minutes at 0-4^{o}C and then centrifuged at 2000xg for 12 minutes in a refrigerated (4^{o}C) centrifuge (Beckman model J6-B, Palo Alto, CA). The supernatant was decanted into scintillation vials containing 15 ml scintillation cocktail (Safety Solve, Mount Prospect, IL). The radioactivity was counted on a Packard Tri Carb 300C liquid scintillation counter (Packard Instrument Co., Downers Grove, IL) for ten minutes or until 2.0 percent statistical accuracy was achieved.

2. Estradiol

The amount of estradiol in the incubation media was determined by a highly specific radioimmunoassay. Standards with known amounts of steroid (0.25-100.0pg/0.025ml, RSL Inc., Carson, CA) were used to

TABLE 2

SPECIFIC COMPONENTS OF RADIOIMMUNOASSAYS

	Prostacyclin	Thromboxane	Progesterone
Standards	9.80-1250pg per 0.05ml, Seragen, Boston, MA	3.12-200pg per 0.10ml, Seragen, Boston, MA	0.05-3.20ng per 0.10ml, RSL Inc., Carson,CA
Radioactive Eicosanoid or Steroid	^{3}H-6-keto PGF1$_{\alpha}$ New England Nuclear, Boston,MA	^{3}H-TXB2, New England Nuclear, Boston,MA	^{3}H-Progesterone, Amersham, Arlington Heights, IL
Antibody	Rabbit anti-6-keto PGF1$_{\alpha}$, Seragen, Boston,MA	Rabbit anti-TXB2, Seragen, Boston,MA	Rabbit anti-Progesterone, RSL Inc., Carson,CA

generate a standard curve from which unknown quantities in samples could be calculated. The assay consisted of competitive binding of radioactive (^{125}I) and natural estradiol to a specific antibody. A second antibody (goat anti-rabbit gamma globulin, RSL Inc., Carson, CA) was added to precipitate the antibody bound antigen. After a 60 minute incubation, all tubes were centrifuged at 1000xg for 20 minutes. The supernatant was discarded and the radioactivity in the precipitate was counted on a Packard Auto-Gamma 800C scintillation counter (Packard Instrument Co., Downers Grove, IL) for five minutes or until 2.0 percent statistical accuracy was achieved.

D. Statistical Analysis

Statistical differences between production curves were assessed by analysis of variance with Student-Newman-Keuls test. Production rates were determined by linear regression analysis of the production curves, with differences between production rates assessed by t-test (Linton and Gallo, 1975) .

IV. RESULTS

A. Patients

There were twelve vaginal deliveries and ten cesarean sections in the normal group (patient information was not available for one member) and twelve vaginal deliveries and three cesarean sections in the preeclamptic group. Table 3 indicates the studies in which specific patients were used. All of the preeclamptic patients and six of the normal patients were primigravid. There were no significant differences ($P > 0.50$) between normal and preeclamptic pregnancies for placental weights (595 $\pm$ 25 vs. 597 $\pm$ 33 gm, respectively, mean $\pm$ SE), fetal weights (3.32 $\pm$ 0.1 vs. 3.45 $\pm$ 0.2 kg), weeks of gestation (39 $\pm$ 1 vs. 38 $\pm$ 1), and time in labor (9.9 $\pm$ 1.8 vs. 9.5 $\pm$ 1.0 hours). Time in labor was not correlated with the production of thromboxane, prostacyclin, progesterone or estradiol ($r = -0.10$; 0.05; -0.17; -0.11, respectively). One minute apgar scores of the fetuses born to the group of normal patients were slightly higher than those born to the group of preeclamptic patients (9.0 $\pm$ 0.2 vs. 8.4 $\pm$ 0.4, $P < 0.10$). However, all fetuses were born healthy. The preeclamptic mothers were slightly younger than the normal mothers (23.0 $\pm$ 1.9 vs. 28.6 $\pm$ 1.1 years, $P < 0.01$) and gained more weight (39.0 $\pm$ 4.0 vs. 29.5 $\pm$ 1.9 lb, $P < 0.05$). One of the preeclamptic patients had severe preeclampsia (systolic/diastolic $\geq$ 170/110 mmHg).

TABLE 3

TABLE OF PATIENTS

PATIENT/DATE			PATIENT DATA USED IN RESULTS SECTION: B. Eicosanoid Studies	C. Steroid Studies	D. Effect of Steroids on Eicosanoids
1.	5-8-83	*P	X		
2.	6-14-83	**N	X		
3.	6-21-83	N	X		
4.	6-28-83	P	X		
5.	8-2-83	P	X		
6.	9-5-83	N	X		
7.	9-26-83	N	X		
8.	9-29-83	N	X		
9.	10-3-83	N	X		
10.	10-6-83	N	X		
11.	10-11-83	P	X	X	X
12.	10-13-83	N		X	
13.	10-18-83	N	X	X	
14.	10-24-83	N		X	
15.	10-22-83	N		X	
16.	10-28-83	P		X	
17.	10-28-83	P	X	X	X
18.	11-3-83	N	X	X	X
19.	11-3-83	N	X	X	X
20.	11-30-83	N	X	X	
21.	12-13-83	N	X		X
22.	1-17-84	P	X	X	X
23.	2-1-84	N	X	X	X
24.	2-22-84	N	X	X	X
25.	2-24-84	P		X	
26.	2-28-84	P	X		X
27.	3-1-84	P	X		X
28.	5-23-84	N	X	X	X
29.	5-24-84	N		X	
30.	6-1-84	P	X	X	X
32.	6-28-84	N	X	X	
33.	7-16-84	P	X	X	X
34.	7-17-84	P		X	
35.	8-21-84	N	X		
36.	8-23-84	P	X		
37.	9-6-84	N	X		
38.	9-7-84	N	X		
39.	10-2-84	P	X		

*P- preeclamptic patient

**N- normal patient

In addition, one of the preeclamptic patients underwent cesarean section at thirty weeks gestation and another had gestational diabetes. Tissue viability as assessed by trypan blue exclusion staining was greater than 95% after 48 h of incubation in both normal and preeclamptic placentas (Behr, 1984).

B. Eicosanoids

1. Placental Production of Thromboxane and Prostacyclin

The production of thromboxane (as estimated by TXB2) from normal versus preeclamptic placentas is shown in Figure 1. The concentration of TXB2 in the incubation media increased progressively during the 48 h of incubation. The production of TXB2 from normal placentas peaked at 363 pg/mg after 48 h exhibiting a production rate of 6.3 $\pm$ 1.5 pg/mg/h (mean $\pm$ SE, n=11). In contrast, the production of TXB2 by preeclamptic placentas was significantly greater ($P<0.0001$) than that by the normal placentas. Peak levels after 48 h averaged 1164 pg/mg. The production rate for the preeclamptic placentas of 22.9 $\pm$ 4.7 pg/mg/h (mean $\pm$ SE, n=10) was significantly greater than that for the normal placentas ($P<0.001$).

The production of prostacyclin for normal and preeclamptic placental tissues, as estimated by 6-keto PGF1α, are shown in Figure 2. The concentrations of 6-keto PGF1α in the incubation media increased progressively and in a linear fashion during the 48 h of incubation.

Figure 1

Production of thromboxane by normal (n=11) versus preeclamptic (n=10) placentas, as estimated by TXB2. Data represent mean ± SE.

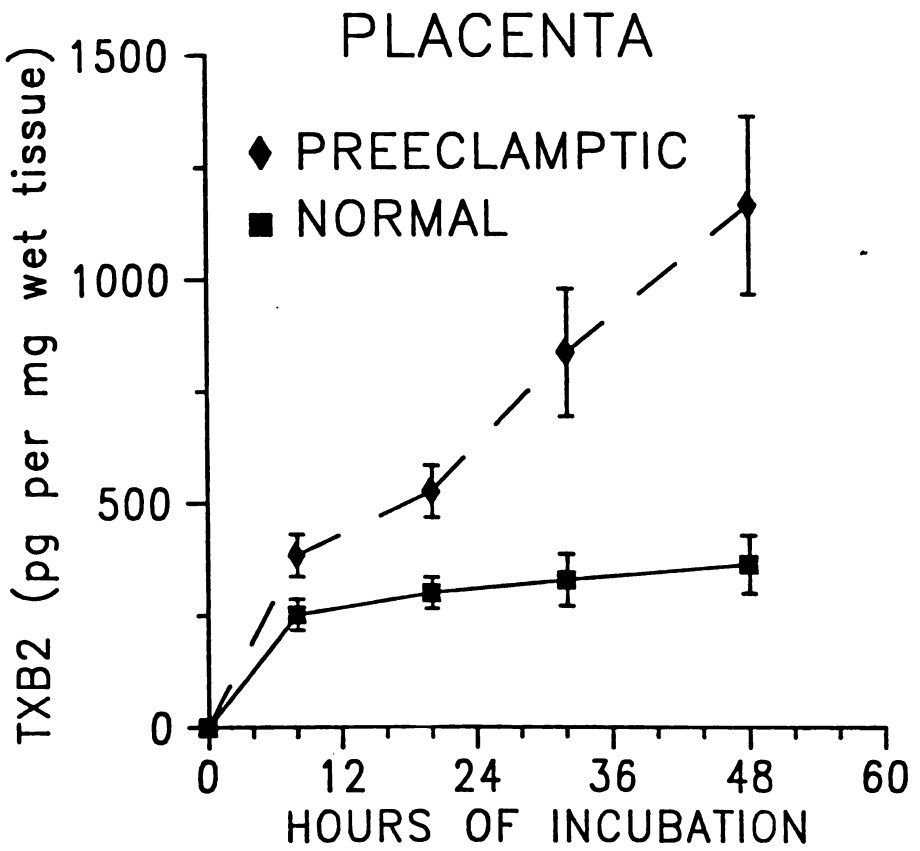

PLACENTA
PREECLAMPTIC
NORMAL
TXB2 (pg per mg wet tissue)
1500
1000
500
0
0
12
24
36
48
60
HOURS OF INCUBATION

Figure 1

Figure 2

Production of prostacyclin by normal (n=11) versus preeclamptic (n=10) placentas, as estimated by 6-keto PGF1α. Data represent mean $\pm$ SE.

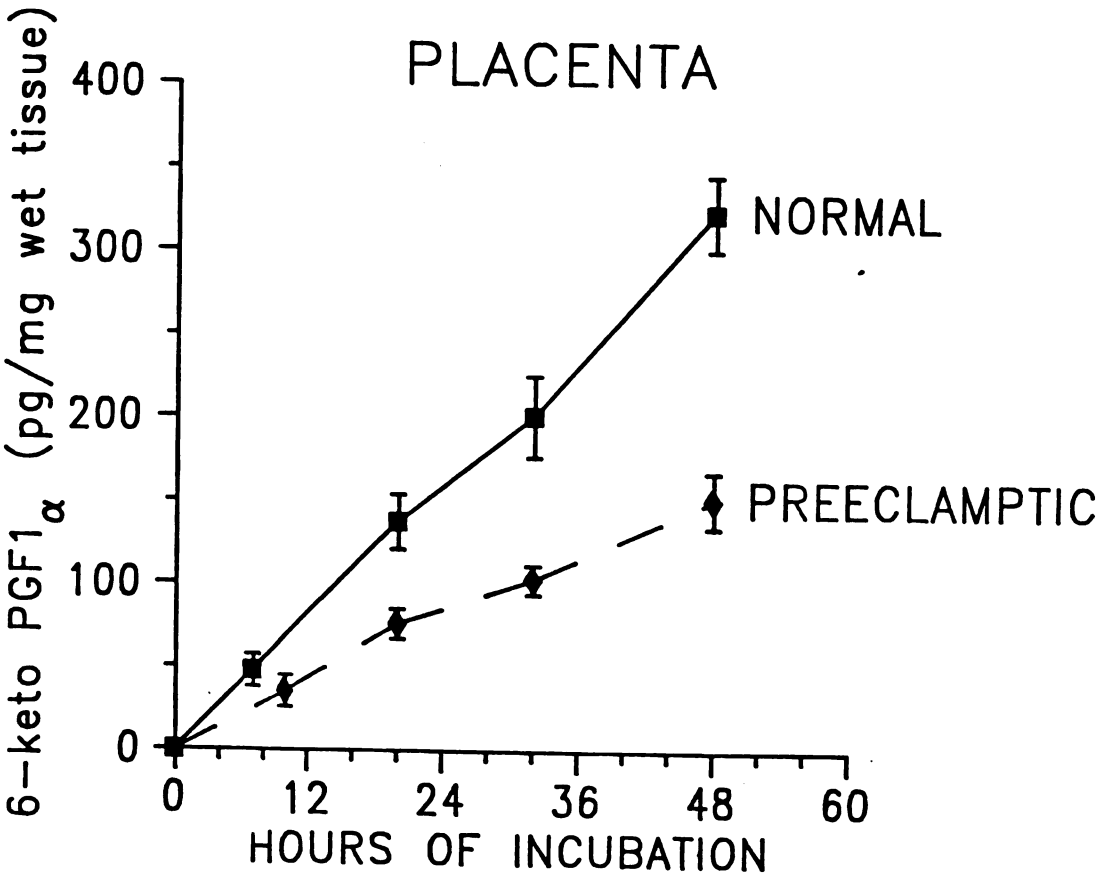

PLACENTA
6-keto PGF1α (pg/mg wet tissue)
400
300
200
100
0
0
12
24
36
48
60
HOURS OF INCUBATION
NORMAL
PREECLAMPTIC

Figure 2

Peak levels after 48 h averaged 322 pg/mg for normal tissue and 151 pg/mg for preeclamptic tissue. The production rate for normal versus preeclamptic placentas was 6.7 ± 0.5 pg/mg/h (mean ± SE, n=11) versus 3.0 ± 0.3 pg/mg/h (n=10), respectively. The production rate of prostacyclin from preeclamptic placentas was significantly lower ($P<0.001$) than that from normal placentas.

2. Validation of Placental Production Rates

a. Thromboxane

To assure that thromboxane was being produced and not just released from placental tissues indomethacin was added to the incubation medium. Addition of indomethacin significantly decreased ($P<0.05$) the production of thromboxane from both normal (Fig. 3) and preeclamptic (Fig. 4) placental tissues. Fifty uM indomethacin completely inhibited thromboxane production; whereas 5 uM indomethacin allowed a slight amount to be produced from normal tissue (0.61 ± 0.57 pg/mg/h, n=3) and preeclamptic tissue (1.58 ± 0.69 pg/mg/h, n=3).

Some tissues were incubated in media enriched with arachidonic acid to assure that the production of thromboxane was not limited due to precursor availability. Addition of arachidonic acid (100 uM) did not significantly affect ($P>0.10$) thromboxane production by either normal (Fig.3) or preeclamptic (Fig. 4) placentas. The production rates in normal placentas with and without

Figure 3

Production of thromboxane by normal placentas with either 100 uM arachidonic acid (AA, n=7) or 5 uM indomethacin (INDO, n=3). Data represent mean± SE.

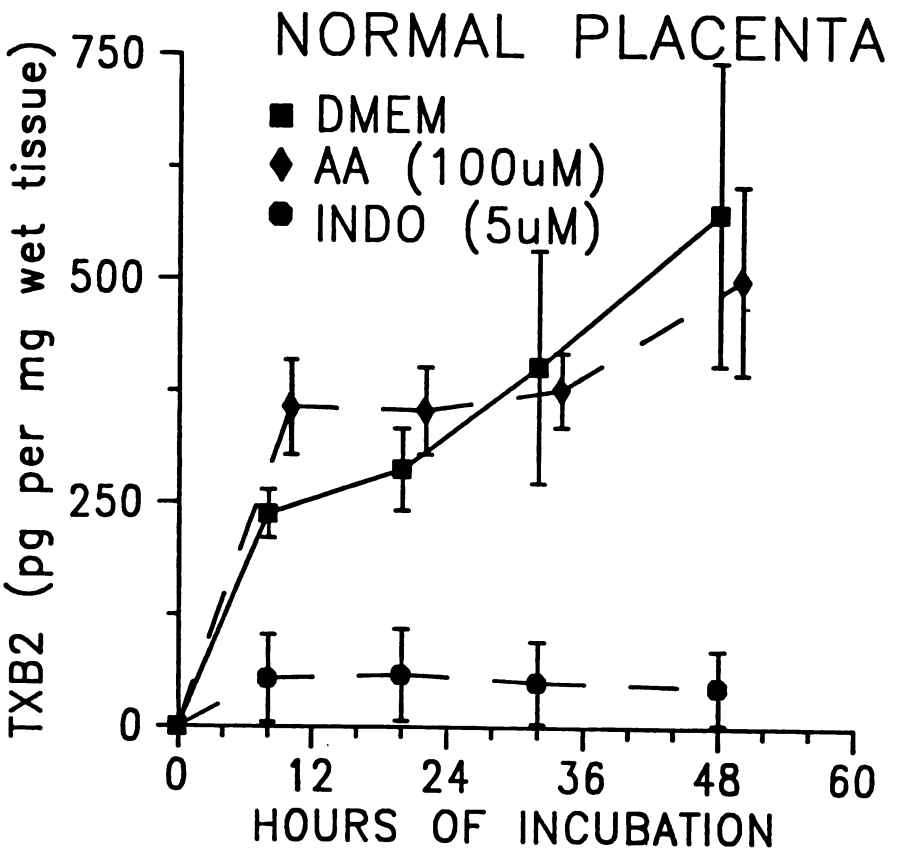

NORMAL PLACENTA
DMEM
AA (100uM)
INDO (5uM)
TXB2 (pg per mg wet tissue)
750
500
250
0
0
12
24
36
48
60
HOURS OF INCUBATION

Figure 3

Figure 4

Production of thromboxane by preeclamptic placentas with either 100 uM arachidonic acid (AA, n=6) or 5 uM indomethacin (INDO, n=3). Data represent mean± SE.

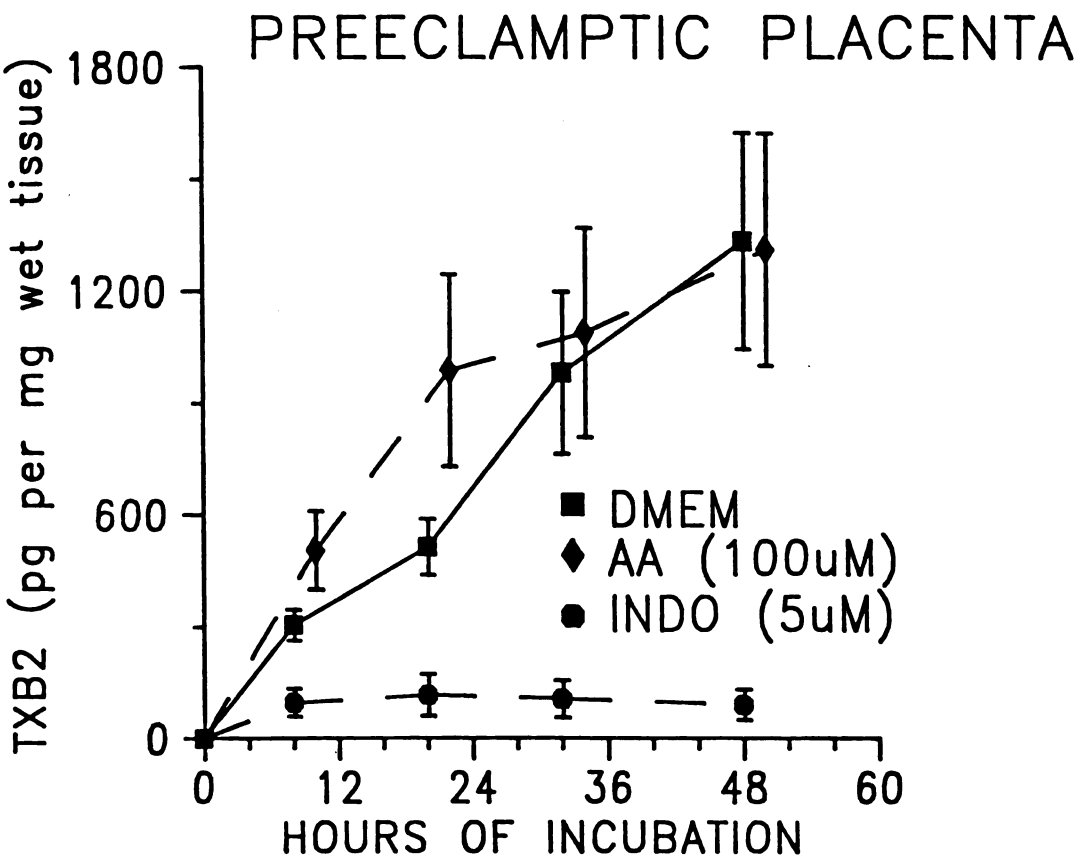

PREECLAMPTIC PLACENTA
TXB2 (pg per mg wet tissue)
1800
1200
600
0
0
12
24
36
48
60
HOURS OF INCUBATION
DMEM
AA (100uM)
INDO (5uM)

Figure 4

arachidonic acid were 8.0 ± 1.8 vs. 10.7 ± 3.8 pg/mg/h (n=7) and in preeclamptic placentas were 25.7 ± 6.7 vs. 27.7 ± 6.9 pg/mg/h (n=6), respectively.

b. Prostacyclin

Validation of the placental production of prostacyclin in this experimental protocol has been previously documented (Behr, 1984). Prostacyclin production is inhibited by indomethacin and not affected by the addition of arachidonic acid in normal and preclamptic placentas, respectively.

3. Thromboxane Production in a Low Oxygen Environment

The production of thromboxane from normal placental tissues was not significantly affected ($P>0.5$) by incubation in a low oxygen environment (n=3, Fig. 5). The production rate in a 95% oxygen environment was 6.12 ± 1.29 pg/mg/h (mean ± SE, n=3) versus 5.76 ± 0.81 pg/mg/h in a 20% oxygen environment. In contrast, the production rate of thromboxane from preeclamptic placentas was significantly decreased ($P<0.10$) in a low oxygen environment (n=3, Fig. 6). Preeclamptic placental tissues produced 13.1 ± 2.2 pg/mg/h in 95% oxygen versus 5.48 ± 2.7 pg/mg/h in 20% oxygen.

Figure 5

The production of thromboxane by normal placentas in low and high oxygen environments (20% vs. 95% O2). Data represent mean ± SE (n=3).

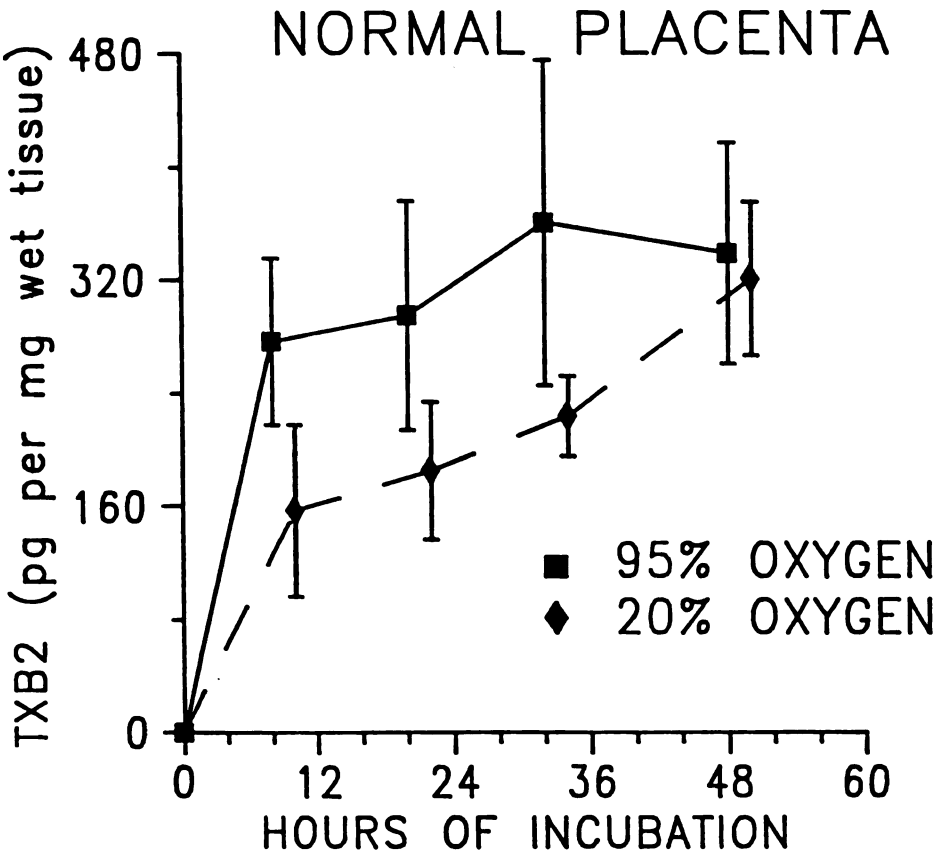

NORMAL PLACENTA
TXB2 (pg per mg wet tissue)
480
320
160
0
0
12
24
36
48
60
HOURS OF INCUBATION
95% OXYGEN
20% OXYGEN

Figure 5

Figure 6

The production of thromboxane by preeclamptic placentas in low and high oxygen environments (20% vs. 95%). Data represent mean ± SE (n=3).

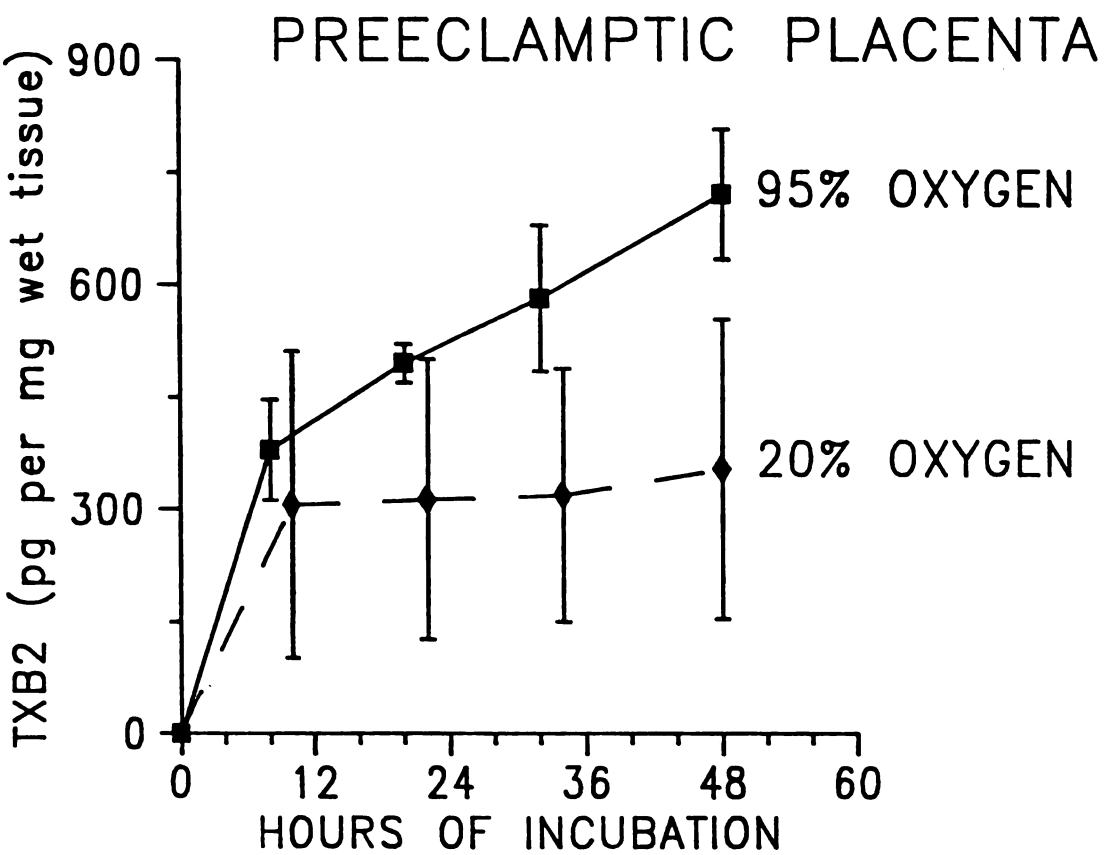

PREECLAMPTIC PLACENTA
TXB2 (pg per mg wet tissue)
900
600
300
0
95% OXYGEN
20% OXYGEN
0
12
24
36
48
60
HOURS OF INCUBATION

Figure 6

4. Thromboxane Synthase Inhibition

Addition of thromboxane synthase inhibitor (0.1 uM) significantly decreased ($P<0.05$) thromboxane production in preeclamptic tissue (n=6, Fig.7) and normal tissue (n=5, Fig. 8). The production rates in preeclamptic placentas with and without thromboxane synthase inhibitor were 3.25 $\pm$ 0.58 vs. 13.7 $\pm$ 2.66 pg/mg/h and in normal placentas were 3.95 $\pm$ 0.52 vs. 8.31 $\pm$ 2.24 pg/mg/h. Thromboxane synthase inhibitor did not significantly affect ($P>0.50$) prostacyclin production in either preeclamptic or normal tissues (Figs. 9 and 10). The production rates of prostacyclin in preeclamptic placentas with and without thromboxane synthase inhibitor were 3.01$\pm$ 0.62 vs. 3.04 $\pm$ 0.32 pg/mg/h (n=6) and in normal placentas were 7.29 $\pm$ 1.18 vs. 6.80 $\pm$ 0.76 pg/mg/h (n=7).

C. Steroids

1. Placental Production of Progesterone

The production of progesterone from normal versus preclamptic placentas is shown in Figure 11. The production rates for normal versus preeclamptic placentas were 0.67 $\pm$ 0.11 ng/mg/h (mean $\pm$ SE, n=8) versus 1.12 $\pm$ 0.13 ng/mg/h (n=8), respectively. The production rate of progesterone from preeclamptic placentas was significantly greater ($P<0.01$) than that from normal placentas.

Figure 7

The production of thromboxane by preeclamptic placentas in media enriched with thromboxane synthase inhibitor (U63557A, 0.1uM) vs. control levels in DMEM. Data represent mean ± SE (n=6).

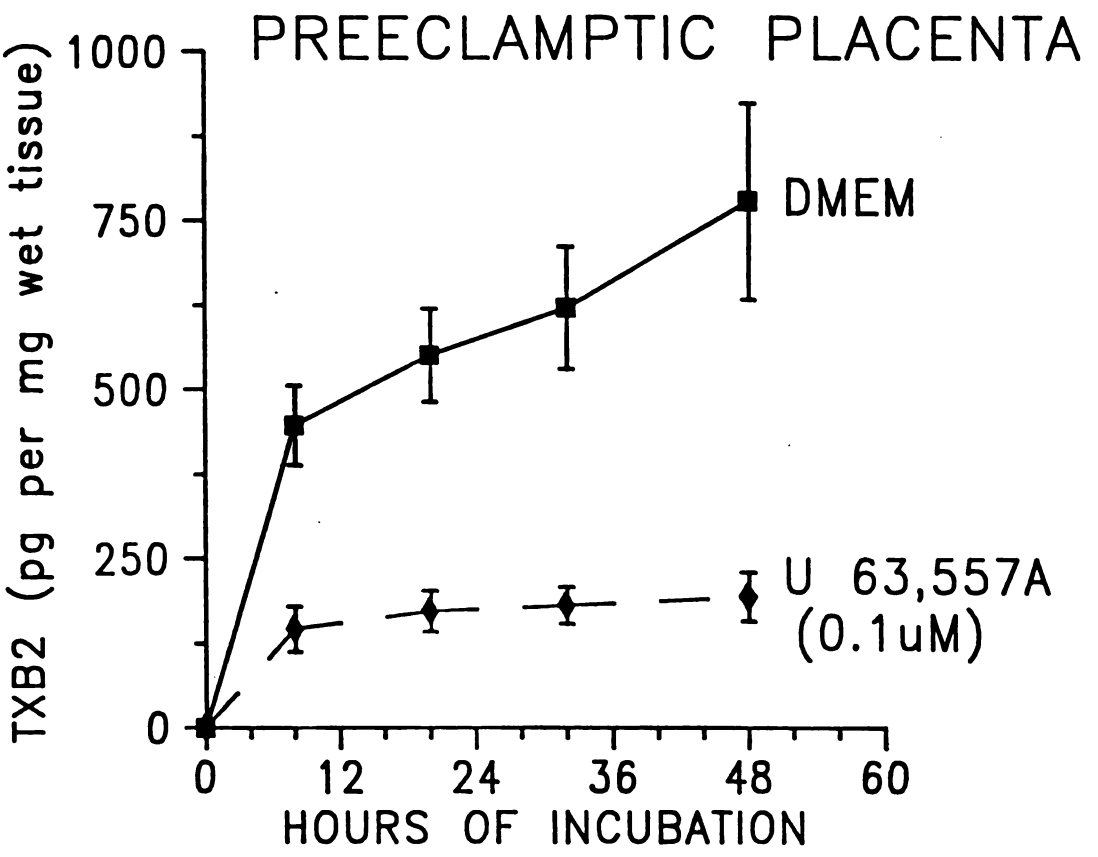

PREECLAMPTIC PLACENTA
TXB2 (pg per mg wet tissue)
1000
750
500
250
0
DMEM
U 63,557A
(0.1uM)
0
12
24
36
48
60
HOURS OF INCUBATION

Figure 7

Figure 8

The production of thromboxane by normal placentas in media enriched with thromboxane synthase inhibitor (U63557A, 0.1uM) vs. control levels in DMEM. Data represent mean ± SE (n=5).

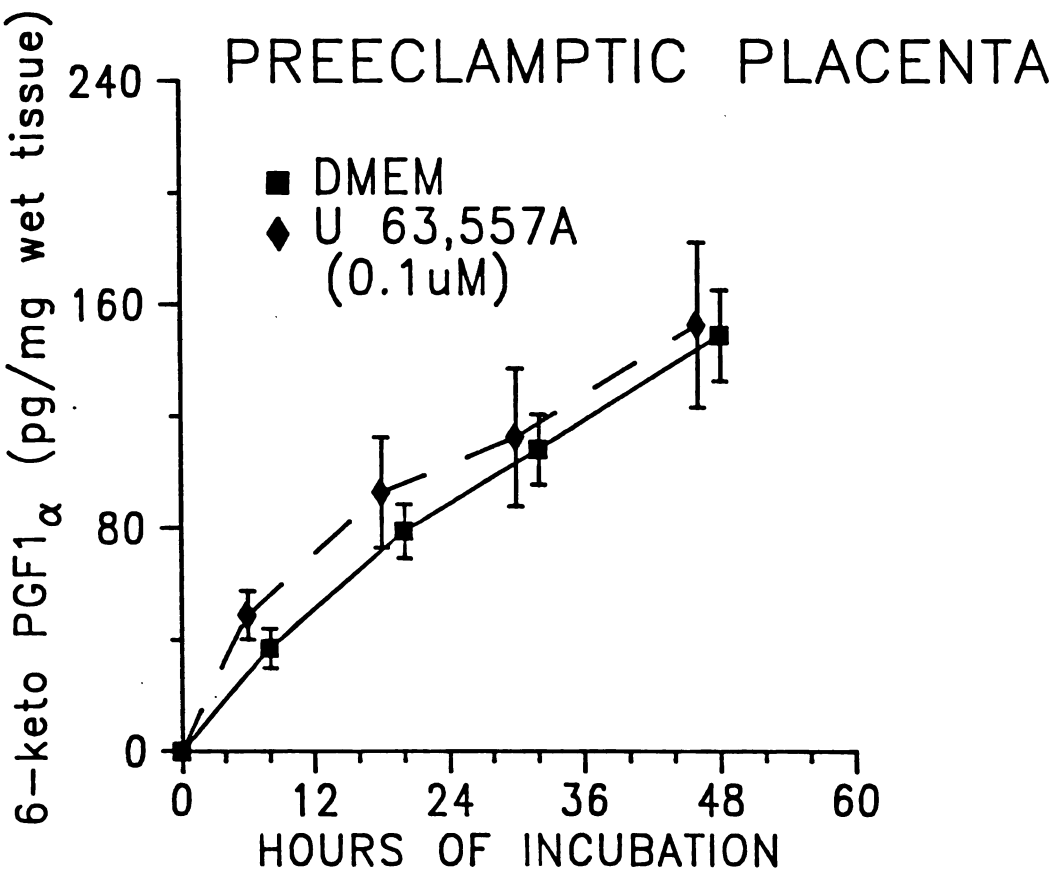

PREECLAMPTIC PLACENTA
DMEM
U 63,557A
(0.1uM)
6-keto PGF1α (pg/mg wet tissue)
240
160
80
0
0
12
24
36
48
60
HOURS OF INCUBATION

Figure 8

Figure 9

The production of prostacyclin by preeclamptic placentas in media enriched with thromboxane synthase inhibitor (U63557A, 0.1uM) vs. control levels in DMEM. Data represent mean ± SE (n=6).

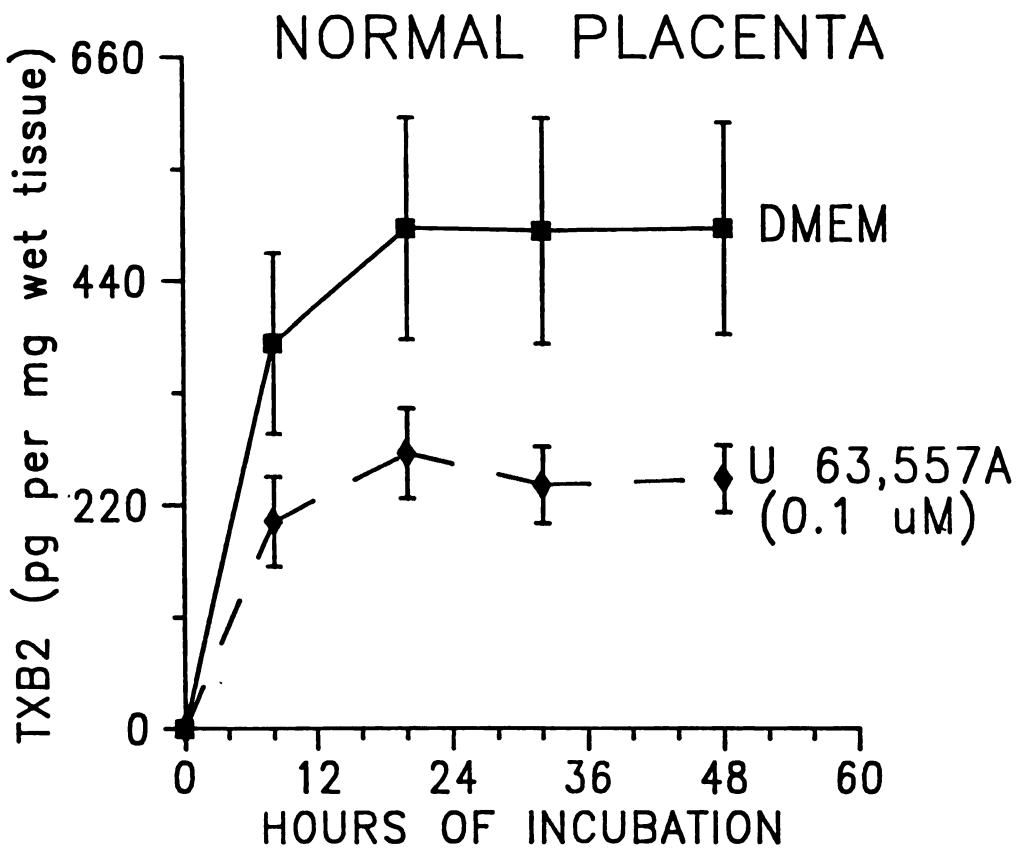

NORMAL PLACENTA
TXB2 (pg per mg wet tissue)
660
440
220
0
0
12
24
36
48
60
HOURS OF INCUBATION
DMEM
U 63,557A
(0.1 uM)

Figure 9

Figure 10

The production of prostacyclin by normal placentas in media enriched with thromboxane synthase inhibitor (U63557A, 0.1uM) vs. control levels in DMEM. Data represent mean ± SE (n=7).

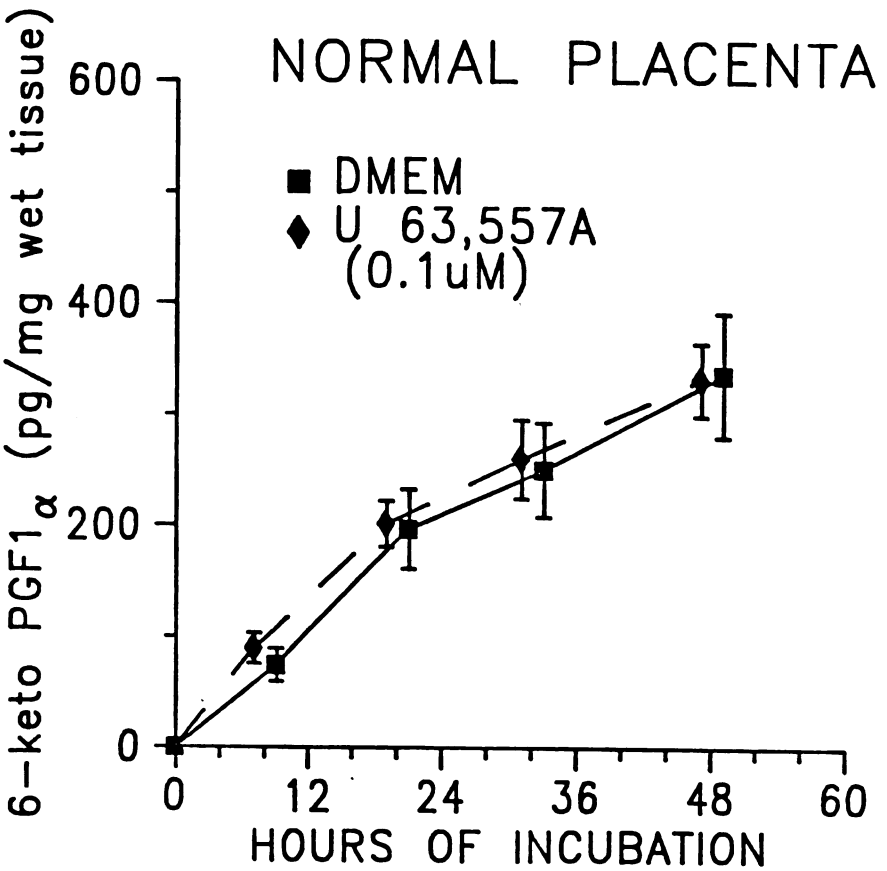

NORMAL PLACENTA
DMEM
U 63,557A
(0.1uM)
6-keto PGF1α (pg/mg wet tissue)
600
400
200
0
0
12
24
36
48
60
HOURS OF INCUBATION

Figure 10

Figure 11

The production of progesterone by normal versus preeclamptic placentas. Data represent mean ± SE (n=8).

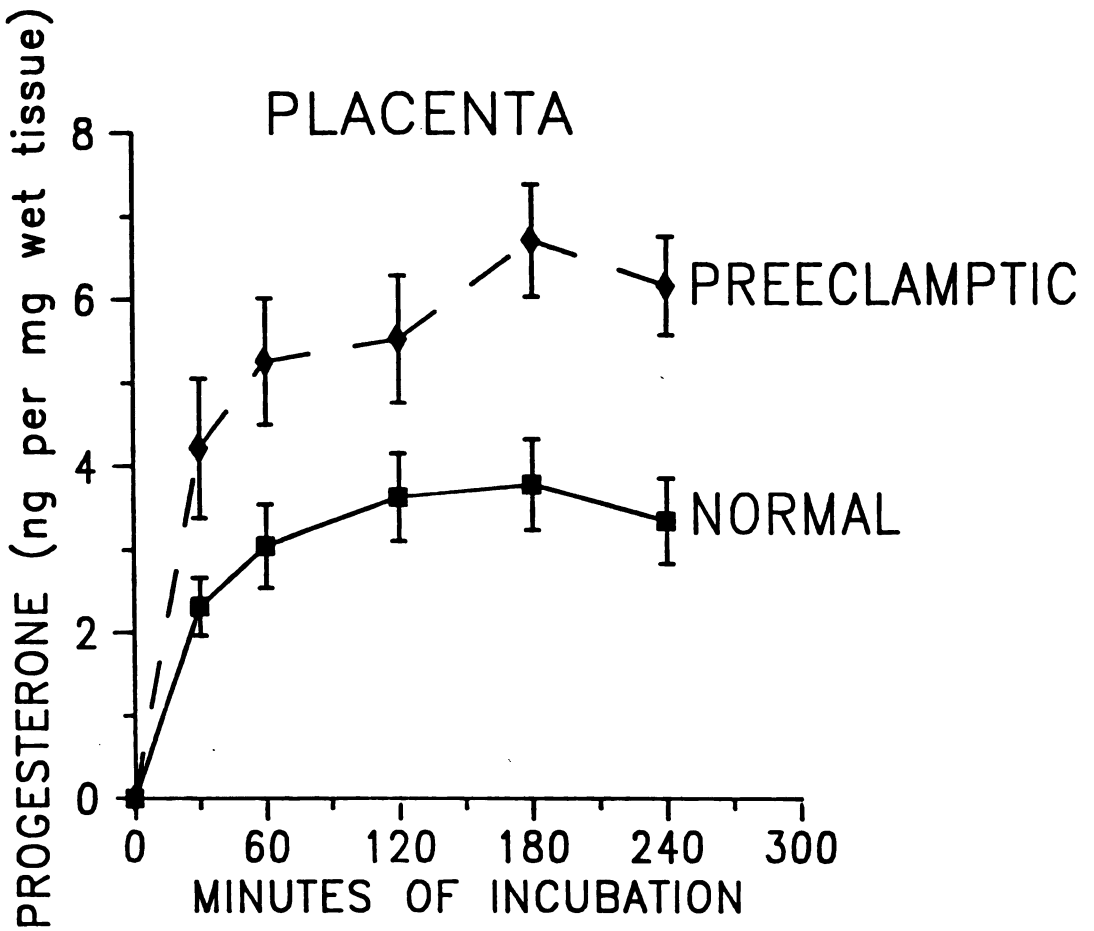

PLACENTA
PREECLAMPTIC
NORMAL
PROGESTERONE (ng per mg wet tissue)
0
2
4
6
8
0
60
120
180
240
300
MINUTES OF INCUBATION

Figure 11

To ascertain if the difference in normal versus preeclamptic progesterone production rates were due to precursor availability, the incubation media was enriched with pregnenolone or pregnenolone sulfate. Addition of pregnenolone (100 uM) to the incubation medium significantly increased ($P<0.01$) the production of progesterone in both normal and preeclamptic tissues (n=5, Fig.12). However, there were no significant differences ($P>0.50$) between the production rates for normal and preeclamptic placentas (9.20 $\pm$ 1.60 vs. 10.0 $\pm$ 1.34 ng/mg/h, respectively). The effects of adding pregnenolone sulfate (100 uM) to the incubation media are shown in Figure 13. The production curve of progesterone from preeclamptic placentas was significantly greater ($P<0.05$) than that from normal placentas when this precursor was added to the incubation media (n=5).

2. Placental Production of Estradiol

The production of estradiol by normal placentas (n=5, Fig.14) is not significantly different ($P>0.50$) from that produced by preeclamptic placentas (n=3, Fig.15). The production rate for normal tissues was 6.09 $\pm$ 0.53 pg/mg/h and the production rate for preeclamptic tissues was 5.31 $\pm$ 0.69 pg/mg/h.

Addition of the major precursor for estradiol, dehydroepiandrosterone sulfate (DHEAS, 100 uM), to the incubation media significantly increased ($P<0.05$) the production

Figure 12

The production of progesterone by normal versus preeclamptic placentas in media enriched with pregnenolone (100 uM). Data represent mean ± SE (n=5).

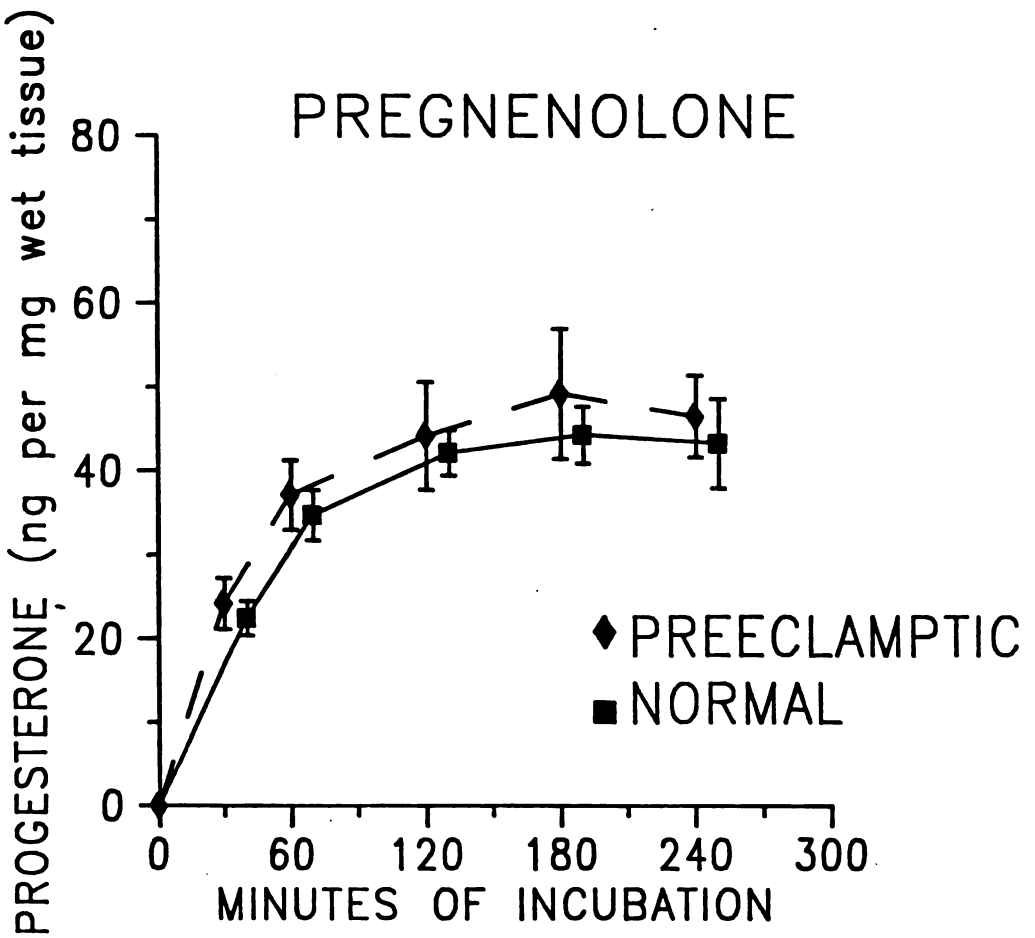

PREGNENOLONE
PROGESTERONE (ng per mg wet tissue)
80
60
40
20
0
0
60
120
180
240
300
MINUTES OF INCUBATION
PREECLAMPTIC
NORMAL

Figure 12

Figure 13

The production of progesterone by normal versus preeclamptic placentas in media enriched with pregnenolone sulfate (100 uM). Data represent mean ± SE (n=5).

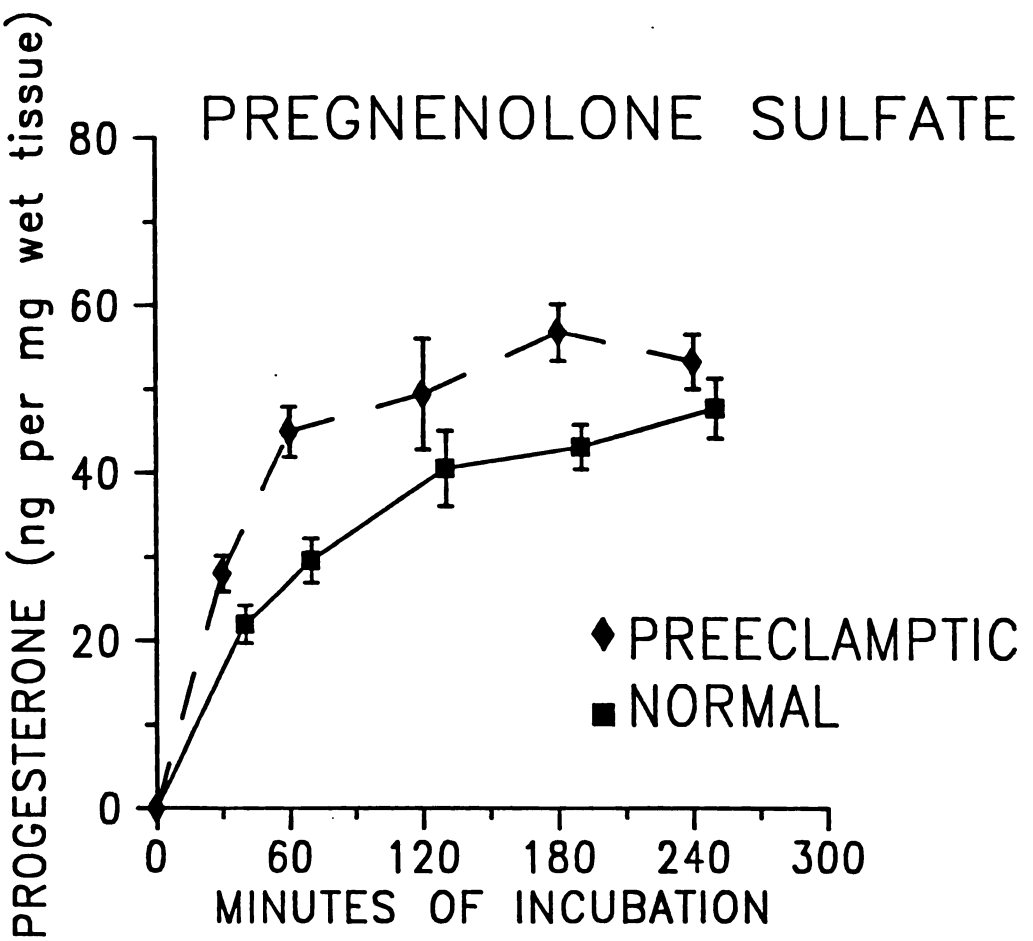

PREGNENOLONE SULFATE
PROGESTERONE (ng per mg wet tissue)
80
60
40
20
0
0
60
120
180
240
300
MINUTES OF INCUBATION
PREECLAMPTIC
NORMAL

Figure 13

Figure 14

The production of estradiol by normal placentas with either dehydroepiandrosterone sulfate (DHEAS, 100 uM, n=5) or dehydroepiandrosterone sulfate (100 uM) and aromatase inhibitor (AI, 100 uM, n=6) added to the incubation media. Data represent mean ± SE .

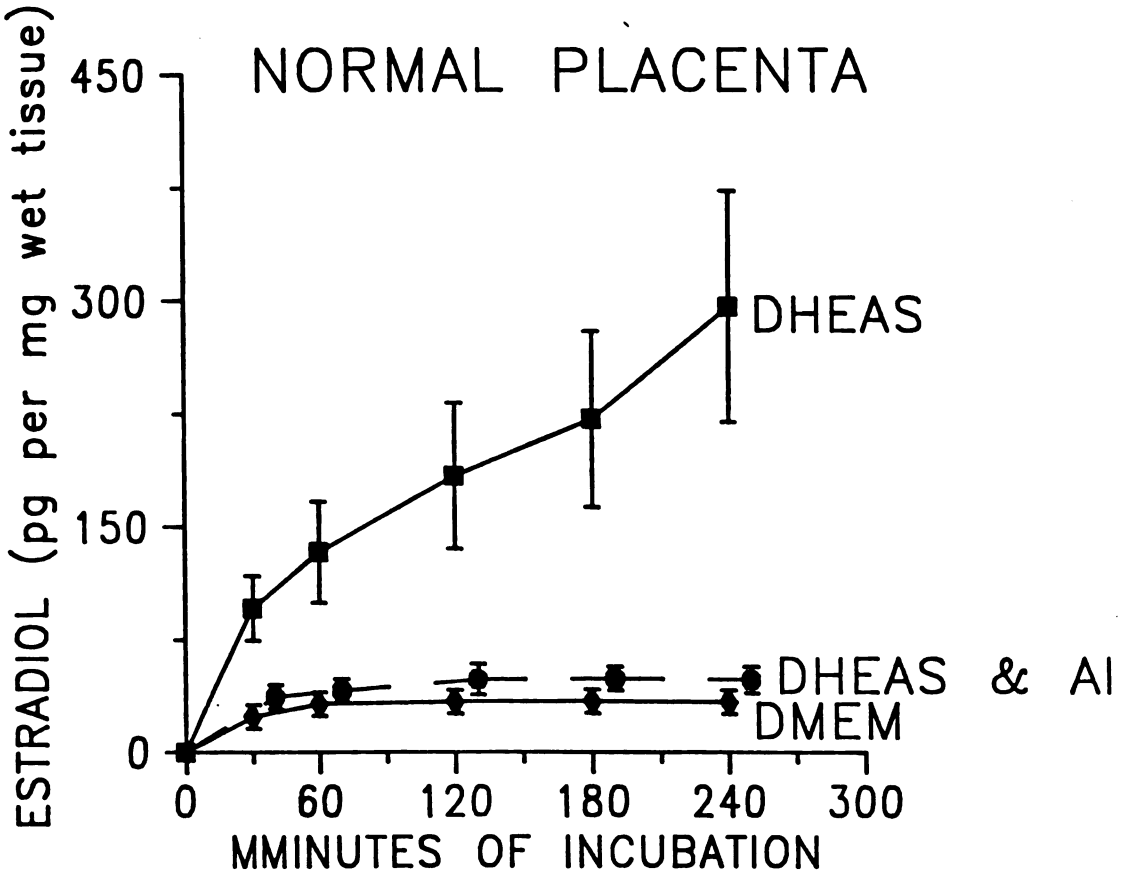

NORMAL PLACENTA
ESTRADIOL (pg per mg wet tissue)
450
300
150
0
DHEAS
DHEAS & AI
DMEM
0
60
120
180
240
300
MMINUTES OF INCUBATION

Figure 14

Figure 15

The production of estradiol by preeclamptic placentas with either dehydroepiandrosterone sulfate (DHEAS, 100 uM) or dehydroepiandrosterone sulfate (100 uM) and aromatase inhibitor (AI, 100 uM) added to the incubation media. Data represent mean ± SE (n=3).

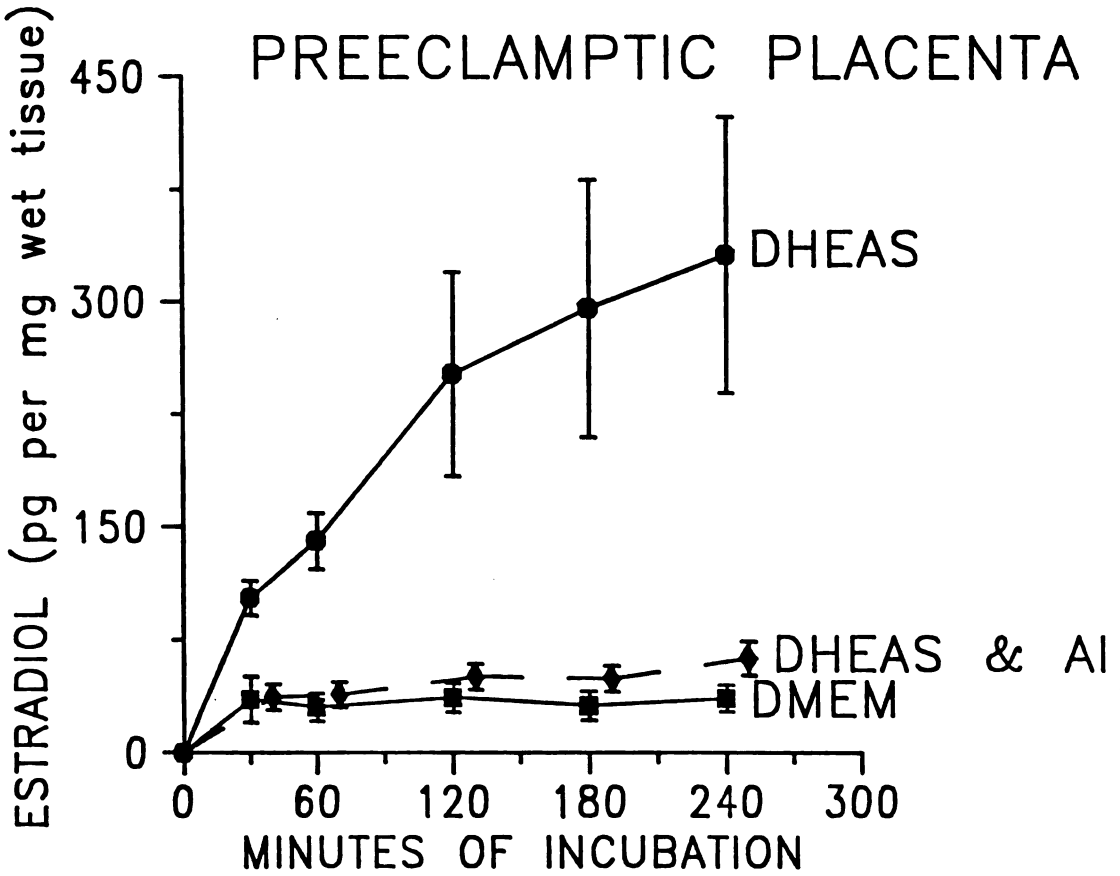

PREECLAMPTIC PLACENTA
ESTRADIOL (pg per mg wet tissue)
450
300
150
0
0
60
120
180
240
300
MINUTES OF INCUBATION
DHEAS
DHEAS & AI
DMEM

Figure 15

of estradiol from both normal (Fig. 14) and preeclamptic (Fig. 15) placentas. The production rates in normal placentas with and without DHEAS were 64.9 ± 17.6 vs. 6.09 ± 0.53 pg/mg/h (n=5) and in preeclamptic placentas were 79.6 ± 25.6 vs. 5.31± 0.69 pg/mg/h (n=3), respectively. There were no significant differences (P>0.50) between normal and preeclamptic placental production rates of estradiol with DHEAS added to the incubation media.

The conversion of DHEAS to estradiol can be blocked by aromatase inhibitor. The production of estradiol from normal (Fig. 14) and preeclamptic (Fig. 15) placentas was significantly decreased (P<0.05) when tissues were incubated in media containing both DHEAS (100 uM) and aromatase inhibitor (100 uM). The production rate of estradiol by normal tissues in media containing both DHEAS and aromatase inhibitor was significantly less than that in media with DHEAS only (8.85 ± 2.53 (n=6) vs. 64.9 ± 17.6 pg/mg/h (n=5), respectively). The production rate of estradiol by preeclamptic tissues in media containing DHEAS and aromatase inhibitor was 11.9 ± 2.02 (n=3) vs. 79.6 ± 25.6 pg/mg/h (n=3) in media with DHEAS only.

D. Effect of Estradiol and Progesterone on Placental Production of Prostacyclin and Thromboxane

Adding estradiol (100 uM) to the incubation media did not significantly alter ($P>0.25$) the production of prostacyclin by normal placentas (n=5, Fig.16) or by preeclamptic placentas (n=6, Fig.17). The production rates with and without estradiol were 5.41 $\pm$ 1.23 vs. 6.49 $\pm$ 1.00 pg/mg/h for normal placentas and 3.23 $\pm$ 0.31 vs. 3.56 $\pm$ 0.33 pg/mg/h for preeclamptic placentas. In contrast, the production of prostacyclin was significantly decreased in normal ($P<0.01$) and preeclamptic ($P<0.10$) placentas when progesterone (100 uM) was added to the incubation media. The production rates with progesterone added were 2.96 $\pm$ 0.66 pg/mg/h (n=5, Fig.16) and 2.54 $\pm$ 0.32 pg/mg/h (n=6, Fig.17) for normal and preeclamptic placentas, respectively. Simultaneous addition of estradiol and progesterone (each 100 uM) also significantly decreased prostacyclin production in normal ($P<0.01$) and preeclamptic ($P<0.05$) placental tissues. In fact, these production rates for normal tissues (1.12 $\pm$ 0.19 pg/mg/h, n=5, Fig.16) and preeclamptic tissues (1.56 $\pm$ 0.59 pg/mg/h, n=6,Fig.17) were significantly lower ($P<0.10$) than with only progesterone added to the incubation media.

The effects of adding estradiol and progesterone on the production of thromboxane in normal placentas are shown in Figure 18 (n=5). In all cases the production rates of

Figure 16

The production of prostacyclin by normal placentas in media enriched with one of the following: estradiol (E2, 100 uM), progesterone (P4, 100 uM), estradiol and progesterone (each 100 uM) vs. control levels in DMEM. Data represent mean ± SE (n=5).

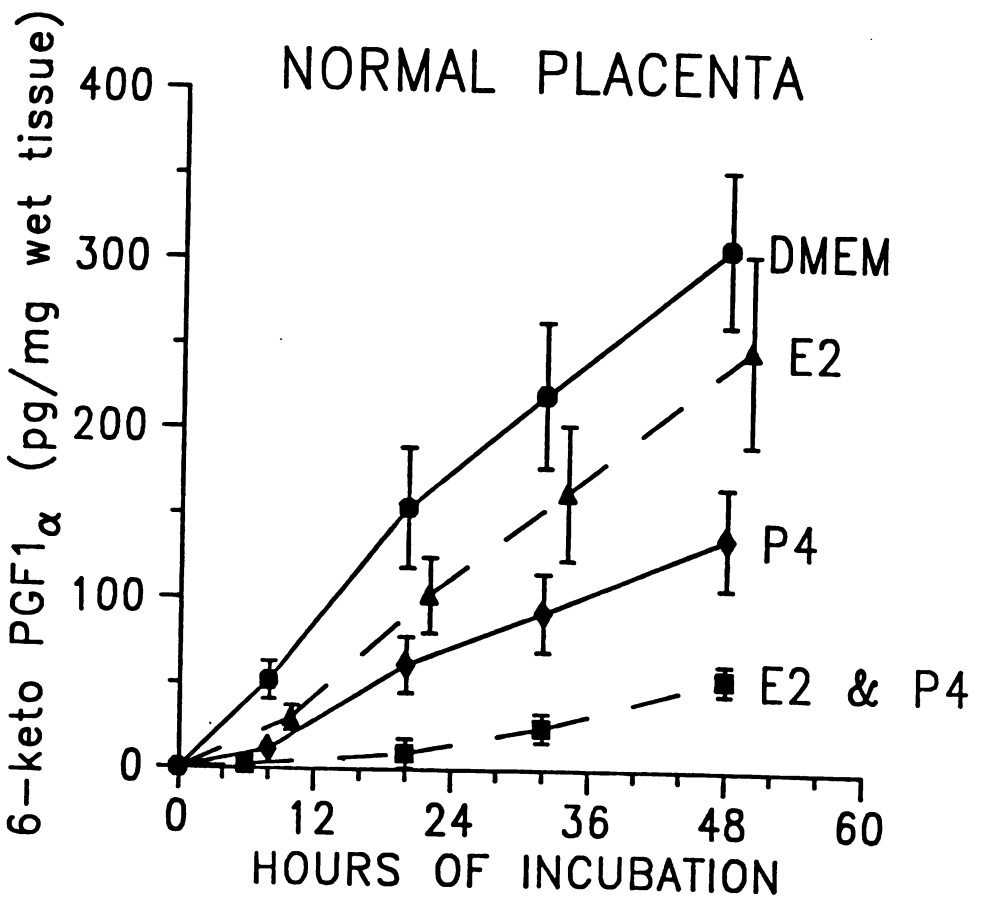

NORMAL PLACENTA
6-keto PGF1α (pg/mg wet tissue)
400
300
200
100
0
0
12
24
36
48
60
HOURS OF INCUBATION
DMEM
E2
P4
E2 & P4

Figure 16

Figure 17

The production of prostacyclin by preeclamptic placentas in media enriched with one of the following: estradiol (E2, 100 uM), progesterone (P4, 100 uM), estradiol and progesterone (each 100 uM) vs. control levels in DMEM. Data represent mean ± SE (n=6).

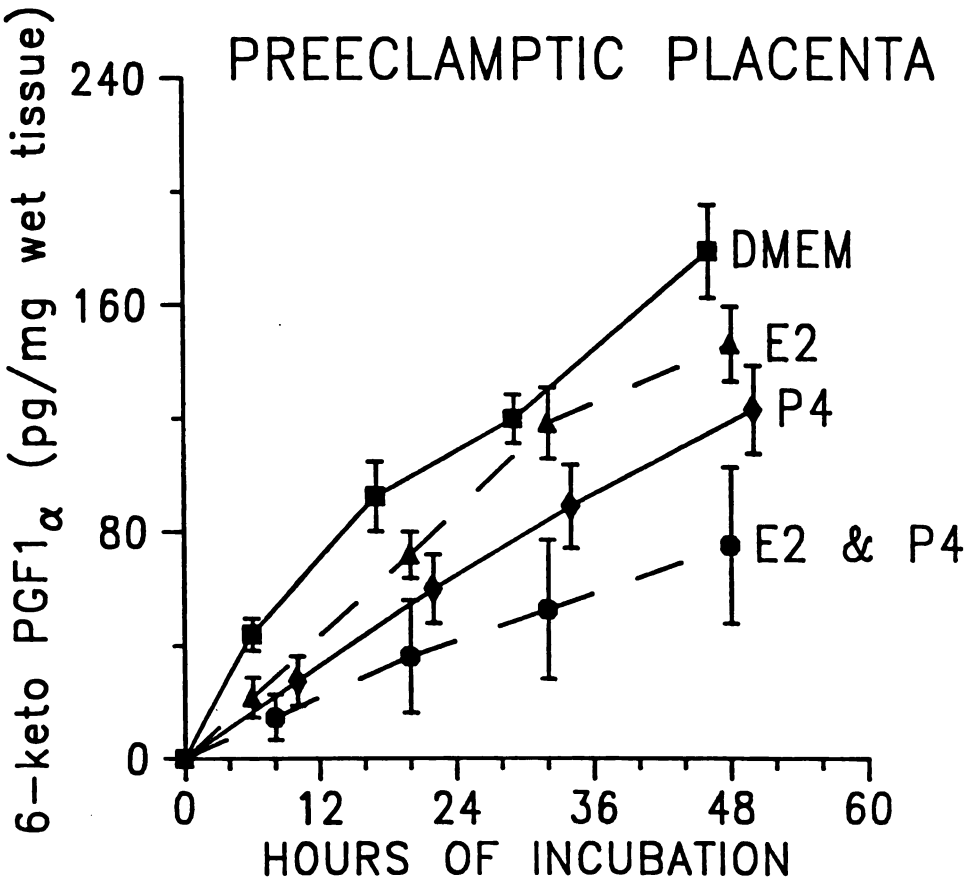

PREECLAMPTIC PLACENTA
6-keto PGF1α (pg/mg wet tissue)
240
160
80
0
0
12
24
36
48
60
HOURS OF INCUBATION
DMEM
E2
P4
E2 & P4

Figure 17

Figure 18

The production of thromboxane by normal placentas in media enriched with one of the following: estradiol (E2, 100 uM), progesterone (P4, 100 uM), estradiol and progesterone (each 100 uM) vs. control levels in DMEM. Data represent mean ± SE (n=5).

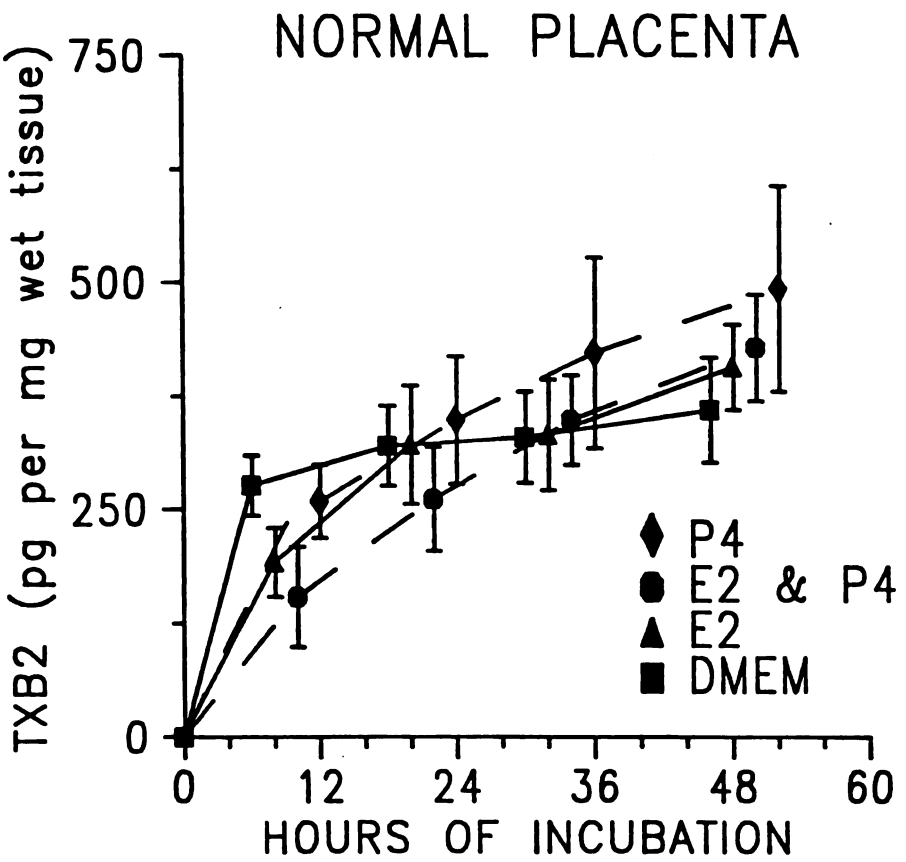

NORMAL PLACENTA
TXB2 (pg per mg wet tissue)
750
500
250
0
0
12
24
36
48
60
HOURS OF INCUBATION
P4
E2 & P4
E2
DMEM

Figure 18

thromboxane were slightly higher in solutions containing one of the steroids than that for DMEM alone (Table 4). However, there were no significant differences (P>0.30) between production curves. Addition of estradiol (100 uM) and/or progesterone (100 uM) to the incubation media did not significantly affect (P>0.50) the production of thromboxane by preeclamptic placentas (n=6, Fig.19). The production rates are listed in Table 5.

TABLE 4

NORMAL PLACENTA THROMBOXANE PRODUCTION

INCUBATION MEDIA	THROMBOXANE PRODUCTION RATES (mean ± SE)
DMEM only	5.93 ± 1.19 pg/mg/h
Estradiol (100 uM)	7.58 ± 1.12 pg/mg/h
Progesterone (100 uM)	9.15 ± 2.50 pg/mg/h
Etradiol (100 uM) and Progesterone (100 uM)	8.53 ± 1.13 pg/mg/h

Figure 19

The production of thromboxane by preeclamptic placentas in media enriched with one of the following: estradiol (E2, 100 uM), progesterone (P4, 100 uM), estradiol and progesterone (each 100 uM) vs. control levels in DMEM. Data represent mean ± SE (n=6).

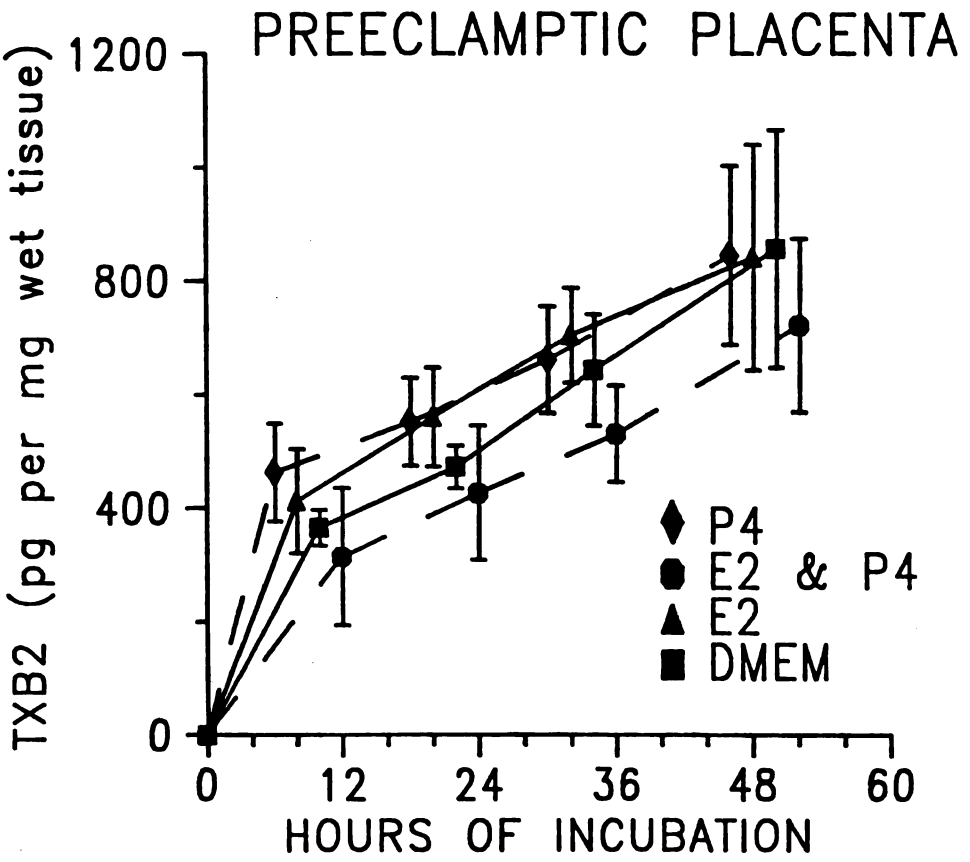

PREECLAMPTIC PLACENTA
TXB2 (pg per mg wet tissue)
1200
800
400
0
0
12
24
36
48
60
HOURS OF INCUBATION
P4
E2 & P4
E2
DMEM

Figure 19

TABLE 5

PREECLAMPTIC PLACENTA THROMBOXANE PRODUCTION

INCUBATION MEDIA	THROMBOXANE PRODUCTION RATES (mean ± SE)
DMEM only	16.1 ± 4.57 pg/mg/h
Estradiol (100 uM)	15.8 ± 4.50 pg/mg/h
Progesterone (100 uM)	15.1 ± 4.04 pg/mg/h
Estradiol (100 uM) and Progesterone (100 uM)	13.4 ± 3.08 pg/mg/h

V. DISCUSSION

A. Eicosanoids

The production rate of thromboxane by preeclamptic placentas was significantly greater than that by normal placentas (Fig. 20). The preeclamptic placenta produced over three times as much thromboxane as the normal placenta. In contrast, the prostacyclin production rate by preeclamptic placentas was less than one-half the production rate of prostacyclin by the normal placenta (Fig. 20). These production rates are graphically depicted in Figure 21. The placenta from the normotensive patient produced approximately equal amounts of thromboxane and prostacyclin, whereas the placenta from the preeclamptic patient produced over seven times as much thromboxane as prostacyclin (Fig. 21).

Because of the vasoactive properties of these eicosanoids, this imbalance could contribute to the increased vasoconstriction, platelet aggregation, and reduced uteroplacental blood flow characteristic of preeclampsia (Chesley, 1978; Dennis et al., 1982; Pritchard et al., 1976). The imbalance could also affect the incidence of preterm labor during preeclampsia because prostacyclin inhibits (Lye and Challis, 1982; Omini et al., 1979; Wilhelmsson et al., 1981; Zahadnik et al., 1983) and thromboxane increases uterine activity (Wilhelmsson et al., 1981).

Most likely the concentrations of prostacyclin and

Figure 20

Ratios of the placental production rates of thromboxane to prostacyclin in normal and preeclamptic pregnancies.

Figure 21

Ratios of the placental production rates of thromboxane to prostacyclin for normal and preeclamptic pregnancies in bar graph form.

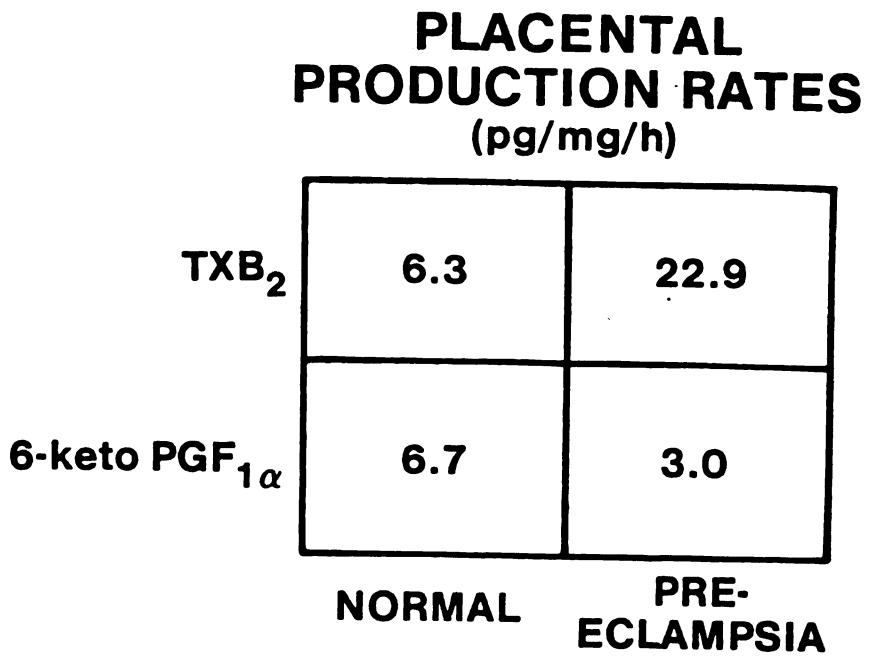


FIGURE 20

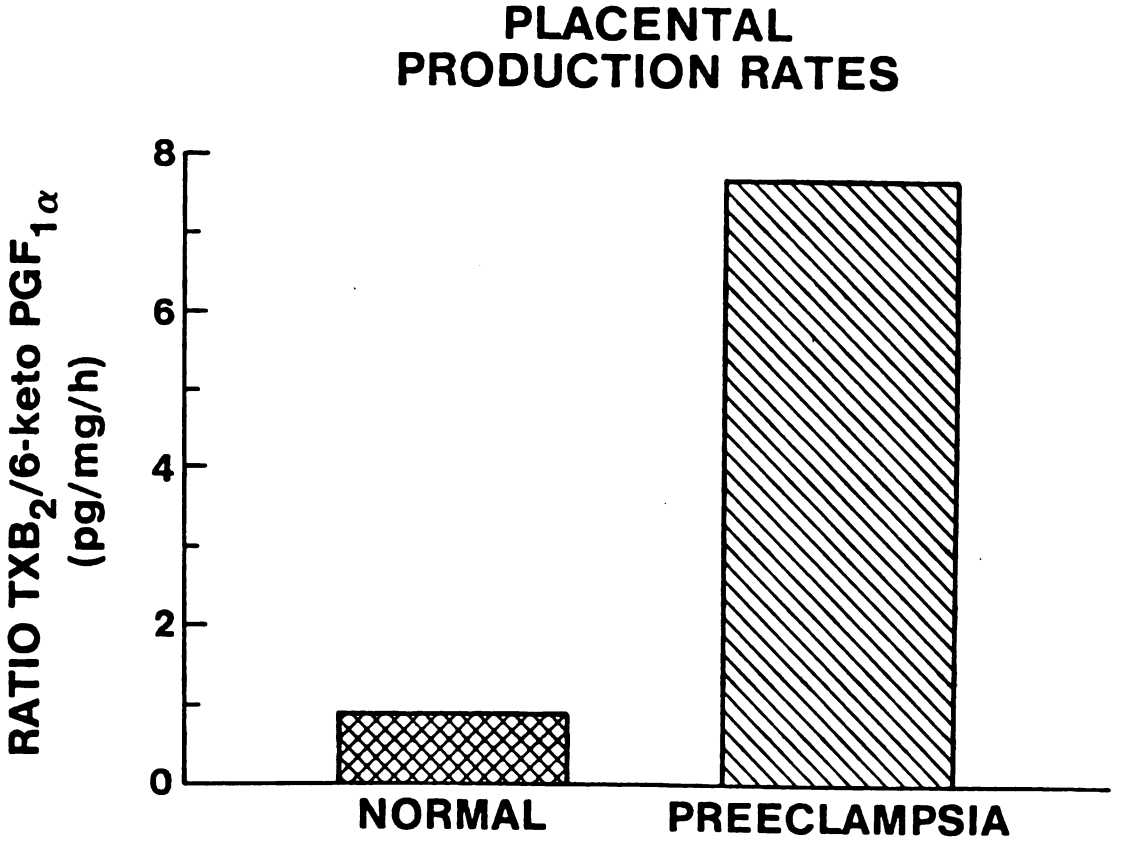


FIGURE 21

thromboxane would be greatest in the placenta because that is the source of production. Glance et al. (1984), utilizing an in vitro placental perfusion system, determined that prostacyclin and thromboxane can alter blood flow in the placental vasculature. Thus the most profound effects of the increased thromboxane/decreased prostacyclin would most probably be within the fetoplacental unit. This could explain the increased vascular congestion and platelet aggregation of preeclamptic placentas (Chesley, 1978; DeWolf et al., 1975; Dennis et al., 1982; Ylikorkala et al., 1981). The imbalance of thromboxane/prostacyclin could reduce the blood flow from the placenta to the fetus because thromboxane constricts and prostacyclin dilates the umbilical artery (Tuvemo et al.,1980). The distribution of blood flow within the fetus might also be affected because the increased ratio of thromboxane to prostacyclin could cause vasoconstriction of the pulmonary vasculature and/or premature closure of the ductus arteriosus (Cassin, 1980; Coceani and Olley, 1980; Makila et al., 1983; Terragno et al., 1980).

Thromboxane and prostacyclin of placental origin is most probably of primary importance in the local regulation of uteroplacental blood flow. However, Martensson and Wallenburg (1984) determined that the concentrations of thromboxane and prostacyclin were greater in the uterine vein than in the maternal periphery. Furthermore, these investigators determined

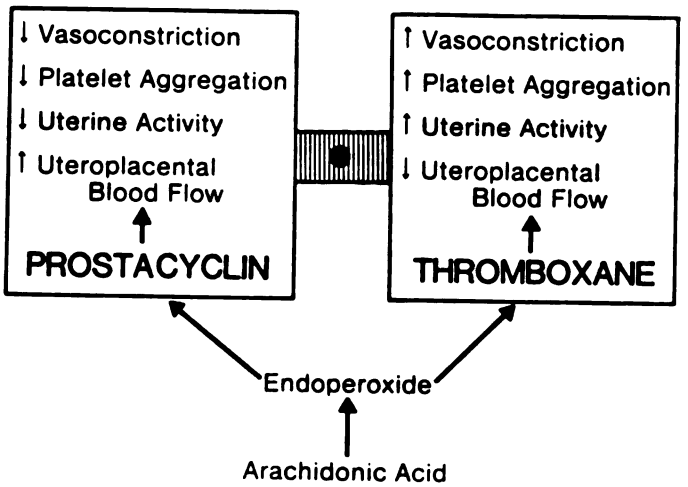
that during preeclampsia there is a significant shift in the ratio of thromboxane to prostacyclin in favor of thromboxane. Thus it is very likely that placental eicosanoid production contributes to maternal systemic levels as well. Therefore, normal pregnancy would be characterized by the placental production of equivalent amounts of prostacyclin and thromboxane with a subsequent balance of their biologic actions (Fig. 22). In contrast, preeclampsia would be characterized by an imbalance in the placental production of these eicosanoids. This imbalance would exacerbate the actions of thromboxane and promote increased vasoconstriction, platelet aggregation and uterine activity concomitant with decreased uteroplacental blood flow (Fig. 22).

The cause of this shift in the arachidonic acid cascade during preeclampsia is unknown. Ogburn et al. (1984) analyzed serum samples from normotensive and preeclamptic patients for total nonesterified fatty acids and total nonesterified arachidonic acid. These authors found that samples from placentas of preeclamptic pregnancies had significantly lower proportions of arachidonic acid in the nonesterified fatty acids and triglycerides than normal placentas. They suggested that a decreased availability of arachidonic acid in the fetoplacental circulation resulted in a decreased production of prostacyclin in preeclampsia. However, if less arachidonic acid is available for conversion to

Figure 22

Comparison of the balance in the biological actions of prostacyclin and thromboxane in normal pregnancy to the imbalance of increased thromboxane/decreased prostacyclin in preeclamptic pregnancies.

NORMAL PREGNANCY



PREECLAMPSIA

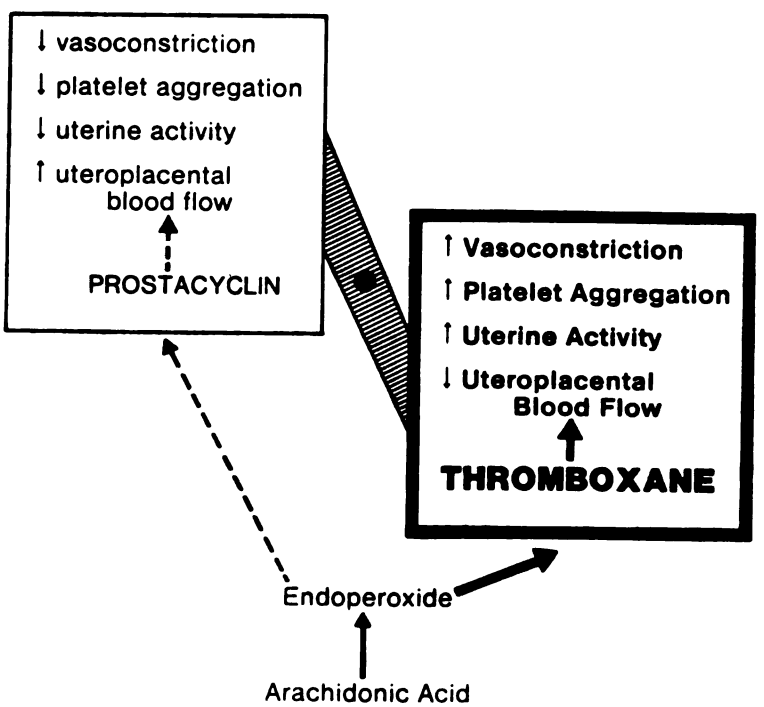


FIGURE 22

prostacyclin then less is available for conversion to thromboxane. Therefore, this does not appear to be a sufficient explanation for a shift in the arachidonic acid cascade. One could infer that perhaps circulating levels of arachidonic acid are not the single determining factor in the production of placental eicosanoids. In fact, M. Behr (1984) speculated that the supply of arachidonic acid was not a limiting factor in placental prostacyclin production because of the abundance of phospholipids in the vascular walls throughout the placenta. In the experimental preparation utilized for this study neither the production rate of thromboxane nor that of prostacyclin (Behr, 1984) were affected by addition of arachidonic acid in either normal or preeclamptic placentas, respectively. Therefore, alterations of eicosanoid production (in vitro) during preeclampsia are not due to precursor availability.

The increased thromboxane/decreased prostacyclin may be due to the amount of enzyme present, or alterations in levels of regulatory proteins/substances effecting prostacyclin synthetase or thromboxane synthase. Both prostacyclin synthetase (DeWitt and Smith, 1983) and thromboxane synthase (Hamberg and Samuelsson, 1974) require oxygen to convert the endoperoxides to prostacyclin or thromboxane, respectively. Because of this, alterations in the oxygen tension perfusing the placental tissues could affect the amount of product formed. Normal

placental tissues incubated in a 20% oxygen environment produced less prostacyclin (Behr, 1984) and the same amount of thromboxane as identical tissues in a 95% oxygen environment. In comparison, preeclamptic placental tissues perfused with 20% oxygen produced the same levels of prostacyclin (Behr, 1984) and less thromboxane than their counterpart perfused with 95% oxygen. The fact that less available oxygen decreases the production of of thromboxane in the preeclamptic placenta suggests that there may be more thromboxane synthase in the preeclamptic placental tissues. Hence, the preeclamptic placenta would have a greater capacity to produce thromboxane. The same could be true for prostacyclin synthetase in the normal placenta. Thus it is possible that alterations in the amount of these enzymes could contribute to the etiology of preeclampsia.

Because preeclampsia is characterized by an increased production of thromboxane and decreased prostacyclin selective inhibition of the thromboxane synthase enzyme could reinstate the balance in the production of these eicosanoids. Previous in vitro studies using a thromboxane synthase inhibitor have significantly decreased thromboxane levels with a simultaneous increase in prostacyclin production (Blackwell et al., 1978; Flower and Cardinal, 1979). In vivo (using a cat), Dusting et al. (1982) induced a five-fold increase in prostacyclin by selective inhibition of thromboxane production. This does not

appear to be the case in the placenta. Addition of a thromboxane synthase inhibitor (U 63557A, Upjohn) significantly decreased the production of thromboxane by both normal and preeclamptic placentas. However, it did not significantly alter the placental production of prostacyclin by either normal or preeclamptic tissues. Therefore, the utilization of a thromboxane synthase inhibitor alone does not appear to be a comprehensive treatment for preeclampsia.

B. Steroids

The production of progesterone by preeclamptic placentas was significantly greater than that by normal placentas. This enhanced progesterone production is not reflected in maternal plasma levels (Allen and Lachelin, 1978; Simmer and Simmer, 1959). However, Walsh et al. (1974) found that levels of progesterone in the uterine vessels were higher than those of the peripheral blood in the primate. So it is likely that progesterone may not be elevated in the peripheral blood during preeclampsia even if the fetoplacental unit produces more.

In vivo the major precursor for progesterone is cholesterol which is converted to pregnenolone (by the 20,22 desmolase complex) then to progesterone (by 3-beta-hydroxysteroid dehydrogenase: Δ 5-4 isomerase enzyme complex [3βHSDH]) (Hagen, 1981). In vitro either preg-

nenolone or pregnenolone sulfate can serve as substrates for progesterone synthesis (Gentz et al., 1984). In this instance pregnenolone sulfate is converted into pregnenolone (by a placental sulfatase) then to progesterone (Hagen, 1981).

Addition of pregnenolone increased the production of progesterone by preeclamptic and normal placentas. This was in agreement with previous investigators (Grimshaw et al., 1983; Little et al., 1971) who determined that exogenous pregnenolone was readily converted to progesterone by placental cells in vitro. However, with pregnenolone added there was no significant difference in the production levels of progesterone by normal and preeclamptic placentas, respectively. This was expected because Diaz-Zagoya and Arias (1981) determined that there was no difference in the mitotic activity of the 3βHSDH between normal and preeclamptic placental tissues. In fact there is an excess of 3βHSDH in the placenta (Little et al., 1971). In contrast, the addition of pregnenolone sulfate resulted in a significant difference in the production curves of progesterone from normal and preeclamptic placentas, respectively. Specifically, the production curve of progesterone by preeclamptic placental tissues was significantly greater than that by normal placental tissues. The enhanced production of progesterone during preeclampsia may be due to the proliferation of the placental sulfatase enzyme, or to the positive activation of

this enzyme by a regulatory substance/protein. Either an increased activity or amount of the sulfatase enzyme during preeclampsia would lead to higher levels of endogenous pregnenolone. Because there is an excess of the 3β HSDH enzyme in the placenta increased available pregnenolone could result in increased levels of progesterone.

The inability to show any differences in the production of estradiol by preeclamptic placentas versus that by normal placentas is consistent with previous plasma and tissue homogenate studies during preeclampsia. The majority of papers on estradiol levels during preeclampsia conclude that estradiol levels are unchanged unless fetal complications are apparent. None of the preeclamptic patients in this study exhibited fetal complications and no differences were found between normal and preeclamptic placental production rates of estradiol. Therefore, the present study corroborates the previous findings and points to no specific role for estradiol in the etiology of preeclampsia.

C. Effect of Estradiol and Progesterone on Placental Production of Prostacyclin and Thromboxane

Progesterone's ability to inhibit prostacyclin has been previously documented in placental cells in monolayer (Myatt et al., 1983) and cultured endometrial cells (Liggins et el., 1980). In the present study enriching the

incubation media with progesterone also significantly decreased the production of prostacyclin by normal and preeclamptic placentas. Similarly, estradiol slightly decreased (but not significantly) placental prostacyclin production from normotensive and preeclamptic patients. This was contrary to the findings of Myatt et al. (1983), who determined that estradiol had a stimulatory effect on prostacyclin production. The discrepencies of results could be due to differences in methodologies; Myatt et al. (1983) utilized trypsinized dispersed cells, whereas my study used mechanically separated fragments of tissue.

Simultaneous addition of progesterone and estradiol resulted in the greatest inhibition of prostacyclin production by normal and preeclamptic placentas. The synergistic effect of these two steroids was consistent with other steroid studies. Genti-Raimondi et al. (1983) determined that estradiol increases levels of progesterone by enhancing the conversion of pregnenolone to progesterone. Younes et al. (1981) isolated receptors for progesterone and estradiol in the cytosol of placental cells. In addition, a previous study (Vu Hai et al., 1977) found that cytosolic progesterone receptors increase in number in response to estradiol. Therefore one would expect an intensification of the effects of progesterone with increased estradiol.

The regulation of thromboxane production from placental cells in vitro by steroids has not been

previously documented. Witter and DiBlasi (1984), studying umbilical endothelial cells, determined that progesterone did not affect thromboxane, but estradiol increased its release from these cells. In comparison, Liggins et al. (1980) found that progesterone decreased and estradiol increased the production of thromboxane from cultured endometrium cells. In our experimental model neither progesterone nor estradiol had any effect on the production of thromboxane by normal or preeclamptic placental tissues. Therefore, increases in thromboxane production during preeclampsia do not appear to be mediated by the actions of these two steroids.

Preeclampsia is characterized by deficient placental production of prostacyclin and enhanced placental production of thromboxane. The fact that progesterone production is increased during preeclampsia and that it effectively decreases the production of prostacyclin implicate its involvement in the etiology of preeclampsia. Progesterone could affect prostacyclin production in at least three ways: 1) it could bind to prostacyclin synthetase directly and act as an allosteric inhibitor; 2) it could bind to a cytosolic or nuclear receptor and alter protein synthesis; 3) it could activate a secondary messenger which could alter the activity of prostacyclin synthetase.

It is likely, from the above data, that progesterone

may directly affect prostacyclin synthesis within the placenta. However, because the placental concentrations of progesterone are not reflected in the peripheral circulation (Walsh et al., 1974) it is possible that other substances are involved in the maternal vascular changes during preeclampsia. The hydroxyeicosatetraenoic acids (HETE's) are attractive substances to fulfill this role. The HETE's are known to be produced by platelets and peripheral blood leukocytes (Borgeat et al., 1982). In addition, they have been implicated in platelet function (Sams et al.,1982), histamine release (Peters et al., 1982),neutrophil and eosinophil chemotaxis (Konig et al., 1982).

Interestingly, preliminary data compiled in our laboratory (Fenner and Walsh, 1985) reveal that placental tissues produce and metabolize the lipoxygenase products, 5-hydroxyeicosatetraenoic acid (5-HETE), 12-HETE and 15-HETE. We have made several interesting observations concerning the HETE's:

1) The preeclamptic placenta produces less 5- and 12-HETE than the normal placenta.

2) The addition of 5- or 12-HETE to the incubation media increased the production of prostacyclin from one preeclamptic patient.

3) The production rates of the HETE's, prostacyclin and thromboxane are different.

4) Inhibition of prostaglandin and thromboxane production increased the production of 12-HETE and prevented the metabolism of 5-and 15-HETE.

5) Addition of exogenous precursor (arachidonic acid) greatly increases the production of the HETE's, with no appreciable effect on the production rates of prostacyclin and thromboxane.

Therefore, our initial data indicate that there may be some interaction between the HETE's and prostacyclin and thromboxane. Although it is too early to make any definite conclusions it is probable that the prostaglandins, thromboxanes and HETE's regulate each other as well as exhibiting other biologic actions. If this is the case, then in all likelihood the HETE's may also be involved in preeclampsia.

VI. SUMMARY AND CONCLUSIONS

A. Eicosanoids

1. During normal pregnancy the placenta produces equivalent amounts of prostacyclin and thromboxane so their biological actions on vascular tone, platelet aggregation and uterine activity would be balanced.

2. In preeclamptic pregnancy the production of thromboxane is heavily favored over that of prostacyclin. Therefore, thromboxane's actions to increase vasoconstriction, platelet aggregation and uterine activity would be exacerbated.

3. Thromboxane synthase inhibitor significantly reduced thromboxane production with no effect on the production rate of prostacyclin in normal and preeclamptic placentas, respectively. Thus thromboxane synthase inhibitor may serve as an adjunct in the treatment of preeclamptic patients in the future.

4. Prostacyclin and thromboxane production, in normal and preeclamptic placentas, can be inhibited with indomethacin. This indicates that both substances are being produced and not just released from placental tissues.

5. Neither the production rate of prostacyclin nor that of thromboxane were significantly affected by addition of the substrate arachidonic acid in either normal or preeclamptic placentas, respectively. Therefore, the production of these eicosanoids is not substrate limited in the placenta.

6. The production of thromboxane in normal placentas is not affected by incubation in a 20% oxygen environment. Thromboxane production in the preeclamptic placenta was significantly reduced in a low oxygen environment.

B. Steroids

1. The production of progesterone from the preeclamptic placenta is significantly greater than that from the normal placenta.

2. The substrate pregnenolone significantly increased the production of progesterone by normal and preeclamptic placentas with no significant differences between these two production rates. This suggests that there is no difference in the activity of 3 HSDH between normal and preeclamptic placentas.

3. The production of progesterone by preeclamptic placentas was significantly greater than from normal placentas with the precursor pregnenolone sulfate added. This

implies an increase in the amount or activity of the placental sulfatase enzyme (which converts pregnenolone sulfate to pregnenolone) in the preeclamptic placenta.

4. There were no significant differences among production curves for estradiol between normal and preeclamptic placentas.

C. Effect of Estradiol and Progesterone on Placental Production of Prostacyclin and Thromboxane.

1. Addition of estradiol to the incubation media did not affect the production of prostacyclin or thromboxane by normal placentas or preeclamptic placentas.

2. The production of thromboxane in normal or preeclamptic placentas was not affected by the addition of progesterone or progesterone plus estradiol to the incubation media.

3. The addition of progesterone or progesterone plus estradiol significantly decreased the production rates of prostacyclin by normal and preeclamptic placentas. Because the preeclamptic placenta produces more progesterone endogenously, it is likely that the local production of progesterone contributes to the decreased production of prostacyclin in the preeclamptic placenta.

BIBLIOGRAPHY

Bibliography

Allen, E. I. and Lachelin., G. C. L.: A comparison of plasma levels of progesterone, oestradiol, unconjugated oestriol and total oestriol with urinary total oestrogen levels in clinical obstetric practice. Br.J. Obstet. Gynecol. 85: 278-292, 1978.

Behr, M. J.: Placental prostacyclin production in pregnancy induced hypertension. Master's Thesis : 1-97, 1984.

Blackwell, G. J., Flower, R. J., Russell-Smith, N., Salmon, J. A., Thorogood, P. B. and Vane., J. R.: I-n-butylimidazole: A potent and selective inhibitor of 'Thromboxane Synthetase'. Br. J. Pharmacol. 64: 436, 1978.

Bodzenta, A., Thomson, J. M. and Poller., L.: Prostacyclin activity in amniotic fluid in pre-eclampsia. Lancet 2: 650, 1980.

Borgeat, P., deLaclos, F., Picard, S., Vallerand, P. and Sirois., P.: Double dioxygenation of arachidonic acid in leukocytes by lipoxygenases. In Leukotrienes and Other Lipoxygenase Products. In: B. Samuelsson and R. Paoletti (eds), Advances in Prostaglandin, Thromboxane and Leukotriene Research. Raven Press, New York, 1982, 45-52.

Brash, A. R., Goodman, R. P. and FitzGerald., G. A.: Endogenous Prostacyclin Production in Human Pregnancy. In: P.J. Lewis (ed), Prostacyclin in Pregnancy. Raven Press, New York, 1983, 71-77.

Briel, R. C. and Lippert., T. H.: Platelet Sensitivity to Prostacyclin in Normal and Complicated Pregnancies. In: P. J. Lewis (ed), Prostacyclin in Pregnancy. Raven Press, New York, 1983, 195-197.

Bussolino, F., Benedetto, C., Massobrio, M. and Camussi., G.: Maternal vascular prostacyclin activity in pre-eclampsia. Lancet 2: 702, 1980.

Carreras, L. O., Defreyn, G., Van Houtte, E., Vermylen, J. and Van Assche., A.: Prostacyclin and pre-eclampsia. Lancet 1: 442, 1981.

Cassin, S.: Role of prostaglandins and thromboxanes in the control of the pulmonary circulation in the fetus and newborn. Seminars in Perinatology 4: 101-107, 1980.

Chesley, L. C.: Hypertensive Disorders in Pregnancy. Appleton-Century-Crofts, New York, 1978, 1-628.

Chew, P. C. T. and Ratnam., S. S.: Plasma estradiol 17-beta as an index of fetoplacental function. Int. J. Gynecol. Obstet. 14: 445-448, 1976.

Coceani, F. and Olley., P. M.: Role of prostaglandins, prostacyclin, and thromboxanes in the control of prenatal patency and postnatal closure of the ductus arteriosus. Seminars in Perinatology 4: 109-113, 1980.

De Wolf, F., Robertson, W. B. and Brosens., I.: The ultrastructure of acute atherosis in hypertensive pregnancy. Am. J. Obstet. Gynecol. 123: 164-174, 1975.

Demers, L. M.: PROSTAGLANDINS. In: G.J. Race (ed), Laboratory Medicine. Harper & Row, Hagerstown, 1980, 1-19.

Dennis, E. J., III, McFarland, K. F. and Hester., L. L., Jr: The preeclampsia-eclampsia syndrome. In: D.N. Danforth (ed), Obstetrics and Gynecology. Harper and Row, Philadelphia, 1982, 455-474.

DeWitt, D. L. and Smith., W. L.: Purification of prostacyclin synthase from bovine aorta by immunoaffinity chromatography. Evidence that the enzyme is a hemoprotein. J. Biol. Chem. 258: 3285, 1983.

Diaz-Zagoya, J. C. and Arias., F.: Synthesis and catabolism of progesterone in placentas from normotensive and severely hypertensive patients before and after parturition. Am. J. Obstet. Gynecol. 141: 637-640, 1981.

Diczfalusy, E.:. Acta Endocr. 10: 373, 1952.

Dusting, G. J., Moncada, S. and Vane., J. R.: Recirculation of prostacyclin (PGI) in the dog. Br. J.

Pharmacol. 64: 315-320, 1978.

Dusting, G. J., Moncada, S. and Vane., J. R.: Prostacyclin: Its Biosynthesis, Actions, and Clinical Potential In Prostaglandins and the Cardiovascular System. In: J.A. Oates (ed), Advances in Prostaglandin, Thromboxane, and Leukotriene Research. Raven Press, New York, 1982, 59-106.

Easterling, W. A. and Talbert., L. M.: Estriol excretion in normal and complicated pregnancies. Amer. J. Obstet. Gynec. 107: 417-422, 1970.

Everett, R. B., Worley, R. J., MacDonald, P. C. and Gant., N. F.: Modification of vascular responsiveness to angiotensin II in pregnant women by intravenously infused 5-alpha-dihydroprogesterone. Am. J. Obstet. Gynecol. 131: 352-357, 1978a.

Everett, R. B., Worley, R. J., Macdonald, P. C. and Gant., N. F.: Effect of prostaglandin synthetase inhibitors on pressor response to angiotensin II in human pregnancy. J. Clin. Endo. and Med. 46: 1007-1009, 1978b.

Farquharson, R. G. and Klopper., A. I.: Progesterone concentrations in maternal and fetal blood. Br. J. Obstet. Gynecol. 91: 133-137, 1984.

Fitzgerald, D. J., Entman, S. S., Mulloy, M. K. and Fitzgerald., G. A.: Reduced biosynthesis of prostacyclin in human pregnancy: A predictor of pregnancy induced hypertension?. In: E.M. Symonds, F.P. Zuspan, and H.C.S. Wallenburg (eds), Hypertension in Pregnancy. Marcel Decker, Inc., New York, 1984, 65.

Flower, R. J. and Cardinal., D. G.: Use of a novel platelet aggregometer to study the generation by, and actions of prostacyclin in whole blood. In: J.R. Vane (ed), Prostacyclin. Raven Press, New York, 1979, 211-220.

Gant, N. F., Whalley, P. J., Everett, R. B., Worley, R. J. and MacDonald., P. C.: Evidence for a Vasodepressor Prostaglandin Deficiency in Pregnancy-Induced Hypertension. In: P.J. Lewis (ed), Prostacyclin in Pregnancy. Raven Press, New York, 1983, 99-108.

Genti-Raimondi, S., Patrito, L. C. and Flury., A.: Effect of estradiol-17-beta on the conversion of pregnenolone to progesterone in human term placenta incubated in vitro. Steroids 41: 467-474, 1983.

Gentz, J., Persson, B., Westin, b. and Zetterstrom., R.: Perinatal Medicine. Praeger, New York, 1984, 51-57.

Glance, D. G., Elder, M. G. and Myatt., L.: The actions of prostaglandins and their modulation of angiotensin II in the isolated perfused human placental cotyledon. In: (ed), Presented at the 4th World Congress of the International Society for the Study of Hypertension in Pregnancy. (Abstract, p.122), Amsterdam, 1984, June 18-21.

Goodman, R. P., Killam, A. P., Brash, A. R. and Branch., R. A.: Prostacyclin production during pregnancy: Comparison of productionduring normal pregnancy and pregnancy complicated by hypertension. Am. J. Obstet. Gynecol. 142: 817-822, 1982.

Gorman, R. R.: Modulation of human patelet function by prostacyclin and thromboxane A2. Fed Proc. 38: 83-88, 1979.

Granstrom, E., Diczfalusy, U., Hamburg, M., Hansson, G., Malmsten, C. and Samuelsson., B.: Thromboxane A2: Biosynthesis and Effects on Platelets. In: J.A. Oates (ed), Advances in Prostaglandin, Thromboxane and Leukotriene Research. Raven Press, New York, 1982, 15-58.

Grimshaw, R. N., Mitchell, B. F. and Challis., J. R. G.: Steroid modulation of pregnenolone to progesterone conversion by human placental cells in vitro. Am. J. Obstet. Gynecol. 145: 234-238, 1983.

Gryglewski, R. J., Korbut, R. and Ocetkiewicz., A.: Generation of prostacyclin by lungs in vivo and its release into the arterial circulation. Nature 273: 765-767, 1978.

Hagen, A. A.: Steroidogenesis in the fetoplacental unit. In: J.R. Givens and G.D. Anderson (eds), Endocrinology of Pregnancy. Year Book Medical Pub., Inc., Chicago, 1981, 99-105.

Hamberg, M. and Samuelsson., B.: Novel transformations of arachidonic acid in human platelets. Proc. Natl. Acad. Sci. USA 71: 3400-3404, 1974.

Hellman, L. M., Kobayashi, M., Fillisti, L. and Lavenhar., M.: Sources of error in sonographic fetal mensuration and estimation ofgrowth. Am. J. Obstet. Gynecol. 99: 662-670, 1967.

Hensby, C. N., Dollery, C. T., Barnes, P. J. and Dargie., H.: Production of 6-oxo-PGF1-alpha by human lungs in vivo. Lancet 2: 1162-1163, 1979.

Ingerman-Wojenski, C., Silver, M. J. and al., e. t.: Bovine endothelial cells in culture produce thromboxane as well as prostacyclin. J. Clin. Invest. 67: 1292, 1981.

Jogee, M., Elder, M. G. and Myatt., L.: Decreased Prostacyclin Production by Placental Cells in Culture from Pregnancies Complicated by Fetal Growth Retardation. In: P.J. Lewis (ed), Prostacyclin in Pregnancy. Raven Press, New York, 1983, 115-118.

Kankaanrinta, T. and Forssell., O.: Some aspects of the urinary excretion of pregnanediol in late pregnancy toxemia. Ann. Chir. Gynaec. Fenn. 50: 434-444, 1961.

Konig, W., Kunau, H. W. and Borgeat., P.: Induction and comparison of the eosinophil chemotactic factor with endogenous hydroxyeicosatetraenoic acids: Its inhibition by arachidonic acid analogs. In Leukotrienes and Other Lipoxygenase Products. In: B. Samuelsson and R. Paoletti (eds), Advances in Prostaglandin, Thromboxane and Leukotriene Research. Raven Press, New York, 1982, 301-314.

Koullapis, E. N., Nicolaides, K. H., Collins, W. P. and al., e. t.: Plasma prostanoids in pregnancy-induced hypertension. Br. J. Obstet. Gynecol. 89: 617, 1982.

Lewis, P. J.: The role of prostacyclin in pre-eclampsia. Br. J. Hosp. Med. 28: 393-395, 1982.

Lewis, P. J., Boylan, P., Friedman, L. A., Hensby, C. N. and Downing., I.: Prostacyclin in preeclampsia. Br. Med. J. 280: 1581-1582, 1980.

Lewis, P. J., Shepherd, G. L., Ritter, J., Chan, S. M., Bolton, P. J., Jogee, M., Myatt, L. and Elder., M. G.: Prostacyclin and pre-eclampsia. Lancet 1: 559, 1981.

Liggins, G. C., Campos, G. A., Roberts, C. M. and Skinner., S. J.: Production rates of prostaglandin F, 6-keto-PGF1-alpha and thromboxane B2 by perifused human endometrium. Prostaglandins 19: 461-477, 1980.

Lindberg, S., Nilsson, B. A. and Johansson., E. D. B.: Plasma progesterone levels in normal and abnormal pregnancies. Acta Obstet. Gynec. Scand. 53: 329-335, 1974.

Linton, M. and Gallo., P. S., Jr: The Practical Statistician. Simplified Handbook of Statistics. Brooks/Cole Pub. Co., Monterey, 1975, 224-235.

Little, B., Billier, R. B., Halla, M., Heinsons, A., Jassani, M., Kline, I. T. and Purdy., R. H.: Pregnenolone production in pregnancy. Am. J. Obstet. Gynec. 111: 505-514, 1971.

Llauro, J. L., Runnebaum, B. and Zander., J.: Progesterone in human peripheral blood before, during, and after labor. Am. J. Obstet. Gynec. 101: 867-873, 1968.

Louros, N. C., Miras, K. and Frangiadakis., L.: Urinary aldosterone and pregnanediol excretion in normal pregnancy and toxaemia of pregnancy. Gebutsh. U. Frauenheilk 20: 848-851, 1960.

Lye, S. J. and Challis., J. R. G.: Inhibition by PGI-2 of myometrial activity in vivo in non-pregnant ovariectomized sheep. J. Reprod. Fert. 66: 311-315, 1982.

Makila, U. M., Kokkonen, E., Viinikka, L. and Ylikorkala., O.: Differential inhibition of fetal vascular prostacyclin and platelet thromboxane synthesis by nonsteroidal anti-inflammatory drugs in humans. Prostaglandins 25: 39-46, 1983.

Makila, U. M., Viinikka, L. and Ylikorkala., O.: Evidence that prostacyclin deficiency is a specific feature in

preeclampsia. Am. J. Obstet. Gynecol. 148: 772-774, 1984.

Martensson, L. and Wallenburg., H. C. S.: Uterine venous concentrations of 6-keto-PGF1-alpha(6-K) in normal pregnant(NP) and pregnancy-induced hypertensive(PIH) women. Presented at the 31st Annual Meeting of the Society for GynecologicInvestigation. March 21-25 : San Francisco (Abstr.#410), 1984.

Masson, G. M.: Plasma oestriol in pre eclampsia. J. Obstet. Gynaec. Brit. Cwlth. 80: 206-209, 1973.

Mather, R. S., Chestnut, S. K., Leaming, A. B. and Williamson., H. O.: Application of plasma estriol estimations in the management of highrisk pregnancies. Am. J. Obstet. Gynecol. 117: 210-219, 1973.

Mather, R. S., Leaming, A. B. and Williamson., H. O.: An assessment of free and conjugated estriol in uncomplicated and complicated pregnancy plasma. Am. J. Obstet. Gynecol. 117: 316-320, 1973.

Mati, J. K. G., Mugambi, M., Odipo, W. S. and Nguli., K.: Prolactin and hypertension. Am. J. Obstet. Gynecol. 127: 616-619, 1977.

Mitchell, M. D., Bibby, J., Hicks, B. R. and Turnbull., A. C.: Specific production of prostaglandin E by human amnion in vitro. Prostaglandins 15: 877-882, 1978.

Mitchell, M. D., Bibby, J. G., Hicks, B. R., Redman, C. W. G., Anderson, A. B. M. and Turnbull., A. C.: Thromboxane B2 and human parturition: cocentrations in the plasma and production in vitro. J. Endocr. 78: 435-441, 1978.

Mitchell, M. D., Bibby, J. G., Hicks, B. R. and Turnbull., A. C.: Possible role for prostacyclin in human parturition. Prostaglandins 16: 931, 1978.

Moncada, S. and Amezcua., J. L.: Prostacyclin, thromboxane A2 interactions in haemostasis and thrombosis. Haemostasis 8: 252-265, 1979.

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

Moncada, S., Higgs, E. A. and Vane., J. R.: Human arterial and venous tissues generate prostacyclin (prostaglandin X), a potent inhibitor of platelet aggregation. Lancet i: 18-19, 1977.

Moncada, S., Korbut, R., Bunting, S. and Vane., J. R.: Prostacyclin is a circulating hormone. Nature 273: 767-768, 1978.

Moncada, S. and Vane., J. R.: The role of prostacyclin in vascular tissue. Fed. Proc. 38: 66-71, 1979a.

Moncada, S. and Vane., J. R.: Pharmacology and endogenous roles of prostagalndin endoperoxides, thromboxane A2, and prostacyclin. Pharmacological Reviews 30: 293-331, 1979b.

Mutschler, F., Kecskes, L. and Kobor., J.: Studies on oestrogen fractions in full-term normal and toxaemic placentae. Zbl. Gynak. 85: 762-764, 1963.

Myatt, L., Jogee, M. and Elder., M. G.: Regulation of Prostacyclin Metabolism in Human Placental Cells in Culture by Steroid Hormones. In: P.J. Lewis (ed), Prostacyclin in Pregnancy. Raven Press, New York, 1983, 119-129.

Needleman, P., Wyche, A. and Raz., A.: Platelet and blood vessel arachidonate metabolism and interactions. J. Clin. Invest. 63: 345-349, 1979.

Neri Serneri, G. G., Abbate, R., Gensini, G. F., Panetta, A., Casolo, G. C. and Carini., M.: TXA2 production by human arteries and veins. Prostaglandins 25: 753-766, 1983.

Ogburn, P. L., Jr, Williams, P. P., Johnson, S. B. and Holman., R. T.: Serum arachidonic acid levels in normal and preeclamptic pregnancies. Am. J. Obstet. Gynecol. 148: 5-9, 1984.

Omini, C., Folco, G. C., Pasargiklian, R., Fano, M. and Berti., F.: Prostacyclin (PGI2) in pregnant human uterus. Prostaglandins 17: 113-120, 1979.

Ostergard, D. R. and Kushinsky., S.: Urinary estriol as an indicator of fetal well-being. Obstet. & Gynec. 38: 74-78, 1971.

Pearlman, W. H. and Cerceo., E. J.:. J. Biol. Chem. 198: 79, 1952.

Pedersen, E. B., Rasmussen, A. B., Johannesen, P., Kornerup, H. J., Kristensen, S., Lauritsen, J. G. and Wohlert., M.: The renin-aldosterone system in pre-eclampsia, essential and transient hypertension during pregnancy, and normotensive pregnant and non-pregnant control subjects. Acta Endocr. 101: 273-280, 1982.

Pederson, E. B., Christensen, N. J., Christensen, P., Johannesen, P., Kornerup, H. J., Kristensen, S., Lauritsen, J. G. and Leysacc., P. P.: Urinary Prostaglandin Excretion, the Renin-Aldosterone System, and Circulating Catecholamines in Preeclampsia. Hypertension 5: 105-111, 1983.

Peters, S. P., Schleimer, R. P., Marone, G., Kagey-Sobotka, A., Siegel, M. I. and Lichtenstein., L. M.: Lipoxygenase products of arachidonic acid: Role in modulation of IgE-induced histamine release. In Leukotrienes and Other Lipoxygenase Products. In: B. Samuelsson and R. Paoletti (eds), Advances in Prostaglandin, Thronboxane and Leukotriene Research. Raven Press, New York, 1982, 315-324.

Pritchard, J. A., Cunningham, F. G. and Mason., R. A.: Coagulation changes in eclampsia: Their frequency and pathogenesis. Am. J. Obstet. Gynecol. 124: 855-864, 1976.

Rahman, S. A., Hingorani, V. and Laumas., K. R.: Biosynthesis of oestrogens and their inter-conversion in human placentae from normal and toxaemic pregnancies. Clin. Endocrinol. 4: 333-341, 1975.

Rakoczi, I., Tihanyi, K., Falkay, G., Rozsa, I., Demeter, J. and Gati., I.: Prostacyclin Production in Trophoblast. In: P.J. Lewis (ed), Prostacyclin in Pregnancy. Raven Press, New York, 1983, 15-23.

Ranta, T., Stenman, U. H., Unnerus, H. A., Rossi, J. and Seppala., M.: Maternal plasma prolactin levels in preeclampsia. Obstet. Gynecol. 55: 428-430, 1980.

Reid, S., Beischer, N. A., Brown, J. B. and Smith., M. A.: Urinary oestriol excretion in pregnancies complicated by proteinurea. Aust. N.Z. J. Obstet. Gynecol. 8: 189-196, 1968.

Remuzzi, G., Marchesi, D., Mecca, G., Misiani, R., Rossi, E., Donati, M. B. and deGaetano., G.: Reduction of fetal vascular prostacyclin activity in pre-eclampsia. Lancet 2: 310, 1980.

Remuzzi, G., Marchesi, D., Zoja, C., Muratore, D., Mecca, G., Misiani, R., Rossi, E. and al., e. t.: Reduced umbilical and placental vascular prostacyclin in severe pre-eclampsia. Prostaglandins 20: 105-110, 1980.

Remuzzi, G., Misiani, D., Muratore, D., Marchesi, D., Livio, M., Schieppati, A., Mecca, G. and al., e. t.: Prostacyclin and human foetal circulation. Prostaglandins 18: 341-348, 1979.

Resnik, R. and Brink., G. W.: Uterine vascular response to prostacyclin in nonpregnant sheep. Am. J. Obstet. Gynecol. 137: 267-269, 1980.

Resnik, R., Brink, G. W. and Plumer., M. H.: The effect of progesterone on estrogen-induced uterine blood flow. Am. J. Obstet. Gynecol. 128: 251-254, 1977.

Salhanick, H. A., Noall, M. W., Zarrow, M. X. and Samuels., L. T.:. Science 115: 708, 1952.

Sams, A. R., Sprecher, H., Sankarappa, S. K. and Needleman., P.: Selective inhibitors of platelet arachidonic acid metabolism: Aggregation independent of lipoxygenase. In Leukotrienes and Other Lipoxygenase Products. In: B. Samuelsson and R. Paoletti (eds), Advances in Prostaglandin, Thromboxane and Leukotriene Research. Raven Press, New York, 1982, 19-28.

Simmer, H. and Simmer., I.: Progesterone in teh peripheral venous blood in patients with late toxaemia of pregnancy. Klin. Wschr. 37: 971-975, 1959.

Speroff, L.: An essay: prostaglandins and toxemia of pregnancy. Prostaglandins 3: 721-728, 1973.

Stuart, M. J., Sunderji, S. G., Yambo, T., Clark, D. A., Allen, J. B., Elrad, H. and Slott., J. H.: Decreased prostacyclin production: a characteristic of chronic placental insufficiency syndromes. Lancet 1: 1126-1128, 1981.

Svensson, J., Strandberg, K., Tuvemo, T. and Hamberg., M.: Thromboxane A2: Effects on airway and vascular smooth muscle. Prostaglandins 14: 425-436, 1977.

Terragno, N. A., Terragno, A. and McGiff., J. C.: Role of prostaglandins in blood vessels. Seminars in Perinatology 4: 85-90, 1980.

Townsley, J. D., Gartman, L. J. and Crystle., C. D.: Maternal serum 17-beta estradiol levels in normal and complicated pregnancies: A comparison with other estrogen indices of fetal health. Am. J. Obstet. Gynecol. 115: 830-834, 1973.

Tulchinsky, D. and Okada., D. M.: Hormones in human pregnancy. IV. Plasma progesterone. Am. J. Obstet. Gynecol. 121: 293-299, 1975.

Tuvemo, T.: Role of prostaglandins, prostacyclin, and thromboxanes in the control of the umbilical-placental circulation. Seminars in Perinatology 4: 91-95, 1980.

Vu Hai, M. T., Logeat, F., Warembourg, M. and Milgrom., E.: Hormonal control of progesterone receptors. Ann. N.Y. Acad. Sci. 286: 199-209, 1977.

Wallenburg, H. C. S. and Rotmans., N.: Enhanced reactivity of the platelet thromboxane pathway in normotensive and hypertensive pregnancies with insufficient fetal growth. Am. J. Obstet. Gynecol. 144: 523-528, 1982.

Walsh, S. W., Behr, M. J. and Allen., N. H.: Placental prostacyclin production in normal and toxemic pregnancies. Am. J. Obstet. Gynecol. 151: 110-115, 1985.

Walsh, S. W., Wolf, R. C. and Meyer., R. K.: Progesterone, progestins and 17-hydroxypregn-4-ene-3,20 dione in the utero-ovarian, uterine and peripheral blood of the pregnant rhesus monkey. Endocrinology 95: 1704-1710, 1974.

Wilhelmsson, L., Wikland, M. and Wiqvist., N.: PGH2, TXA2 and PGI2 have potent and differentiated actions on human uterine contractility. Prostaglandins 21: 277-285, 1981.

Willocks, J.: The use of ultrasonic cephalometry. Proc. Roy. Soc. Med. 55: 640, 1962.

Willocks, J., Donald, I., Duggan, T. C. and Day., N.: Foetal cephalometry by ultrasound. J. Obstet. Gynecol. Br. Commonw. 71: 11-20, 1964.

Witter, F. R. and DiBlasi., M. C.: Effect of steroid hormones on arachidonic acid metabolites of endothelial cells. Obstet. Gynecol. 63: 747-751, 1984.

Wurterle, A.: The excretion of oestrogens in late toxaemia of pregnancy. Z. Gebutsh. Gynak. 159: 287-307, 1962.

Yamaguchi, M. and Mori., N.: 6-keto prostaglandin F1-alpha, thromboxane B2, and 13,14-dihydro- 15-keto prostaglandin F concentrations of normotensive and preeclamptic patients during pregnancy, delivery, and the postpartum period. Am. J. Obstet. Gynecol. 151: 121-127, 1985.

Ylikorkala, O., Jouppila, P., Kirkinen, P. and Viinikka., L.: Maternal thromboxane, prostacylclin, and umbilical blood flow in humans. Obstet. Gynecol. 63: 677-680, 1984.

Ylikorkala, O., Jouppila, P., Makila, U., Malarainen, L. and : Prostacyclin in Hypertensive Pregnancies. In: E.M. Symonds, F.P. Zuspan, and H.C.S. Wallenburg (eds), Fourth World Congress of the Society for the Study of Hypertension in Pregnancy. Marcel Decker Inc., Amsterdam-The Neth., 1984, 45.

Ylikorkala, O., Kirkinen, P. and Viinikka., L.: Maternal plasma prostacyclin concentrations in pre-eclampsia and other pregnancy complications. Br. J. Obstet. Gynecol. 88: 968-972, 1981.

Ylikorkala, O., Makila, U. M. and Viinikka., L.: Amniotic fluid prostacyclin and thromboxane in normal, preeclamptic, and some other complicated pregnancies. Am. J. Obstet. Gynecol. 141: 487-490, 1981.

Ylikorkala, O., Makila, U. M. and Viinikka., L.: Effect of vacuum curettage on the concentrations of plasma 6-keto-prostaglandin F1-alpha and serum thromboxane B2. Br. J. Obstet. Gynecol. 90: 251-254, 1983.

Ylikorkala, O. and Viinikka., L.: Thromboxane A2 in pregnancy and puerperium. Br. Med. J. 281: 1601-1602, 1980.

Younes, M. A., Besch, N. F. and Besch., P. K.: Estradiol and progesterone binding in human term placental cytosol. Am. J. Obstet. Gynecol. 141: 170-174, 1981.

Zahradnik, H. P., Schoening, R. and Breckwoldt., M.: Prostaglandins: Correlation to Human Myometrial Activity In Vitro. In: P.J. Lewis (ed), Prostacyclin in Pregnancy. Raven Press, New York, 1983, 147-151.

APPENDICES

APPENDIX A

Prostacyclin Radioimmunoassay

Placental production of prostacyclin was estimated by radioimmunoassay (RIA) for the concentration of a stable breakdown product, 6-keto PGF1$_\alpha$ in the incubation media. 6-keto PGF1$_\alpha$ is generated by nonenzymatic degradation of prostacyclin.

REAGENTS:

All reagents used in the assay were diluted with phosphate buffered saline (PBSG - 0.01 M phosphate, 0.09% saline, 0.1% gelatin, 0.01% sodium azide at pH 7.4) (Table 6). Standards (9.88pg/0.05 ml - 1250pg/0.05 ml) were made by adding known amounts of 6-keto PGF1$_\alpha$ to PBSG in 50 ml conical centrifuge tubes. Fresh standards were made every six months. Disposable glass tubes (12mm X 75mm) were used for the RIA. Rabbit antibody (Seragen, Boston, MA) highly specific for 6-keto PGF1$_\alpha$ (Table 7) was diluted 1:20 in PBSG. The original volume of radioactive 6-keto PGF1$_\alpha$ (New England Nuclear, Boston, MA; 0.25 ml of acetonitrile:water, 9:1, with 0.025 mCi of labeled 6-keto PGF1$_\alpha$) was diluted with an additional 1.0 ml of acetonitrile:water (9:1) to minimize loss of the radioactive prostaglandin due to evaporation and adsorbtion on the vial during storage. For each assay trace was diluted in PBSG to a concentration of approximately 6000 cpm/0.05 ml solution. Dextran-coated charcoal (Table 6) was utilized in each RIA to separate

TABLE 6

Table of Solutions

0.1 M Phosphate buffer
2.76 g $NaH_2PO_4 \cdot H_2O$
11.35 g Na_2HPO_4
9.0 g NaCl
0.1 g gelatin
1000.0 ml grade 1 water

Charcoal Solution
2.5 g Norit A charcoal (Fisher Scientific Co., Fair Lawn, NJ)
0.25 g Dextran T (Pharmacia Fine Chemicals, Uppsala, Sweden)
500.0 ml of 0.01 M PBSG

TABLE 2

Rabbit-anti-6-keto PGF1$_\alpha$ Cross Reactivity at 50% B/Bo*

6-keto PGF1$_\alpha$	(100%)
PGF1$_\alpha$	7.8%
6-keto PGE1$_\alpha$	6.8%
PGF2	2.2%
PGE1	0.7%
PGE2	0.6%
PGD2	<0.01%
PGA2	<0.01%
PGA1	<0.01%
PGB1	<0.01%
PGB2	<0.01%
Thromboxane B2	<0.01%
15-keto PGF2$_\alpha$	<0.01%
15-keto PGE2	<0.01%
DHKE2	<0.01%
DHKF2$_\alpha$	<0.01%

*Data from Seragen

the bound from the free fraction.

PROCEDURE:

A Micromedic automatic pipet was used to add all reagents to the glass tubes. Fifty microliters of sample or standard were pipeted into tubes and diluted with 150 ul of PBSG (Table 8). The final incubation volume (300 ul) was reached by simultaneously adding 50 ul of antibody and 50 ul of trace to each tube (Table 8). The total amount of radioactivity in each tube was determined by the counts per minute produced by 50 ul of trace in 250 ul of PBSG (Table 8). A zero dose tube (Bo tube) was made by adding 50 ul of trace and 50 ul of antibody into 200 ul of PBSG (Table 8). Nonspecific binding in the assay was determined from the counts per minute generated by a charcoal stripped total count tube (Table 8). All tubes were vortexed and incubated for 18-24 h at 4°C.

Following incubation 1.0 ml of dextran-coated charcoal, pre-cooled to 0-4°C, was added to all tubes except the total count tubes. The total count tubes received 1.0 ml PBSG so that quenching was equal for all tubes. Charcoal was used to separate labeled 6-keto $PGF1_{\alpha}$ that was not bound by the antibody during the incubation period. The charcoal solution was kept on ice and mixed continuously. Immediately after charcoaling all the tubes were vortexed and incubated for 15 minutes at 4°C. At the end of this time period all the assay tubes were centrifuged at 2000 x g (Beckman Model J-6B refrigerated

TABLE 8

Prostacyclin Radioimmunoassay Protocol

Tube	Buffer (ul)	Trace (ul)	Antibody (ul)	recieve charcoal on day 2 of assay
Total count tube	250	50	--	NO
zero dose tube	200	50	50	YES
non-specific binding tube	250	50	--	YES
standards (50 ul)	150	50	50	YES
samples (50 ul)	150	50	50	YES

centrifuge, Palo Alto, CA) for 12 minutes. The supernatant was decanted into scintillation vials containing 15 ml of scintillation cocktail (Safety Solve, Mount Prospect, IL) The entire volume of each total count tube was decanted into a scintillation vial. The radioactivity in each assay tube was counted on a Packard Tri Carb 300C liquid scintillation counter (Packard Instrument Co., Downers Grove, IL) for ten minutes or until 2.0 percent accuracy was achieved.

Standards were plotted as a percentage of the zero dose tubes (% B/Bo). The sample concentration could then be calculated from the standard curve using the percent binding of each sample obtained experimentally.

Reliability of the Assay

Specificity: A highly specific antibody for 6-keto PGF1 was used in this assay (Table 7). Therefore sample extraction was not necessary and the incubation media was assayed directly.

Test of Parallelism: A patient sample, with an unknown quantity of 6-keto $PGF1_{\alpha}$, was serially diluted three times with DMEM and processed in each assay. These were plotted as a percent of the zero dose and assessed for parallelism to the standard curve (Figure 23).

Sensitivity: The concentration two standard deviations away from the zero dose level, the least detectable concentration, was 2.72 pg $\pm$ 0.62 (mean $\pm$ SE, n=10).

Figure 23

Representative binding of the 6-keto $PGF1_{\alpha}$ standards. Plotted are the percentage of the total binding for each of the eight standards (mean ± SE, n=10). Also graphed are the serial dilutions (mean ± SE, n=5): P12.5 = 12.5 ul of sample, P25 = 25 ul of sample, P50 = 50 ul of sample.

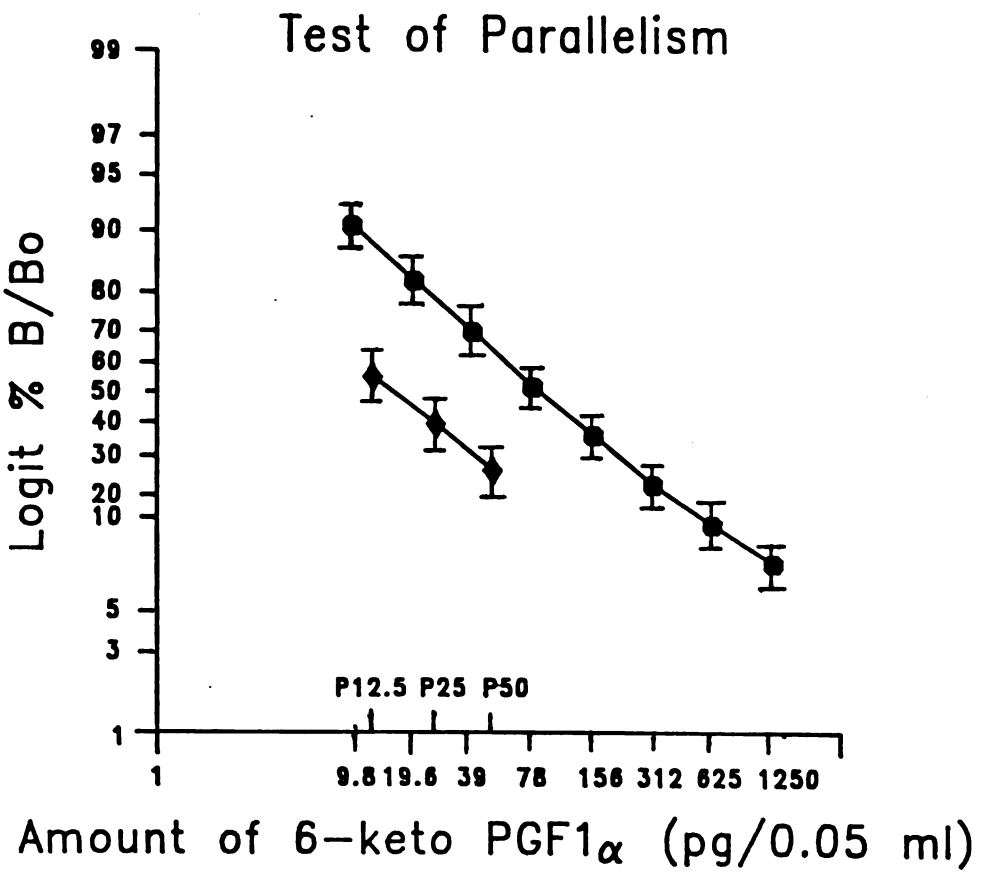

Test of Parallelism
Logit % B/Bo
99
97
95
90
80
70
60
50
40
30
20
10
5
3
1
P12.5 P25 P50
1
9.8 19.6 39 78 156 312 625 1250
Amount of 6-keto PGF1α (pg/0.05 ml)

Figure 23

Precision of the assay: Within- and between-assay variations were calculated according to the methods of Rodbard for each of the three serial dilutions (Table 9). Within-assay variations ranged from 4.79 to 6.70% and between-assay variations ranged from 10.8 to 14.8%.

Accuracy: To determine the accuracy of the assay, varying concentrations of 6-keto PGF1α were added to DMEM. Within- and between-assay variations were calculated according to the methods of Rodbard for each spiked sample (Table 10). Within-assay variations ranged from 5.7 to 13.5% and between-assay variations ranged from 8.2 to 22%. The recovery values for the spiked samples are plotted in Figure 24. Unspiked DMEM samples were also run in each assay and were equivalent to a zero dose response (mean $\pm$ SE, 110 $\pm$ 1.32%, n=5).

TABLE 2

Precision of the 6-keto PGF1$_\alpha$ Radioimmunoassay

Volume of standard assayed (ul)	Mean (pg)	df	Within-assay SD	Within-assay variation =CV	Between-assay SD	Between-assay variation =CV
12.5	75.0	5	3.59	4.79	11.1	14.8
25	157	5	8.68	5.53	16.9	10.8
50	290	5	19.4	6.70	42.8	14.8

TABLE 10

Accuracy of the 6-keto PGF1$_\alpha$ Radioimmunoassay

Amount of 6-keto PGF1$_\alpha$ added (pg)	Amount of 6-keto PGF1$_\alpha$ recovered (pg)	df	Within-assay SD	Within-assay variation =CV	Between-assay SD	Between-assay variation =CV
625	609	12	50.0	8.20	55.5	9.10
312	316	7	20.3	6.41	29.5	9.30
156	140	12	10.4	7.42	17.3	12.3
78	69	7	3.93	5.70	5.65	8.20
39	26.8	12	3.60	13.5	5.94	22.0

Figure 24

Test of accuracy of the 6-keto PGF1α assay. Known amounts of 6-keto PGF1α added were plotted against the amount recovered in the assay (mean $\pm$ SE).

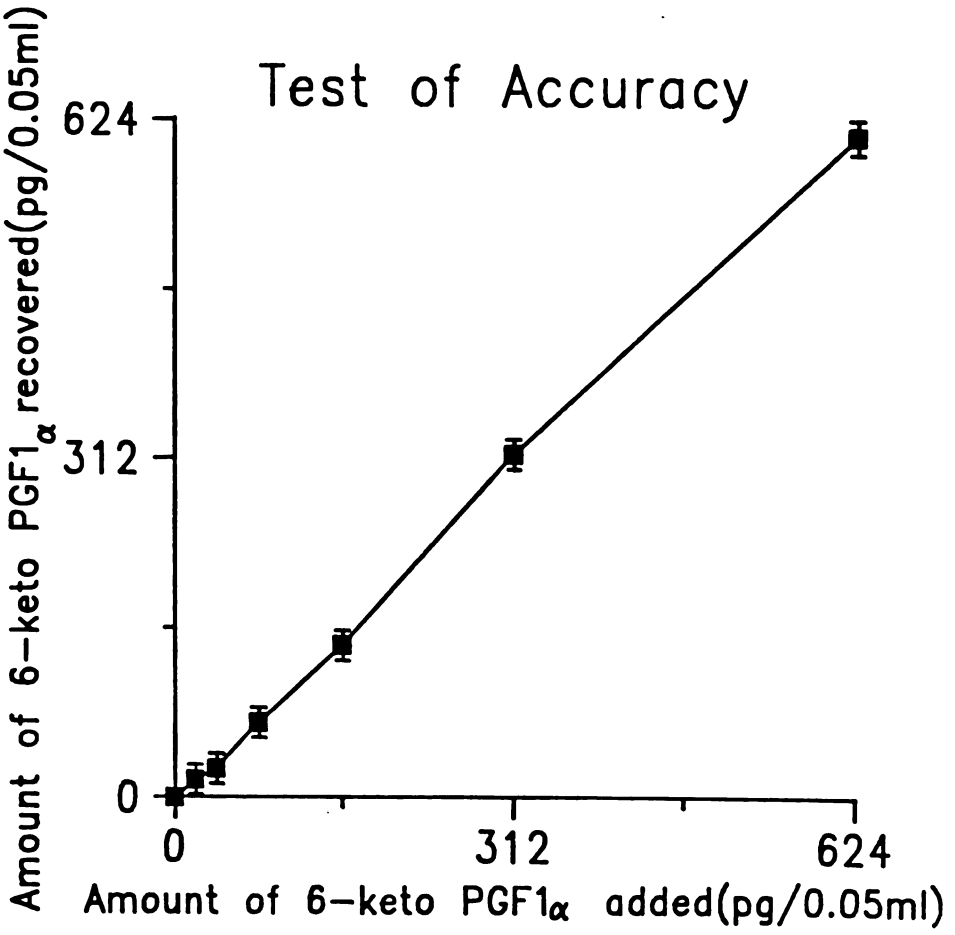

Test of Accuracy
624
312
0
0
312
624
Amount of 6-keto PGF1α recovered(pg/0.05ml)
Amount of 6-keto PGF1α added(pg/0.05ml)

Figure 24

APPENDIX B

Thromboxane Radioimmunoassay

Placental production of thromboxane was estimated by radioimmunoassay (RIA) for the concentration of a stable breakdown product, TXB2, in the incubation media. TXB2 is generated by nonenzymatic degradation of thromboxane.

REAGENTS:

All reagents used in the assay (except the antibody) were diluted with phosphate buffered saline (PBSG - 0.01 M phosphate, 0.09% saline, 0.1% gelatin, 0.01% sodium azide at pH 7.4) (Table 6). The antibody was diluted with a bovine gamma globulin-phosphate buffer (BGG-phosphate-0.01 M phosphate at pH 7.4, 0.9% sodium chloride, 0.1% bovine gamma globulin and 0.1% sodium azide). Fresh standards (3.12pg/0.01 ml - 200pg/0.01 ml) were made for each assay by adding known amounts of TXB2 to PBSG in 5 ml glass test tubes. Disposable glass tubes (12mm X 75mm) were used for the RIA. Rabbit antibody (Seragen, Boston, MA) highly specific for TXB2 (Table 11) was diluted 1:35 in BGG-phosphate buffer. The original volume of radioactive TXB2 (New England Nuclear, Boston, MA; 0.25 ml of ethanol with 0.025 mCi of labeled TXB2) was diluted with an additional 1.0 ml of ethanol to minimize loss of the radioactive prostaglandin due to evaporation and adsorbtion on the vial during storage. For each assay trace was diluted in PBSG to a concentration of approximately 6000 cpm/0.10 ml solution. Dextran-coated charcoal (Table 6)

TABLE 11

Rabbit-anti-TXB2 Cross Reactivity at 50% B/Bo*

Thromboxane B2	(100%)
PGD2	<0.1%
PGE1	<0.1%
PGE2∝	<0.1%
PGF1∝	<0.1%
6-keto PGE1∝	<0.1%
PGF2	<0.1%
PGA2	<0.1%
PGA1	<0.1%
PGB1	<0.1%
PGB2	<0.1%
DHKE2	<0.1%
DHKF2∝	<0.1%
5-HETE	<0.1%
12-HETE	<0.1%
15-HETE	<0.1%

*Data from Seragen

was utilized in each RIA.

PROCEDURE:

A Micromedic automatic pipet was used to add all reagents to the glass tubes. One hundred microliters of standard and no buffer or 10 ul of sample with 90 ul of PBSG were pipeted into assay tubes (Table 12). The final incubation volume (300 ul) was reached by simultaneously adding 100 ul of antibody and 100 ul of trace to each tube (Table 12). The total amount of radioactivity in each tube was determined by the counts per minute produced by 100 ul of trace in 200 ul of PBSG (Table 12). A zero dose tube (Bo tube) was made by adding 100 ul of trace and 100 ul of antibody into 100 ul of PBSG (Table 12).Nonspecific binding in the assay was determined from the counts per minute generated by a charcoal stripped total count tube (Table 12). All tubes were vortexed and incubated for 18-24 h at 4°C.

Following incubation 1.0 ml of dextran-coated charcoal, pre-cooled to 0-4°C, was added to all tubes except the total count tubes. The total count tubes received 1.0 ml PBSG so that quenching was equal for all tubes. Charcoal was used to separate labeled TXB2 was not bound by the antibody during the incubation period. The charcoal solution was kept on ice and mixed continuously. Immediately after charcoaling all the tubes were vortexed and incubated for 15 minutes at 4°C. At the end of this time

TABLE 12

Thromboxane Radioimmunoassay Protocol

Tube	Buffer (ul)	Trace (ul)	Antibody (ul)	recieve charcoal on day 2 of assay
Total count tube	200	100	--	NO
zero dose tube	100	100	100	YES
non-specific binding tube	200	100	--	YES
standards (100 ul)	--	100	100	YES
samples (10 ul)	90	100	100	YES

period all the assay tubes were centrifuged at 2000 x g (Beckman Model J-6B refrigerated centrifuge, Palo Alto, CA) for 12 minutes. The supernatant was decanted into scintillation vials containing 15 ml of scintillation cocktail (Safety Solve, Mount Prospect, IL). The entire volume of each total count tube was decanted into a scintillation vial. The radioactivity in each assay tube was counted on a Packard Tri Carb 300C liquid scintillation counter (Packard Instrument Co., Downers Grove, IL) for ten minutes or until 2.0 percent accuracy was achieved.

Standards were plotted as a percentage of the zero dose tubes (% B/Bo). The sample concentration could then be calculated from the standard curve using the percent binding of each sample obtained experimentally.

Reliability of the Assay

Specificity: A highly specific antibody for TXB2 was used in this assay (Table 11). Therefore sample extraction was not necessary and the incubation media was assayed directly.

Test of Parallelism: A patient sample, with an unknown quantity of TXB2, was serially diluted three times with DMEM and processed in each assay. These were plotted as a percent of the zero dose and assessed for parallelism to the standard curve (Figure 25).

Figure 25

Representative binding of the TXB2 standards. Plotted are the percentage of the total binding for each of the six standards (mean ± SE, n=7). Also graphed are the serial dilutions (mean ± SE, n=5): P2.5 = 2.5 ul of sample, P5.0 = 5.0 ul of sample, P10.0 = 10.0 ul of sample.

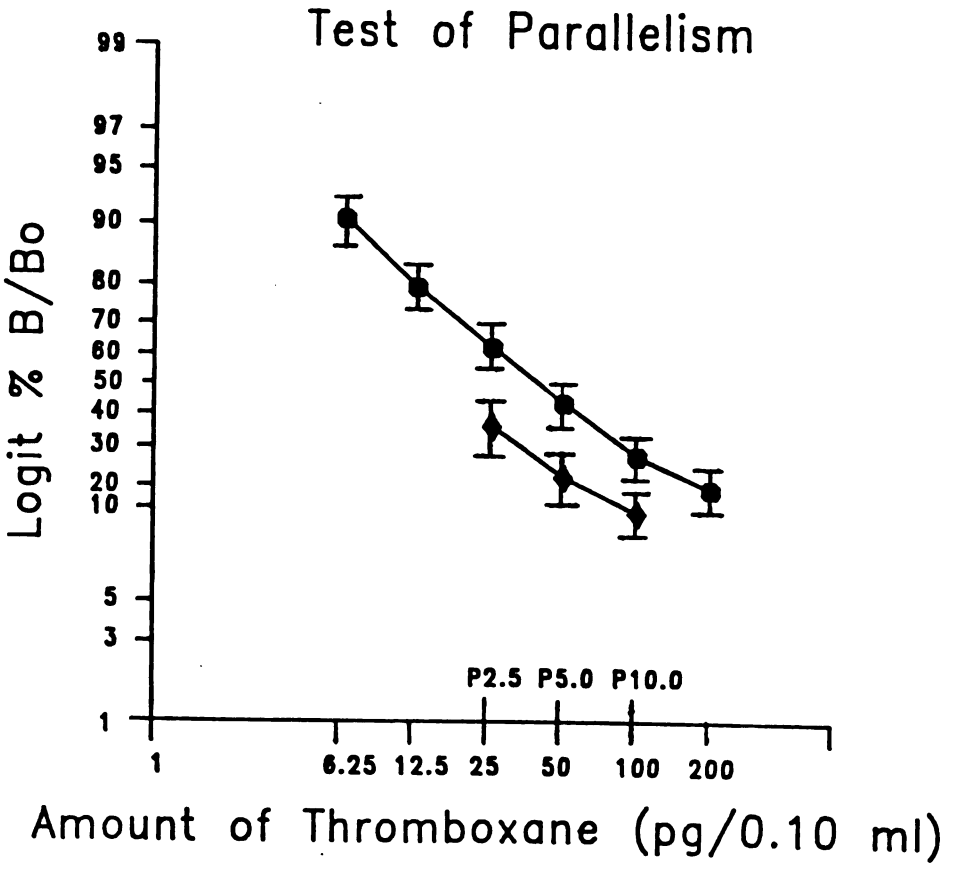

Test of Parallelism
Logit % B/Bo
99
97
95
90
80
70
60
50
40
30
20
10
5
3
1
P2.5 P5.0 P10.0
1
6.25 12.5 25 50 100 200
Amount of Thromboxane (pg/0.10 ml)

Figure 25

Sensitivity: The concentration two standard deviations away from the zero dose level, the least detectable concentration, was 2.33pg ± 1.06 (mean ± SE, n=7).

Precision of the assay: Within- and between-assay variations were calculated according to the methods of Rodbard for each of the three serial dilutions (Table 13). Within-assay variations ranged from 7.21 to 10.1% and between-assay variations ranged from 10.7 to 14.2%.

Accuracy: To determine the accuracy of the assay, varying concentrations of TXB2 were added to DMEM. Within- and between-assay variations were calculated according to the methods of Rodbard for each spiked sample (Table 14). Within-assay variations ranged from 6.03 to 8.04% and between-assay variations ranged from 9.67 to 13.8%. The recovery values for the spiked samples are plotted in Figure 26. Unspiked DMEM samples were also run in each assay and were equivalent to a zero dose response (mean ± SE, 110 ± 3.95%, n=4).

TABLE 13

Precision of the TXB2 Radioimmunoassay

Volume of standard assayed (ul)	Mean (pg)	df	Within-assay SD	Within-assay variation =CV	Between-assay SD	Between-assay variation =CV
2.5	178	5	12.8	7.21	25.2	14.2
5.0	329	5	33.4	10.1	44.2	13.4
10.0	585	5	43.7	7.47	62.8	10.7

TABLE 14

Accuracy of the TXB2 Radioimmunoassay

Amount of TXB2 added (pg)	Amount of TXB2 recovered (pg)	df	Within-assay SD	Within-assay variation =CV	Between-assay SD	Between-assay variation =CV
50	59.0	7	3.55	6.03	8.14	13.8
100	114	7	9.17	8.04	11.0	9.67

Figure 26

Test of accuracy of the TXB2 assay. Known amounts of TXB2 added were plotted against the amount recovered in the assay (mean ± SE).

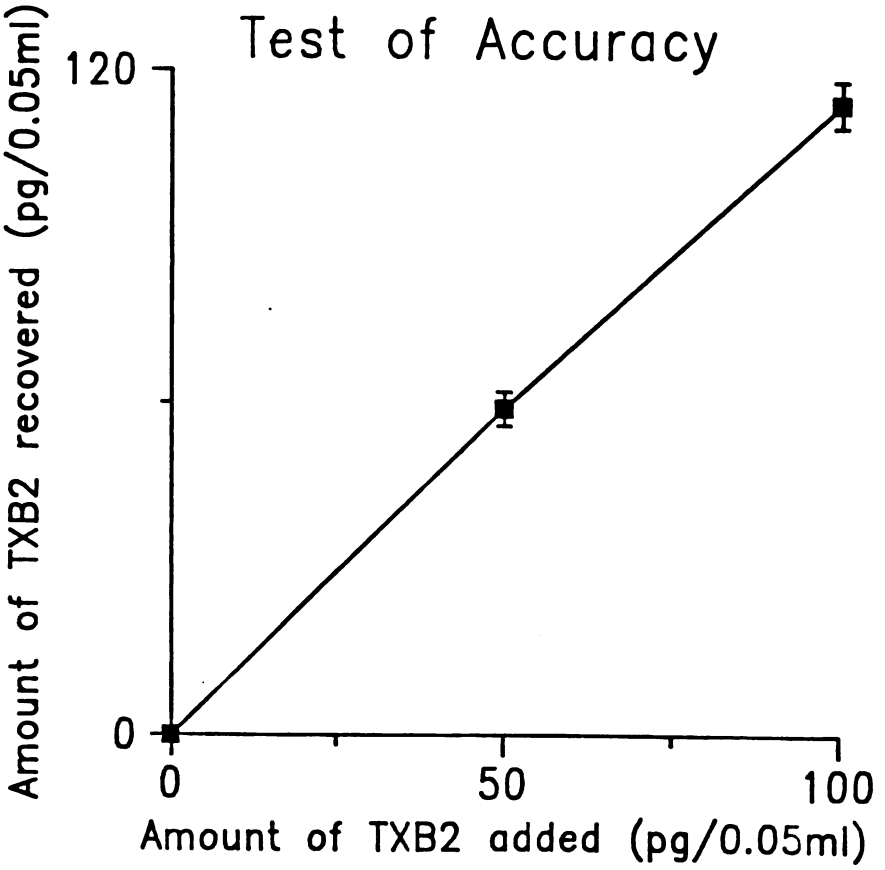

Test of Accuracy
120
0
Amount of TXB2 recovered (pg/0.05ml)
0
50
100
Amount of TXB2 added (pg/0.05ml)

Figure 26

APPENDIX C

Progesterone Radioimmunoassay

Placental production of progesterone was estimated by direct radioimmunoassay (RIA) for the concentration of progesterone in the incubation media.

REAGENTS:

All reagents used in the assay were diluted with phosphate buffered saline (PBSG - 0.01 M phosphate, 0.09% saline, 0.1% gelatin, 0.01% sodium azide at pH 7.4) (Table 6). Fresh standards (0.05ng/0.01 ml - 3.20ng/0.01 ml) were made for each assay by adding known amounts of progesterone to PBSG in 5 ml glass test tubes. Disposable glass tubes (12mm X 75mm) were used for the RIA. Rabbit antibody (RSL Inc., Carson, CA) highly specific for progesterone (Table 15) was diluted 1:10,500 in PBSG. The original volume of radioactive progesterone (Amersham, Arlington Heights, IL; 0.25 ml of ethanol with 0.025 mCi of labeled progesterone) was diluted with an additional 1.0 ml of ethanol to minimize loss of the radioactive prostaglandin due to evaporation and adsorbtion on the vial during storage. For each assay trace was diluted in PBSG to a concentration of approximately 10,000 cpm/0.10 ml solution. Dextran-coated charcoal (Table 6) was utilized in each RIA.

TABLE 15

Rabbit-anti-progesterone Cross Reactivity at 50% B/Bo*

Progesterone	(100%)
Deoxycortisone	1.5%
Pregnenolone	0.8%
20α-Dihydroprogesterone	0.3%
Cholesterol	<0.01%
Pregnenolone Sulfate	<0.01%
17α-Hydroxypregnenolone	<0.01%
17α-Hydroxyprogesterone	<0.01%
11-Deoxycortisol	<0.01%
Cortisol	<0.01%
Corticosterone	<0.01%
Aldosterone	<0.01%
Androstenedione	<0.01%
Testosterone	<0.01%
5α-Dihydrotestosterone	<0.01%
Dehydroepiandrosterone	<0.01%
Androsterone	<0.01%
Ethiocholanolone	<0.01%
Estradiol-17β	<0.01%
Estradiol-17α	<0.01%
Estrone	<0.01%
Estradiol	<0.01%

*Data from RSL, Inc.

PROCEDURE:

A Micromedic automatic pipet was used to add all reagents to the glass tubes. One hundred microliters of standard or 2.5 ul of sample were diluted to a total volume of 500 ul with PBSG (Table 16). The final incubation volume (700 ul) was reached by simultaneously adding 100 ul of antibody and 100 ul of trace to each tube (Table 16). The total amount of radioactivity in each tube was determined by the counts per minute produced by 100 ul of trace in 600 ul of PBSG (Table 16). A zero dose tube (Bo tube) was made by adding 100 ul of trace and 100 ul of antibody into 500 ul of PBSG (Table 16). Nonspecific binding in the assay was determined from the counts per minute generated by a charcoal stripped total count tube (Table 16). All tubes were vortexed and incubated for 1 at 20 °C or for 24 h at 4°C.

Following incubation 200 ul of dextran-coated charcoal, pre-cooled to 0-4°C, was added to all tubes except the total count tubes. The total count tubes received 200 ul PBSG so that quenching was equal for all tubes. Charcoal was used to separate labeled progesterone that was not bound by the antibody during the incubation period. The charcoal solution was kept on ice and mixed continuously. Immediately after charcoaling all the tubes were vortexed and incubated for 20 minutes at 4°C. At the end of this time period all the assay tubes were centrifuged at 2000 x g (Beckman Model J-6B refrigerated centrifuge,

TABLE 16

Progesterone Radioimmunoassay Protocol

Tube	Buffer (ul)	Trace (ul)	Antibody (ul)	recieve charcoal on day 2 of assay
Total count tube	600	100	--	NO
zero dose tube	500	100	100	YES
non-specific binding tube	600	100	--	YES
standards (100 ul)	400	100	100	YES
samples (2.5 ul)	497.5	100	100	YES

Palo Alto, CA) for 15 minutes. The supernatant was decanted into scintillation vials containing 15 ml of scintillation cocktail (Safety Solve, Mount Prospect, IL). The entire volume of each total count tube was decanted into a scintillation vial. The radioactivity in each assay tube was counted on a Packard Tri Carb 300C liquid scintillation counter (Packard Instrument Co., Downers Grove, IL) for ten minutes or until 2.0 percent accuracy was achieved.

Standards were plotted as a percentage of the zero dose tubes (% B/Bo). The sample concentration could then be calculated from the standard curve using the percent binding of each sample obtained experimentally.

Reliability of the Assay

Specificity: A highly specific antibody for progesterone was used in this assay (Table 15). Therefore sample extraction was not necessary and the incubation media was assayed directly.

Test of Parallelism: A patient sample, with an unknown quantity of progesterone was serially diluted three times with DMEM and processed in each assay. These were plotted as a percent of the zero dose and assessed for parallelism to the standard curve (Figure 27).

Figure 27

Representative binding of the progesterone standards. Plotted are the percentage of the total binding for each of the nine standards (mean ± SE, n=5). Also graphed are the serial dilutions (mean ± SE, n= 4): P2.5 = 2.5 ul of sample, P5.0 = 5.0 ul of sample, P10.0 = 10.0 ul of sample.

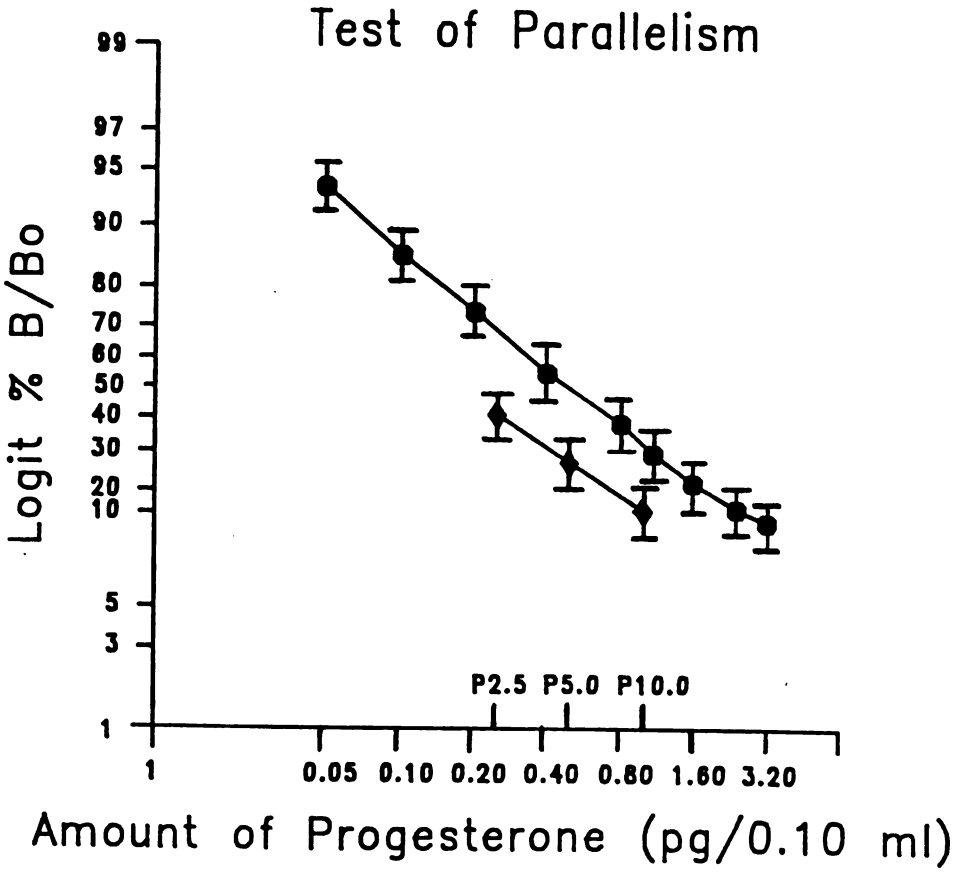

Test of Parallelism
Logit % B/Bo
99
97
95
90
80
70
60
50
40
30
20
10
5
3
1
P2.5 P5.0 P10.0
1
0.05 0.10 0.20 0.40 0.80 1.60 3.20
Amount of Progesterone (pg/0.10 ml)

Figure 27

Sensitivity: The concentration two standard deviations away from the zero dose level, the least detectable concentration, was 0.016ng ± 0.007 (mean ± SE, n=5).

Precision of the assay: Within- and between-assay variations were calculated according to the methods of Rodbard for each of the three serial dilutions (Table 17). Within-assay variations ranged from 1.62 to 5.57% and between-assay variations ranged from 3.54 to 13.8%.

Accuracy: To determine the accuracy of the assay, varying concentrations of progesterone were added to DMEM. Within- and between-assay variations were calculated according to the methods of Rodbard for each spiked sample (Table 18). Within-assay variations ranged from 4.60 to 12.9% and between-assay variations ranged from 11.1 to 14.9%. The recovery values for the spiked samples are plotted in Figure 28. Unspiked DMEM samples were also run in each assay and were equivalent to a zero dose response (mean ± SE, 106 ± 3.69%, n=5).

TABLE 12

Precision of the Progesterone Radioimmunoassay

Volume of standard assayed (ul)	Mean (ng)	df	Within-assay SD	Within-assay variation =CV	Between-assay SD	Between-assay variation =CV
2.5	0.58	4	0.01	2.43	0.02	3.54
5.0	1.21	4	0.06	5.57	0.05	3.93
10.0	2.22	4	0.04	1.62	0.31	13.8

TABLE 18

Accuracy of the Progesterone Radioimmunoassay

Amount of Proges. added (ng)	Amount of Proges. recovered (ng)	df	Within-assay SD	Within-assay variation =CV	Between-assay SD	Between-assay variation =CV
0.4	0.34	3	0.04	12.9	0.04	11.1
1.4	1.37	5	0.08	5.80	0.20	14.9
1.8	1.83	5	0.08	4.60	0.22	12.2

Figure 28

Test of accuracy of the progesterone assay. Known amounts of progesterone added were plotted against the amount recovered in the assay (mean ± SE).

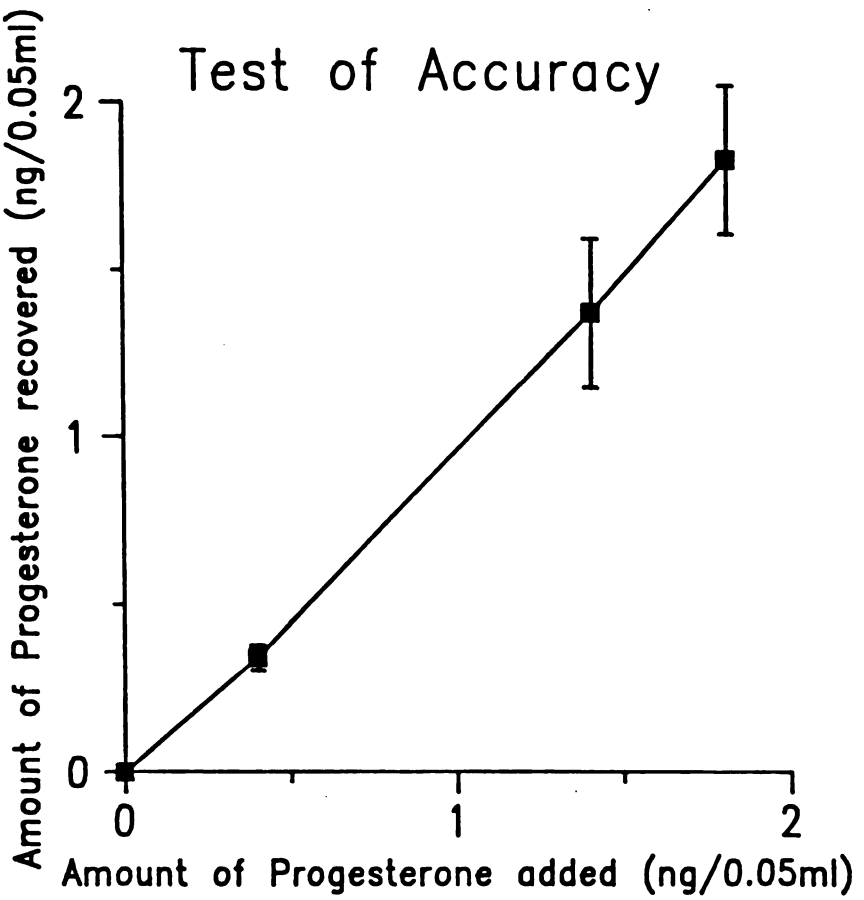

Test of Accuracy
Amount of Progesterone recovered (ng/0.05ml)
2
1
0
0
1
2
Amount of Progesterone added (ng/0.05ml)

Figure 28

APPENDIX D

Estradiol Radioimmunoassay

Placental production of estradiol was estimated by direct radioimmunoassay (RIA) for the concentration of estradiol in the incubation media.

REAGENTS:

This assay was obtained from RSL Inc. (Carson, CA) as a complete kit (catalog #1018). All reagents in the assay were used as provided by RSL Inc. Disposable glass tubes (12mm x 75mm) were used in each assay. Rabbit antibody highly specific for 17β-estradiol (Table 19) was utilized in the assay.

PROCEDURE:

Twenty-five microliters of standard or sample were diluted with 0.1 ml of sex binding globulin inhibitor (SBGI) solution (Table 20). The initial incubation volume (1.125 ml) was reached by adding 0.5 ml of anti-estradiol and 0.5 ml of trace to each tube (Table 20). All tubes were vortexed and incubated at 37^{o}C for 1 h. The total amount of radioactivity in each tube was determined by the counts per minute produced by 0.5 ml of trace in 0.5 ml of assay buffer and 0.1 ml of SBGI solution (Table 20). A zero dose tube (Bo tube) was made by adding 0.5 ml of trace, 0.5 ml of anti-estradiol and 0.1 ml of SBGI solution (Table 20). Nonspecific binding in the assay was determined from the counts per minute generated by a total

TABLE 19

Rabbit-anti-estradiol Cross Reactivity at 50% B/Bo*

Estradiol, 17β	(100%)
Estrone	15.0%
Estriol	1.15%
Estradiol-17α	0.68%
Progesterone	<0.01%
Deoxycortisone	<0.01%
Pregnenolone	<0.01%
20α-Dihydroprogesterone	<0.01%
Cholesterol	<0.01%
Pregnenolone Sulfate	<0.01%
17α-Hydroxypregnenolone	<0.01%
17α-Hydroxyprogesterone	<0.01%
11-Deoxycortisol	<0.01%
Cortisol	<0.01%
Corticosterone	<0.01%
Aldosterone	<0.01%
Androstenedione	<0.01%
Testosterone	<0.01%
5α-Dihydrotestosterone	<0.01%
Dehydroepiandrosterone	<0.01%
Androsterone	<0.01%
Ethiocholanolone	<0.01%

*Data from RSL, Inc.

TABLE 20

Estradiol Radioimmunoassay Protocol

Tube	Buffer (ml)	Trace (ml)	Anti-estradiol (ml)	receive SBGI* solution (0.1 ml)	receive second antibody (0.1 ml)
Total count tube	0.5	0.5	--	--	NO
zero dose tube	--	0.5	0.5	YES	YES
non-specific binding tube	0.5	0.5	--	YES	YES
standards (25 ul)	--	0.5	0.5	YES	YES
samples (25 ul)	--	0.5	0.5	YES	YES

*SBGI=sex binding globulin inhibitor

count tube which received the second antibody (Table 20). All tubes were vortexed and incubated for 1-24 h at 4°C.

Following the initial incubation 0.1 ml of second antibody, goat anti-rabbit gamma globulin, was added to all tubes except the total count tubes (Table 20). The second antibody was added to precipitate the antibody bound antigen. All test tubes were vortexed and incubated at 37°C for another 60 minutes. After this incubation all tubes were centrifuged at 1000 x g (Beckman Model J6-B centrifuge, Palo Alto, CA) for 20 minutes. The supernatant was discarded (except in the case of the total count tubes) and the radioactivity in the precipitate was counted on a Packard Auto-Gamma 800C scintillation counter (Packard Instrument Co., Downers Grove, IL) for five minutes or until 2.0 percent standard deviation was achieved.

Standards were plotted as a percentage of the zero dose tubes (% B/Bo, Figure 29). The sample concentration could then be calculated from the standard curve using the percent binding of each sample obtained experimentally.

Reliability of the Assay

Specificity: A highly specific antibody for progesterone was used in this assay (Table 19). Therefore sample extraction was not necessary and the incubation media was assayed directly.

Figure 29

Representaive binding of the estradiol standards. Plotted are the percentage of the total binding for each of the seven standards (mean ± SE, n=5).

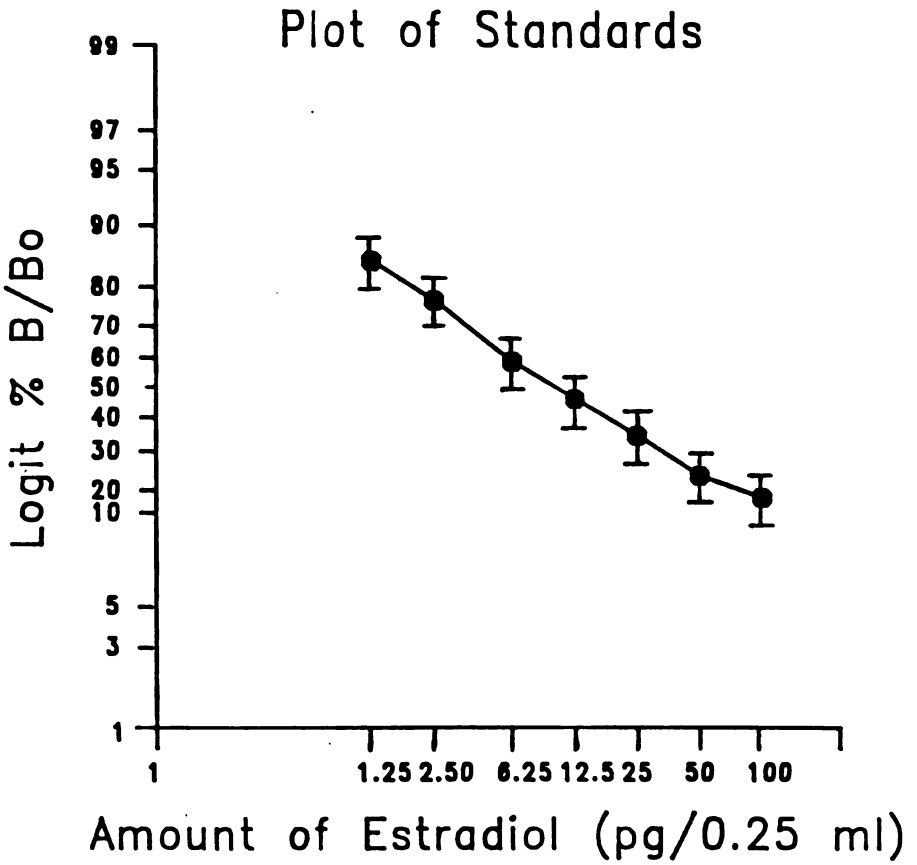

Plot of Standards
Logit % B/Bo
99
97
95
90
80
70
60
50
40
30
20
10
5
3
1
1
1.25
2.50
6.25
12.5
25
50
100
Amount of Estradiol (pg/0.25 ml)

Figure 29

Test of Parallelism: Serial dilutions were not performed for the estradiol RIA.

Sensitivity: The concentration two standard deviations away from the zero dose level, the least detectable concentration, was 0.16pg ± 0.05 (mean ± SE, n=5).

Precision of the assay: These calculations were not possible for the serial dilutions but were calculated on the spiked samples (Table 21).

Accuracy: To determine the accuracy of the assay, varying concentrations of estradiol were added to DMEM. Within- and between-assay variations were calculated according to the methods of Rodbard for each spiked sample (Table 21). Within-assay variations ranged from 7.26 to 13.5% and between-assay variations ranged from 4.64 to 9.40%. The recovery values for the spiked samples are plotted in Figure 30. Unspiked DMEM samples were also run in each assay and were equivalent to a zero dose response (mean ± SE, 99 ± 3.0%, n=3).

TABLE 21

Precision and Accuracy of the Estradiol Radioimmunoassay

Amount of Estradiol added (pg)	Amount of Estradiol recovered (pg)	df	Within-assay SD	Within-assay variation =CV	Between-assay SD	Between-assay variation =CV
5.0	3.43	3	0.46	13.5	0.32	9.40
30	24.5	3	1.78	7.26	1.14	4.64

Figure 30

Test of accuracy of the estradiol assay. Known amounts of estradiol added were plotted against the amount recovered in the assay (mean ± SE).

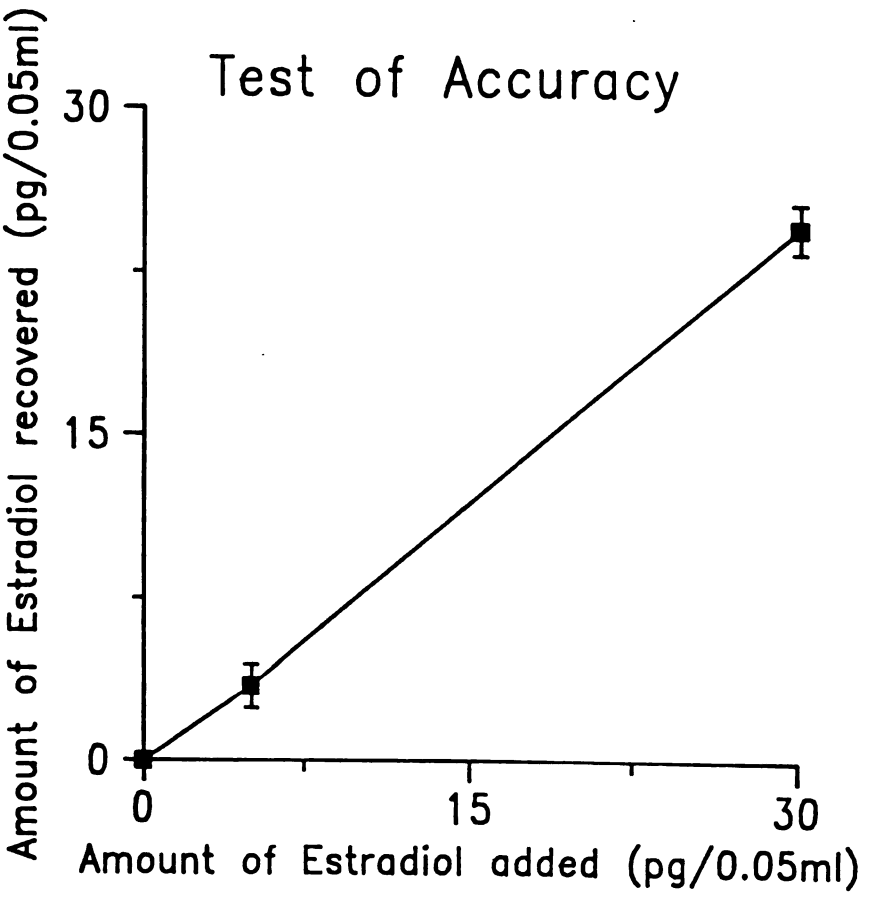

Test of Accuracy
Amount of Estradiol recovered (pg/0.05ml)
30
15
0
0
15
30
Amount of Estradiol added (pg/0.05ml)

Figure 30