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VASOACTIVE PROSTANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCIES

BY

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ABSTRACT

The role of vasoactive prostanoids in the pathogenesis of the hypertensive diseases in pregnancy is currently under debate. Studies have demonstrated a generalised deficiency of vasodilator prostanoids, particularly prostacyclin and a disturbance of the vasodilator to vasoconstrictor balance in pre-eclampsia.

The first part of this thesis reviews the literature with regard to the pathophysiology of gestational hypertension and the factors and mechanisms which may be responsible for the causation of the disease, with particular reference to the role of the prostanoids.

In order to study the prostanoids in normal pregnancy and pregnancy complicated by gestational and chronic essential hypertension, a single maternal blood sample and 4 hr urine collection were obtained from 168 women for measurement by radioimmunoassay of the levels of PGE and the stable metabolites of $PGF_{2\alpha}$, prostacyclin and thromboxane.

Maternal plasma levels of the thromboxane metabolite TxB_2, were significantly lower in both groups of hypertensive women than in normal pregnancy. The plasma levels of PGE, PGFM (a metabolite of $PGF_{2\alpha}$) and 6 keto $PGF_{1\alpha}$ (a prostacyclin metabolite) in pregnancy complicated by hypertension were comparable to normal pregnancy. In plasma there was however some evidence of disturbance of the balance of the vasodilator to vasoconstrictor prostanoids in pregnancy complicated by both chronic and gestational hypertension, with an excess of vasodilators. This was demonstrated by a trend to higher ratios of the vasodilator prostanoids PGE and 6 keto $PGF_{1\alpha}$ to the vasoconstrictor TxB_2. The changes were similar in both groups of hypertensive women.

No significant differences were found in maternal urinary prostanoid excretion or the ratio of vasodilator to vasoconstrictor prostanoids in the urine between normal pregnancy and pregnancy with either chronic or gestational hypertension. The urinary excretion of PGE was however negatively correlated to the diastolic blood pressure.

The levels of each prostanoid in plasma had a positive correlation with each of the others and similarly the urinary excretion of each prostanoid had a positive correlation with each of the others. The plasma level of each prostanoid and the urinary excretion of the corresponding prostanoid were however not related.

The urinary excretion of TxB_2 and 6 keto $PGF_{1\alpha}$ both had a statistically significant negative correlation to serum oestradiol, hpL and progesterone in all the groups studied. The plasma PGF level had a significant positive correlation with the serum oestradiol in chronic hypertensive women but not in the other study groups.

There were no significant differences in maternal plasma prostanoid levels in relation to intrauterine growth retardation (IUGR, fetal birthweight below 10th percentile for gestational age), although there were trends to an excess of vasodilator prostanoids in patients with smal babies (birthweight below 25th percentile).

Maternal urinary excretion of PGFM was significantly higher in women with IUGR infants and there was a non-significant trend for these women to have a higher urinary excretion of TxB_2. Women with babies weighing below the 25th percentile for gestational age had a relative deficiency of vasodilator prostanoids in the urine.

In a second study the levels of 6 keto $PGF_{1\alpha}$ and TxB_2 in amniotic fluid obtained by amniocentesis and umbilical arterial blood were compared between 14 normotensive and 16 hypertensive pregnancies in women delivered by elective Caesarean Section. Amniotic fluid TxB_2 levels increased significantly with gestational age. Although amniotic fluid 6 keto $PGF_{1\alpha}$ also increased with gestational age, the effect was not statistically significant. Umbilical arterial blood 6 keto $PGF_{1\alpha}$ and TxB_2 levels were not affected by gestational age.

Amniotic fluid 6 keto $PGF_{1\alpha}$ levels were significantly lower in pregnancies complicated by hypertension but TxB_2 levels were similar in the two groups.

Umbilical arterial blood 6 keto $PGF_{1\alpha}$ levels were comparable between normotensive and hypertensive pregnancies whereas TxB_2 levels were significantly lower in hypertensive pregnancies.

Rapid intravenous infusion of fluid in patients with severe pre-eclampsia produces vasodilatation and a fall in blood pressure. The effect of plasma volume expansion on the plasma 6 keto $PGF_{1\alpha}$ and TxB_2 levels in patients with pregnancy hypertension was investigated in a controlled study of 21 patients. There was a significant increase in

plasma 6 keto $PGF_{1\alpha}$ and a significant fall in plasma TxB_2 after plasma volume expansion, whereas control patients demonstrated no change in prostanoid metabolites.

The final part of this study involved a randomised controlled study of the effect of antiplatelet therapy on maternal plasma 6 keto $PGF_{1\alpha}$ and TxB_2 levels and the effect of this treatment administered from the second trimester of pregnancy, on the incidence of pre-eclampsia. The women were randomised to treatment with low dose aspirin, a combination of low dose aspirin and dipyridamole or no treatment. Aspirin 81mg daily had no effect on plasma 6 keto $PGF_{1\alpha}$ or TxB_2 levels. Aspirin 81mg daily in combination with dipyridamole 200mg daily suppressed TxB_2 levels after 1 week of treatment but the differences were no longer apparent after 4 weeks. Although the incidence of pre-eclampsia was lowest in the women taking aspirin only, there were no significant differences in the incidence of pre-eclampsia between the patients on either of the treatments and the controls. Women taking aspirin however had significantly higher fetal birthweight percentiles than both other groups. Women taking aspirin and dipyridamole had a significantly higher incidence of perinatal problems.

The studies described in this thesis contribute to knowledge to the role of vasoactive prostanoids in the hypertensive disorders of pregnancy.

STATEMENT OF CANDIDATE

I DECLARE THAT THE WORK ON WHICH THIS THESIS IS BASED, IS ORIGINAL (EXCEPT WHERE ACKNOWLEDGEMENTS INDICATE OTHERWISE), AND THAT NEITHER THE WHOLE WORK NOR ANY PART OF IT HAS BEEN, IS BEING OR IS TO BE SUBMITTED FOR ANOTHER DEGREE IN THIS OR ANY OTHER UNIVERSITY.

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PART 1

INTRODUCTION

The hypertensive disorders or "toxaemias" of pregnancy have fascinated clinicians and researchers since ancient times and many theories have been advanced to explain the primary pathology. Few of the hypotheses have been supported by as much scientific evidence as the concept that abnormalities in synthesis or metabolism of vasoactive prostanoids may be involved in the pathophysiology of pre-eclampsia.[1]

Normal pregnancy is associated with dramatic changes in cardiovascular function with increased plasma volume and cardiac output and a fall in total peripheral resistance. This results in a reduction in blood pressure but a marked increase in blood flow in many vascular beds particularly the uterus and kidneys. Blood pressure and blood flows to different organs in pregnancy are primarily controlled by the vascular resistance in the tissues. Alterations in the interactions of agents regulating vascular tone are largely responsible for the physiological changes in pregnancy.

[1]The term prostanoids is used for the 20 carbon fatty acid derivatives of arachidonic acid and includes the prostaglandins and thromboxanes.

Pre-eclampsia is characterised by generalised vasospasm in the mother with elevation of the blood pressure, a failure of the normal expansion of plasma volume and impaired regional perfusion. Cardiac output in pre-eclampsia is similar to that in normal pregnancy so factors controlling vascular reactivity are likely to play an important role in the pathophysiology of the disease.

Other recognised features of pre-eclampsia are a loss of the refractoriness to pressor agents exhibited by normal pregnant women and in some cases activation of the coagulation system.

Prostaglandins and thromboxanes have multiple often opposing biological actions and those derived from arachidonic acid are the most important physiologically. Prostanoids are produced in all tissues except red blood cells and have important effects on vascular reactivity either directly or by interaction with other local or circulating vasoactive agents. Prostacyclin is believed to be the most important vascular prostanoid although all the major prostanoids have vasodilator or vasoconstrictor effects.

The maternal, placental and fetal tissues produce large amounts of prostanoids in pregnancy and they play major

roles in initiation and maintenance of uterine contractions, as well as the control of blood flow. The primary pathology of pre-eclampsia results from the presence of the trophoblast as the disease resolves rapidly after delivery. Abnormalities in placentation and placental prostanoid production with a deficiency of vasodilator prostanoids, particularly prostacyclin, are found in pre-eclampsia. It is unclear however whether the prostacyclin deficiency results in defective placentation or the defective placentation causes the prostacyclin deficiency.

A deficiency of vasodilator or an excess of vasoconstrictor prostanoids or a disturbance in the vasodilator to vasoconstrictor ratio have been demonstrated in a number of maternal and fetal tissues in pre-eclampsia, but the findings are not entirely consistent. Theoretically, such an imbalance could explain many of the features of pre-eclampsia. Prostacyclin, for example, is a vasodilator, a potent inhibitor of platelet aggregation and stimulator of renin production, hence its deficiency could account for hypertension, uteroplacental ischaemia, platelet aggregation and increased sensitivity to the effects of angiotensin II.

Although the concept of a generalised imbalance of vasoconstrictor and vasodilator prostanoids in pre-eclampsia is generally accepted, the majority of the evidence has come from uteroplacental or fetal tissues. The relationship of uteroplacental ischaemia to maternal hypertension is however far from clear, particularly as a similar deficiency of prostacyclin production has been demonstrated in umbilical vessels and placental tissues from pregnancies complicated by intrauterine growth retardation without hypertension. The in vitro activity of prostanoids in peripheral maternal blood vessels has been poorly investigated due to their relative inaccessibility, but measurements of prostanoids in plasma and urine in pre-eclampsia have yielded contradictory results and little is known of the role of prostanoids in chronic hypertension in pregnancy.

The manifestations of gestational hypertension vary considerably from one woman to another. Not only are there marked gradations of disease from very mild to severe and life threatening but the specific pattern of the maternal physiological abnormalities varies greatly between individuals. These differences cause difficulties in investigations of the underlying pathophysiology and may account for some of the inconsistencies in published reports.

The balance of the different prostanoids in different vascular beds and their interaction with other vasoactive substances particularly products of the renin angiotensin system, may be important determinants of vascular tone and vascular resistance in different organs. An abnormality in the balance of the different vasoconstrictor and vasodilator prostanoids and their relation with other vasoactive agents may be more important than the individual biological activity of any particular prostanoid.

The first part of this thesis addresses the role of prostanoids in maternal plasma and urine in gestational hypertension of varying severity, in chronic hypertension and in normal pregnancy. Particular attention has been given to the relationships of the levels of the different prostanoids to the severity of the disease, the level of blood pressure and the growth of the fetus. The interrelationships of the different prostanoids in plasma and urine and between plasma and urine levels has also been studied. As prostanoids may also have interactions

with the placental hormones, the relationships of maternal prostanoids to the plasma levels of oestradiol, human placental lactogen and progesterone have also been investigated.

The prostanoids, particularly prostacyclin, are believed to be important regulators of uteroplacental blood flow. In pre-eclampsia and intrauterine growth retardation (IUGR) the uteroplacental flow may be decreased and this could be mediated by a decrease in vasodilator prostanoids. The production of prostacyclin by umbilical vessels in vitro is reduced in patients with pre-eclampsia. High levels of prostanoids in the fetus suggest that they may act as circulating hormones, but there have been few studies of fetal plasma prostanoid levels.

Amniotic fluid prostanoids are another means of assessing intrauterine prostanoid production and have the advantage of accessibility.

Although amniotic fluid prostanoids in relation to pre-eclampsia and IUGR have been studied by a number of workers the results are inconsistent.

In the second part of this thesis therefore the effect of maternal hypertension on fetal prostanoids was studied by measurement of umbilical arterial plasma and amniotic fluid prostanoids in normal and hypertensive pregnancy.

Artificial expansion of the plasma volume has been used in pre-eclampsia in an attempt to reverse some of the physiological abnormalities of the disease. Rapid intravenous infusion of fluid results in vasodilatation with a drop in the blood pressure, particularly the diastolic. The third part of the thesis addressed the question of whether this vasodilatation was mediated by alterations in prostacyclin and thromboxane.

Although the mechanism of prostacyclin deficiency or thromboxane excess in pre-eclampsia is not understood, attempts have been made by a number of workers to correct this imbalance by the use of prostacyclin infusion, thromboxane synthetase inhibitors and dietary supplementation with fatty acid precursors. The use of antiplatelet drugs such as aspirin and dipyridamole to prevent pre-eclampsia appear to have been more successful. These drugs may correct the platelet

abnormality and additionally optimise the prostacyclin-thromboxane balance. The precise mode of action of these drugs in prevention of pre-eclampsia however is not understood. The final part of the thesis examined the possible effect of low dose aspirin and a low dose aspirin/dipyridamole combination on plasma prostacyclin and thromboxane metabolite levels in the hope that the maternal plasma levels may reflect generalised changes in prostanoid synthesis.

The physiological changes in pre-eclampsia are well documented but the underlying pathology is less well understood. It is possible that there are different forms of hypertensive disorders in pregnancy with differing pathophysiology and underlying aetiology. One form is pre-eclampsia, characterised by the development of hypertension and proteinuria. It occurs most commonly in primigravidae and a generalised reduction in vasodilator prostaglandin production may be an important aetiological factor. Another form of the disease characterised by gestational hypertension without proteinuria may represent a less severe form of pre-eclampsia or may, particularly in multigravidae, be due to latent essential hypertension. Chronic essential hypertension may also complicate pregnancy and is associated with an increased propensity to the

development of proteinuric pre-eclampsia. These different forms of hypertension in pregnancy give rise to major difficulties in investigations into the aetiology. One aim of this thesis was to investigate the abnormalities in maternal prostanoid synthesis in pregnant patients with various hypertensive disorders to assess if any abnormalities relate specifically to pre-eclampsia or if they are related rather to the hypertension itself.

The prostanoids are locally produced in the tissues and it is likely that prostanoid synthesis and metabolism vary between different vascular beds. A further aim of this thesis was to test this hypothesis by measurement of prostanoid levels in maternal and fetal blood, maternal urine and amniotic fluid.

The third aim of the thesis was to assess the role of plasma prostanoids in the vasoconstriction commonly seen in hypertensive pregnant patients and the final objective was to assess the effectiveness of aspirin and dipyridamole in the correction of the imbalance of prostacyclin and thromboxane.

It was hoped by these investigations to further knowledge of the underlying pathophysiology of the hypertensive disorders in pregnancy.

PART 2

PATHOPHYSIOLOGY OF HYPERTENSIVE DISEASES IN PREGNANCY AND THE ROLE OF PROSATANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCY

2.1 INTRODUCTION

Hypertension is the commonest complication of pregnancy with an incidence of "toxaemia of pregnancy" of 9.9% in 1976 in the United Kingdom. (DHSS 1980). As there has been no agreement on the classification and terminology used in hypertensive diseases of pregnancy, it is difficult to arrive at a true estimate of the incidence. A review of deliveries in the Cape Peninsula Maternity Services which defined hypertension as a diastolic blood pressure (DBP) of 90mmHg or more on two or more occasions revealed a high incidence of hypertension in this population. Hypertension in some form was present in 30.3% of Africans, 32.6% of Cape Coloureds and 45.2% of Whites. (Knutzen and Davey 1977). The incidence of hypertension occurring for the first time antenatally was 15.8%. (Davey 1985). Hypertensive disorders remain an important cause of maternal and perinatal morbidity and mortality. The outcome of the pregnancy is dependant upon the severity of the hypertension, development of proteinuria and the time of onset of these clinical signs.

Chamberlain et al (1978) reviewing the 1970 British Births survey suggested an increased incidence of growth retardation and increased perinatal mortality in mothers with raised arterial pressure. Page and Christianson (1976) in a study of more than 14,000 singleton pregnancies found an increased incidence of stillbirths and growth retardation if the mean arterial pressure in the mid trimester exceeded 90mmHg. They also demonstrated an increased tendency for later development of pre-eclampsia in those patients confirming the findings of earlier studies (MacGillivray 1961). The development of severe hypertension and proteinuria is associated with a marked increase in risk to the mother (Redman 1980) and a three to seven fold increase in perinatal mortality (Walters 1966, Dunlop 1966). In view of these risks the best approach to the management of pre-eclampsia would be prevention but achievement of this goal is at present limited by a lack of understanding of the primary pathogenesis of the condition.

The aetiology of the hypertensive disease of pregnancy is poorly understood despite intensive investigation. The study of these diseases has been hampered by difficulties in classification. The definition of eclampsia has never been in doubt but there have been differences of opinion regarding nomenclature and classification of the condition preceding the convulsions. It is now possible that the hypertensive disorders of pregnancy encompass a number of different diseases with differing aetiology and that the underlying pathophysiology leading to the hypertension may also vary. The failure of many studies to differentiate between chronic and gestational hypertension, between mild and severe disease or between proteinuric and non proteinuric hypertension makes the results difficult to interpret and may account for some of the inconsistencies in the findings. The definitions of hypertension used in this study are stated in Appendix 1.

2.2 PATHOPHYSIOLOGY OF HYPERTENSION IN PREGNANCY

2.2.1 Cardiovascular changes and vascular reactivity

2.2.1.1 Arterial hypertension

Pre-eclampsia is classically considered to be a triad of hypertension, oedema and proteinuria. Hypertension is usually the earliest feature of the disease (Redman 1984). Normal pregnancy is characterised by decreased peripheral resistance in the second trimester which produces a fall in blood pressure in spite of increased cardiac output. (MacGillivray et al 1969). Arterial blood pressure is determined by the cardiac output and peripheral resistance. Total peripheral resistance is dependant upon a number of factors including (a) the degree of vasoconstriction of the resistance vessels, (b) the filling of the vascular system which is dependant on blood volume and the constriction and elasticity of the venous capacitance vessels and (c) the blood viscosity.

The drop in blood pressure in normal pregnancy appears to occur due to peripheral vasodilatation as a result of a decrease in arteriolar resistance which may be an effect of increased circulating progesterone, a decreased sensitivity to circulating vasoconstrictors, or increased production of the vasodilator prostaglandins E_2 and prostacyclin.

In pre-eclampsia hypertension is caused by arteriolar vasoconstriction associated with a reduced plasma volume (Gallery et al 1979) and the arterial reactivity is altered so that the normal responsiveness to infused angiotensin II is lost (See 2.2.1.4.)

In addition there is a loss of the normal circadian rhythm and in severe pre-eclampsia the blood pressure may be highest at night (Redman et al 1976(a)).

2.2.1.2 Plasma Volume

In hypertensive pregnant patients there is evidence of a failure of plasma volume expansion and an increase in total peripheral resistance. Plasma volume contraction has been demonstrated in pregnant patients with either pre-eclampsia or chronic hypertension (Gallery et al 1979). A low plasma volume however appears to be confined to those women with growth retarded babies and is related to poor fetal outcome. (Soffronoff et al 1977, Campbell and MacGillivray 1977, Arias 1975).

2.2.1.3 Peripheral Resistance and Blood Flow

Total peripheral resistance is dependant upon the total resistance to blood flow to all the tissues and organs of the body. In hypertensive pregnancy vasoconstrictor tone is increased with an increase in total peripheral resistance. However, it appears that peripheral resistance is not uniform but differs between different vascular beds with the result that the blood flow may be more reduced in some organs than in others. For example, the effective renal plasma flow is reduced by an average of 22% in pre-eclampsia (Chesley 1978) whereas uteroplacental blood flow is decreased by 50-65% (Gant and Worley 1980), but there are large individual variations. These differences in the degree of impairment of perfusion to different vascular beds suggest that local vasoactive substances may be important. Many of the effects of hypertension in pregnancy are mediated by alterations in the flow of blood to the various organs and tissues of the body.

2.2.1.4 Renin-angiotensin system in pregnancy

The renin-angiotensin system is stimulated in normal pregnancy (Gant et al 1983) and has important effects on the blood pressure control. Renin production in pregnancy appears to occur not only in the kidney but also in the chorion, although the significance of placental renin is not known, as much of it is

biologically inactive (Skinner et al 1975). Glance et al (1984) suggested conversion of angiotensin I to angiotensin II occurred in the placenta and may be important in controlling fetoplacental blood flow. They later suggested (Glance et al 1985) that prostaglandins E_2 and prostacyclin stimulated by renin release could modulate the vasoactive effects of angiotensin II (AII) on the fetal vasculature.

Plasma renin and AII levels are elevated in normal pregnancy (Brown et al 1963, Weir et al 1976) but this rise in AII does not cause a rise in blood pressure as there is a parallel decline in vascular responsiveness to its effects (Gant et al 1976). The reason for the increase in renin and AII in pregnancy is not known, but it may be to compensate for the increase in vasodilator substances such as prostaglandins, kinins or progesterone (Everett et al 1978, Bay and Ferris 1979, Gant et al 1983). It does not seem to be causally related to increased oestrogen and progesterone or impaired sodium balance in pregnancy (Editorial Lancet 1982).

In pre-eclampsia plasma renin concentration (Weir et al 1976) and plasma renin activity (Symonds 1983) are lower than in normal pregnancy, which is not easily explained in the presence of hypertension. The levels of renin are related to the effective blood volume and vascular capacity. In normal pregnancy plasma renin levels may be

high to maintain filling of the expanded vascular space. A failure of the normal plasma volume expansion is found in some patients with hypertension in pregnancy and could be explained by reduced production of substances of the renin-angiotension system.

Gant et al (1973) demonstrated that primigravidae with pre-eclampsia have an increased sensitivity to infused AII, which is evident even before the onset of hypertension. Patients who subsequently develop pre-eclampsia require a lower dose of AII to elicit a pressor response. Several factors may be involved in the increased response to AII in pre-eclampsia. In non-pregnant subjects pressor responsiveness to exogenous AII depends principally on endogenous levels (Chinn and Dusterdieck 1972). Blood volume deficit may also increase the sensitivity to AII. The third determinant of the pressor response to AII is the vascular smooth muscle responsiveness. In an elegant series of experiments Gant et al (1976) were able to show that rapid expansion of plasma volume in **normotensive pregnant patients by infusion of intravenous saline did not alter the vascular reactivity to AII despite stimulation of**

plasma renin levels. They concluded that the major determinant of pressor responsiveness to AII in normotensive pregnant patients is refractoriness to its action or vascular smooth muscle resistance.

The discovery that vasodilator prostaglandins are produced by blood vessels (see 2.3.1) led to speculation that these prostaglandins may influence the vascular responsiveness to vasoactive agents and account for the differences in response between normal pregnancy and pre-eclampsia. It has been demonstrated in animals (Terragno et al 1974, Speroff and Dorman 1977) that AII increases PGE production by the human uterus and increases uterine blood flow. This effect can be blocked by prostaglandin inhibitors. There is also evidence that PGE may account for the reduced sensitivity to AII in normal pregnancy as PGE_2 infusion suppresses the pressor response to exogenous AII (Broughton Pipkin et al 1982). The reduced vascular reactivity which normal pregnant women exhibit towards infused AII is completely abolished if they are given indomethacin, a prostaglandin synthesis inhibitor (Everett et al 1978). It is thus possible that angiotensin-prostaglandin interactions may participate in the regulation of uterine and renal blood flow and of maternal blood pressure (Elder et al, 1985). These observations led to the hypothesis that a deficiency of vasoactive prostaglandins resulting either from a deficiency of precursors, reduced synthesis or abnormal

biological activity may play a role in the pathogenesis of pre-eclampsia.

2.2.2 Fluid balance in normal and hypertensive pregnancy

Part of the weight gain of pregnancy is accounted for by retention of water (MacGillivray and Campbell 1980) and major changes occur in the handling of sodium and water. Sodium is retained in proportion to the increase in extracellular water in normal pregnancy (Davey 1985). Whilst salt and water retention are common features of pre-eclampsia, oedema is a common finding in normotensive pregnant patients and the presence of oedema does not necessarily imply the presence of pre-eclampsia (MacGillivray and Campbell 1980). The cause of the pathological oedema sometimes seen in pre-eclampsia is not known. As in normal pregnancy, total body sodium and water retention occur in parallel, but an increase in intracellular sodium and water is a feature of pre-eclampsia (Weissberg et al 1983). The mechanism and significance of this are unclear.

A number of factors are known to affect sodium and hence water balance in pregnancy. Sodium retention in pregnancy may be related to increased levels of oestrogens, aldosterone and prolactin whereas sodium excretion may be stimulated by the increase in glomerular filtration rate and progesterone (Lindheimer and Katz

1973). In patients with pre-eclampsia the balance of this maintenance of sodium equilibrium may be upset, leading to retention of sodium and water. A reduction in glomerular filtration rate occurs in some patients with pre-eclampsia (Chesley 1978) which could reduce the excretion of sodium. Despite the apparent effects of hormones on sodium and water retention (Korda and Horvatz 1979) no real relationship has been found between alterations of the various hormones and salt and water metabolism in normal and hypertensive pregnancy (MacGillivray 1982).

2.2.3 The coagulation system in pre-eclampsia

The development of fibrin deposition in the small blood vessels in women dying of eclampsia has been known since 1893 when Schmorl noted fibrin deposits in the renal glomeruli of women dying of eclampsia. This was subsequently confirmed by McKay et al (1953) and a difference in pattern demonstrated between uncomplicated pre-eclampsia and eclampsia. Eclampsia is associated with disseminated intravascular coagulation (DIC) and although typical DIC may be found in some cases of pre-eclampsia, the DIC associated with pre-eclampsia and gestational hypertension is usually a more chronic process.

In severe pre-eclampsia the most frequent finding is of increased fibrin deposition and fibinolysis with

elevation of fibrin degradation products, cryofibrinogen and factor VIII levels (Howie et al 1971). The platelet count may also be reduced in pre-eclampsia (Howie et al 1971, Bonnar et al 1971) and a falling platelet count has been described on an early feature of superimposed pre-eclampsia in chronic hypertensive patients (Redman et al 1978).

In mild pre-eclampsia the changes in coagulation and fibrinolysis are less marked than in severe cases (Howie et al 1971) but in some patients even with imminent eclampsia there may be no detectable abnormality (Chesley 1978).

Pregnant chronic hypertensives were found by Howie et al (1971) to have no detectable abnormality of coagulation, fibrinolysis or platelet function. Although Wodziki and Coopland (1977) in a small study demonstrated thrombocytopaenia in patients with hypertension only, the consensus of opinion is that the coagulation system is unaffected unless pre-eclampsia supervenes. (Giles and Inglis 1981, Gibson et al 1982).

It appears that at least some patients with pre-eclampsia have a chronic low grade DIC and that this tends to become more obvious with increasing severity of the disease. The fact that not all pre-eclamptic patients demonstrate activation of the coagulation system implies that this is not the primary aetiological factor. It seems likely that there are no disorders of coagulation in chronic essential hypertension and that the abnormal values in pre-eclampsia are probably not, therefore, a direct effect of the hypertension. This again underlines the differing pathological mechanism in the different hypertensive diseases of pregnancy.

In addition to the decrease in the absolute platelet count, abnormalities of platelet function have also been demonstrated in some pre-eclamptic patients. The platelets of patients with severe pre-eclampsia are less responsive than normal to aggregating agents (Howie et al 1971, Whigham et al 1978). The latter authors suggested that this lack of responsiveness to aggregating agents may be indicative of previous platelet aggregation which then rendered the platelets less sensitive to further aggregating stimuli. Platelet activation in pre-eclampsia has also been confirmed by the higher circulating levels of the specific platelet protein thromboglobulin (Douglas et al 1982) which is a product of the platelet release reaction. Since prostacyclin is

a powerful antiaggregator of platelets and thromboxane stimulates platelet aggregation (see 2.4) it is possible that alterations in the relative amounts of these two prostanoids may play a role in the initiation of the coagulation abnormalities in gestational hypertension. (Wallenburg 1987).

2.2.4 The kidney and liver in hypertensive pregnancy

2.2.4.1 Renal Function

Changes in renal blood flow and function may occur in patients with hypertension in pregnancy. The severity of impairment of renal function seems to be correlated to the severity of the hypertensive process. Many patients with mild essential or gestational hypertension have entirely normal renal blood flow and function, whereas patients with severe pre-eclampsia and eclampsia frequently have abnormal renal function. In normal pregnancy the glomerular filtration rate (GFR) increases by an average of 50%. In patients with pre-eclampsia the GFR is 32% lower than normal pregnant women (Chesley and Duffus 1971(a)). In chronic hypertensive pregnant patients, low plasma volumes have been correlated with low creatinine clearance values, particularly if they have superimposed pre-eclampsia (Arias 1975). In uncomplicated essential hypertension however, renal function is generally not impaired (Knuppel et al 1985).

Uric acid clearance is decreased in pre-eclampsia and

serum uric acid levels are elevated. These changes have been found to correlate to the severity of the renal pre-eclamptic lesion (Pollack and Nettles 1960), poor fetal outcome (Redman et al 1976) and with the decrement in plasma volume which may occur in pre-eclampsia (Beaufils et al 1981). Although it has been suggested (Redman et al 1976) that hyperuricaemia is a specific feature of pre-eclampsia, elevated serum urate levels are also found in chronic hypertension (Messerli et al 1980). Hyperuricaemia does however provide a sensitive index of renal impairment, particularly of renal tubular function (Moore and Redman 1987).

The main pathological renal changes in pre-eclampsia occur in the glomeruli (Sheehan and Lynch 1973). The characteristic glomerular lesions, termed glomerular capillary endotheliosis by Spargo et al (1959), involves swelling of the intracapillary cells of the glomeruli, together with mesangial cell proliferation and subendothelial deposits in the basement membrane (Sheehan and Lynch 1973). The lesions have been claimed to be pathognomonic of pre-eclampsia (Pollack and Nettles 1960) but Robson (1977) considers that these lesions are similar to those found in other diseases in which intravascular coagulation plays a major role.

The appearance of proteinuria implies glomerular damage and leakage and hence represents a more severe form of

pregnancy hypertension (Redman 1984). Acute renal failure is a rare but recognised complication of gestational hypertension (Knuppel et al 1985).

2.2.4.2. Liver Function

Liver dysfunction is a late manifestation of pre-eclampsia. Liver changes are found in 60-70% of women dying of eclampsia or severe pre-eclampsia (Davey 1985). Early liver dysfunction may be detected by elevation of circulating hepatic enzymes (Shukla et al 1978). The pathology of these lesions is usually periportal haemorrhage and necrosis (Sheehan and Lynch 1973) and it has been considered that these lesions are manifestations of vasoconstriction and disseminated intravascular coagulation (DIC) (since coagulation disturbances are common in patients with liver involvement) (Weinstein 1982).

2.2.5 AETIOLOGY OF PRE-ECLAMPSIA

As long ago as 1916 Zweifel termed pre-eclampsia the disease of theories and despite intensive investigation and numerous hypotheses the aetiology of the hypertensive disorders of pregnancy is unknown, although some of the confusion may have arisen from the assumption that all patients with hypertension in pregnancy have a single disease with a common aetiology and pathology. The various abnormalities which have been demonstrated in patients with gestational hypertension have led to speculation that any one of these may be the primary abnormality.

Genetic factors are known to predispose to the development of pre-eclampsia and homozygosity for a single recessive gene is thought to be responsible (Cooper and Liston 1979, Chesley 1984).

There are differences in the incidence of pre-eclampsia in different racial groups but the implication of this is difficult to judge in view of differing socio-economic backgrounds and standards of medical care in different populations. (MacGillivray 1983).

Certain features of pregnancy hypertension have in recent years led to a growing interest in the concept that the disease may be involved in a disturbance of the normal

mechanism of fetus-host response. The high incidence of pre-eclampsia in hydatidiform mole and twin pregnancy (MacGillivray 1983), an increased incidence in pregnancies of a new paternity (Feeney 1980) and the fact that delivery of the placenta results in rapid reversal of the physiological changes, support an immunological mechanism. However there are certain features which are not consistent with this theory, including the familial pattern, variations between racial groups and the presence of hypertension, oedema and convulsions. (MacGillivray 1983, Thurnau 1985).

MacGillivray 1981 concluded that pre-eclampsia can be considered a disease of cascades, commencing with an abnormality of the immune system. As already noted there are certain aspects of pre-eclampsia which cannot easily be explained by a defect of the immunological system as the primary aetiological factor. The theory of abnormality of the prostaglandins as a primary initiating factor is very attractive, as will be discussed later, and abnormal production, metabolism or response to the prostanoids could well explain the abnormalities found in

gestational hypertension. Defective prostanoid synthesis or metabolism may also be important in the pathogenesis of superimposed pre-eclampsia in chronic hypertensives or intrauterine growth retardation in normotensive patients (See 2.6, 2.7).

2.3 PROSTANOID SYNTHESIS, METABOLISM AND BIOLOGICAL ACTIVITY

2.3.1 Introduction

It is now over 50 years since the prostaglandins were first discovered but a full understanding of the roles of prostaglandins in health and disease has still not been reached.

In 1930 Kurzrok recognised that semen could affect the contractility of the human uterus in some cases stimulating intensive uterine activity. Goldblatt (1935) and Von Euler (1936) independently identified an acidic lipid in seminal fluid that contracted uterine smooth muscle and lowered the blood pressure. Von Euler believed this substance to arise from the prostate and therefore named them prostaglandins.

Advances in chemical techniques in the 1950's allowed identification of the chemically related substances now known as the classic or primary prostaglandins. Bergstrom recognised that the biological activity was due to highly active lipid soluble unsaturated fatty acids and recognised the presence of more than one active compound (Bergstrom and Sjovall 1957, 1960, and Bergstom et al 1962) identifying PGE_1, PGE_2, PGE_3, PGF_1 and PGF_2. It was later confirmed that they derived from polyunsaturated fatty acids (Bergstrom et al 1964, Van Dorp et al 1964).

Utilisation of bioassay techniques allowed some of the biological activities of the primary prostaglandins to be defined and some of the effects of the prostaglandins led to speculation that they may have a role in the aetiology of gestational hypertension (Speroff 1973, Speroff and Dorfman 1977). It was noted that prostaglandins of the E series are increased in normal pregnancy and that as they are potent vasodilators (by relaxing arteriolar smooth muscle) may account for the drop in blood pressure seen in normal pregnancy. It was therefore postulated that a deficiency of this vasodilator activity or an excess of the vasoconstrictor $PGF_{2\alpha}$ may account for the vasoconstriction in pre-eclampsia.

The subsequent discovery of two new groups of the prostaglandin family namely thromboxanes (Hamberg et al 1975) and prostacyclines (Moncada et al 1976(a), Moncada et al 1976(b), Moncada et al 1977) with even more potent vasoactivity, opened up new areas of research into pre-eclampsia. The observation that the principal metabolite of arachidonic acid in vascular tissues is prostacyclin and in platelets thromboxane, has led to the belief that these substances may be even more important than the primary prostaglandins in pre-eclampsia (Terragno and Terragno 1979). It has also been suggested that prostanoids may play a role in other conditions characterised by generalised or local vasoconstriction

such as chronic hypertension and intrauterine growth retardation.

I will now briefly review the chemistry and production of the prostanoids and discuss the considerations which influenced the choice of method of assay and techniques of sampling of blood and other fluids. The known physiological activities of each prostanoid will be reviewed, followed by the evidence regarding their roles in normal pregnancy.

2.3.2 Chemistry, Synthesis and Metabolism of the Prostaglandins

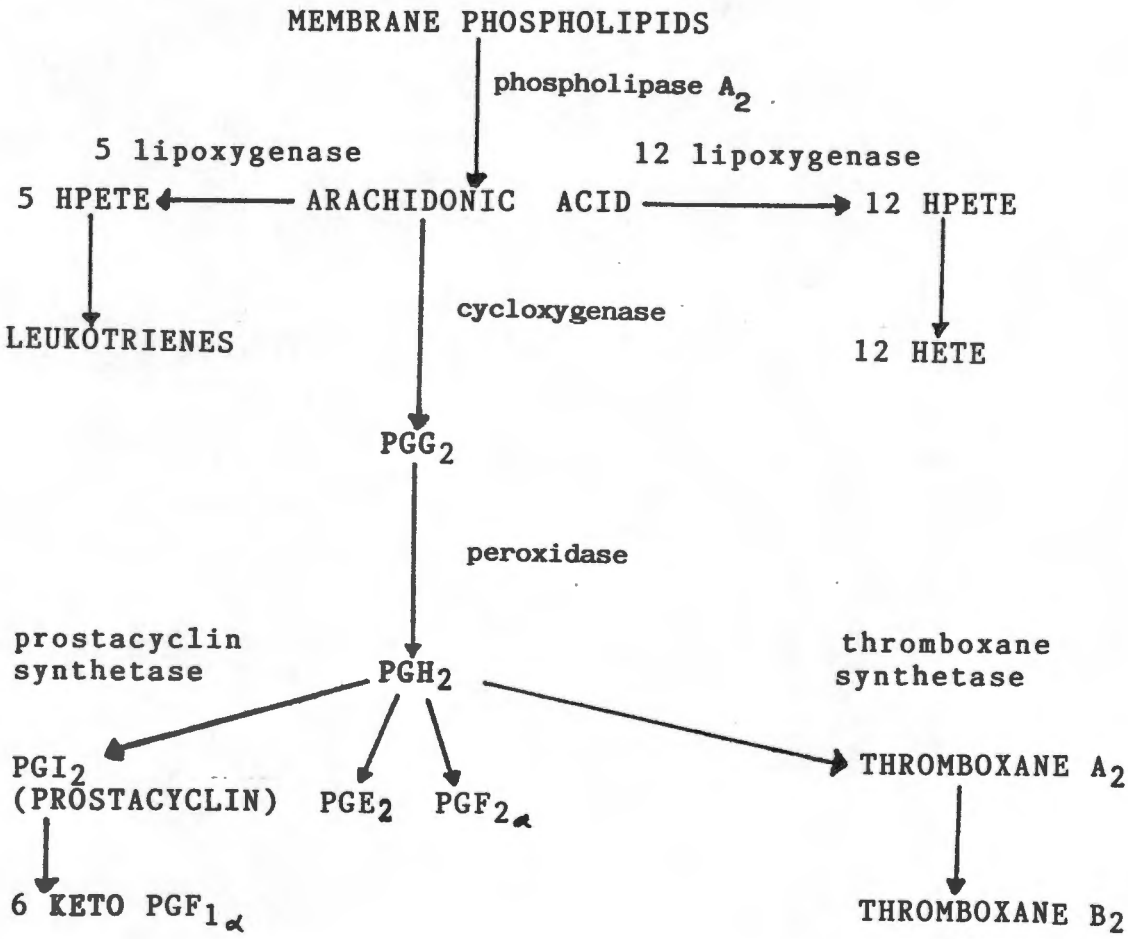
Prostaglandins are a family of substances derived from polyunsaturated fatty acids with a wide range of biological effects. They are fatty acids containing 20 carbon atoms consisting of a cyclopentane ring and 2 side chains and are derived from membrane phospholipid locally in the tissues. The different prostaglandins are given the letters A - I and subscripts 1 - 3, the letters referring to the type of ring substitution and the subscript to the degree of unsaturation of the fatty acid. Three polyunsaturated fatty acids serve as precursors for synthesis of prostaglandins of the 1, 2 and 3 series, dihomolinolenic acid, arachidonic acid and eicosapentanoic acid respectively. As arachidonic acid is present in the largest amounts prostaglandins of the 2 series are preferentially produced (Poyser 1984) and it

has been doubted that the other fatty acids have any physiological importance in the human (Keirse 1979).

The pathway of biosynthesis of the prostaglandins of series 2 and related compounds is summarised in figure 2.1. Free arachidonic acid is released from membrane phospholipids by phospholipase A_2. The first step in the synthesis of PGE_2, $PGF_{2\alpha}$, prostacyclin and thromboxane A_2 is the formation of the cyclic endoperoxides PGG_2 and PGH_2. These are formed by the action of an enzyme, cyclo-oxygenase, on arachidonic acid. PGG_2 and PGH_2 are very unstable but appear to have biological activity (Samuelsson et al 1975). The cyclic endoperoxides are converted to PGE_2, $PGF_{2\alpha}$, prostacyclin and thromboxane A_2 (and also PGA, PGB, PGC and PGD) by the action of specific enzymes, although PGE can also by synthesised non enzymatically. Prostacyclin and thromboxane A_2 are very unstable with half lives of 2 - 3 minutes and 30s respectively (Moncada and Vane 1979) and are rapidly hydrolysed to 6 keto $PGF_{1\alpha}$ and thromboxane B_2 (TxB_2). As the thromboxanes have an oxane rather than a cyclopentane structure they are not strictly considered prostaglandins and the term prostanoids has been used to include all "prostaglandin-like" derivatives of cyclic endoperoxides.

FIGURE 2.1

BIOSYNTHESIS OF PROSTAGLANDINS AND RELATED COMPOUNDS BY THE ARACHIDONIC ACID PATHWAY



(ADAPTED FROM RAMWELL 1980)

HPETE - HYDROPEROXYTETRANOIC ACIDS

HETE - HYDROXYTETRANOIC ACIDS

Arachidonic acid is also metabolised by lipoxygenase enzymes to another group of biologically active compounds hydroperoxytetranoic acids (HPETEs) which can be converted to hydroxytetranoic acids (HETEs). The formation of leukotrienes from 5HPETE is also initiated by lipoxygenase activity.

In order to be available for prostaglandin synthesis, arachidonic acid has to be in the free form. The action of phospholipase A_2 in generating free arachidonic acid from tissue phospholipids has been considered a rate limiting step in the biosynthesis of prostaglandins (Kunze and Vogt 1971) as tissue levels are not normally high enough for prostaglandin synthesis to occur (Poyser 1981). Substrate availability is not the only requirement for prostaglandin synthesis and the activity of the prostaglandin synthetase enzyme system may also have an important regulatory role (Keirse 1979).

The ubiquitous nature of prostaglandins is illustrated by the fact that, with exception of red blood cells, all tissues possess the cyclooxygenase enzymes and thus have the capacity for prostanoid production (Vane et al 1982) although the major products tend to be tissue specific (see below).

Biosynthesis and metabolism of the prostaglandins occur very rapidly (Green 1979). Several prostanoids may be formed simultaneously and as they are synthesised from a common precursor, their relative rates of production may be interdependant. These complexities of prostanoid biochemistry have made it difficult to assess their rates of production and the interaction of their biological effects has complicated the evaluation of their physiological roles.

The primary prostaglandins are metabolised enzymatically by oxidation of the 15 hydroxyl group on the side chain to a keto group followed by reduction of the 13-14 double bond. Most tissues are able to carry out these transformations but the lung, liver and kidney are the main sites of metabolism (Ramwell et al 1980). The lungs in particular have a very high metabolic activity with 95-99% of the circulating PGE_2 and $PGF_{2\alpha}$ being taken up in one passage through the lungs (Ferriera and Vane 1967, Piper et al 1970) although prostacyclin is not inactivated in the pulmonary circulation (Dusting et al 1978). Further degradation of the primary prostaglandins occurs by B and w oxidation to dicarboxylic acids, which are the major urine metabolites of PGE_2 and $PGF_{2\alpha}$ (Granstrom and Kindahl 1982, Green et al 1981).

Prostacyclin is unstable at physiological pH undergoing spontaneous hydrolysis to 6 keto $PGF_{1}\alpha$. It has been possible however to study the metabolism of infused prostacyclin because of its lack of inactivation by the lungs (Rosenkranz et al 1980). A large number of metabolites have been identified from which it appears that prostacyclin is usually metabolised by conversion to 6 keto $PGF_{1}\alpha$ followed by B oxidation to dinor metabolites (Rosenkranz et al 1980).

Thromboxane A_2 is similarly rapidly hydrolysed to TxB_2 in aqueous solutions and a large number of urinary metabolites of TxB_2 have been identified. The major metabolite is 2-3 dinor TxB_2.

As most tissues possess the capacity to synthesise most prostanoids there has been much interest in the mechanisms which regulate the preferential production of prostanoids in different tissues and under different circumstances. The availability of free arachnidonic acid is the initial rate limiting step but other factors are also important as the cyclic endoperoxides are central to the production of all the biologically active prostaglandins. The non steroidal anti-inflammatory drugs such as aspirin, inhibit cyclooxygenase activity and prevent conversion of arachidonic acid to PGG_2 by a competetive reversible blockade of the substrate site on

the enzyme and also by a time dependant irreversible blockade (Poyser 1984).

In vitro several compounds such as copper have been shown to direct cyclic endoperoxide metabolism towards certain prostaglandins, enzymatically or non-enzymatically (Samuelsson et al 1975). Natural and synthetic glucocorticoids inhibit prostaglandin and thromboxane synthesis by stimulating the formation and release of a protein "macrocortin" which inhibits the action of phospholipase A_2. (Blackwell 1980).

Another possible way in which predominance of a particular pathway can be achieved in vivo is that there are 2 mechanisms for the formation of PgE_2 from PGG_2 (Samuelsson et al 1975) and the dominance of one or the other appears to depend upon the predominant pathway in a particular tissue.

Endogenous inhibitors and stimulators of prostaglandin release have been described (Saeed et al 1977, Brennecke et al 1982, Saeed and Mitchell 1982). In animals an infusion of angiotensin II stimulates prostacyclin release (Gryglewski et al 1980). Brennecke et al (1982) described endogenous inhibitors of prostaglandin synthesis (EIPS) in plasma but their subsequent work (1984) suggests that EIPS do not play a role in the control of prostaglandin production during pregnancy.

Arachidonic acid is liberated from membrane phospholipids by the activation of phospholipases which can be initiated by changes in their chemical environment or by mechanical stimulation (Morris et al 1981). The actual process of sampling and the handling and treatment of the sample may therefore cause the release of prostanoids leading to erroneous results in assays. These factors are considered in the section on assay techniques.

In vivo, the catabolism of the prostaglandins to inactive compounds may be important in controlling the levels of physiologically active prostaglandins and may be an important factor in determining the balance of active prostaglandins in a tissue (Bakhle 1983).

2.3.3 Assay methods for Prostanoids

At the present time three methods of assay of prostaglandins are widely used. These are bioassay, gas chromatography with mass spectrometry (GCMS) and radioimmunoassay (RIA).

2.3.3.1 Bioassay

When Bergstrom elucidated the structure of the first prostaglandins (Bergstrom and Sjovall 1957, 1960, Bergstrom et al 1962, 1964) bioassay methods were the only available means of assessing the production of the prostaglandins and their biological activity.

Bioassay methods have the advantage of actual measuring the biological activity and played a major role in the investigation of the physiological actions of the prostaglandins. They were of critical importance in the discovery of rat aorta contracting substance (Piper and Vane 1969) which was subsequently identified as thromboxane (Hamberg et al 1975) and of prostacyclin (Moncada et al 1976(a), 1976(b)). One type of bioassay is based on the fact that different members of the prostaglandin family differ both quantitatively and qualitatively in their effects on smooth muscle from different organs. The blood bathed organ technique of cascade superfusion bioassay described by Vane (see Vane 1983 for a review of the technique) has been widely used in the evaluation of the roles of the various prostanoids in pregnancy. (See 2.4, - 2.7). Another type of bioassay that has been widely used in prostaglandin research is based on the effects of prostacyclin in inhibiting and thromboxane in stimulating platelet aggregation. (Bunting et al 1978, Wallenberg and Rotmans

1982a).

Bioassay has the advantage of instantaneity and therefore permits the detection and measurement of the biological activity of chemically unstable compounds (Vane 1983). The disadvantage of bioassay techniques is that they are difficult to set up and may be non specific (Levine 1970) because of the presence of other biologically active substances in the sample.

2.3.3.2 Gas chromatography mass spectrometry (GCMS)

Gas chromatography coupled with mass spectrometry has the advantage of great specificity and this technique was used to elucidate the chemical structure of a number of prostaglandins and their metabolites. It was of particular importance in the discovery of the thromboxanes (Hamberg et al 1975). The technique however is time consuming, expensive and not readily applicable to the clinical situation. Although it is a useful research tool (Hensby 1977) the necessary equipment is not available to most workers. GCMS however is the most specific method of assay currently available and hence has become a method against which other assays are compared.

2.3.3.3 Radioimmunoassay (RIA)

RIA for prostaglandins was first described for PGE_1 and $PGF_{2\alpha}$ by Levine and van Vunakis in 1970 and since that time numerous radioimmunoassays for various prostaglandins, thromboxanes and their metabolites have been developed (reviewed by Granstrom 1978(a), and Granstrom et al 1978(b)).

Radioimmunoassay is based on the competition of radioactively labelled and unlabelled antigen for the same binding sites on the antibody specifically directed against a particular compound. The necessary reagents are specific antibody, standard unlabelled prostanoid and radioactive prostanoid, together with a method of separating the antibody-antigen complex from free antigens. As the prostanoids are not antigenic they are first bound to an antigenic molecule, the most commonly used being bovine serum albumin. The most commonly used radioactive labelling is with the ^{3}H isotope which has a longer half life than the alternative ^{125}I. The unlabelled prostanoids are now available commercially. The assay procedure is described in Appendix 6.

Most of the early research concentrated on assays for the primary prostaglandins but it has now become apparent that these are relatively unstable (Granstrom 1978) and Granstrom et al 1978(b) and most research is now based on the assay of the more stable metabolites. Due to the extreme instability of prostacyclin and thromboxane A_2 assays of their metabolites 6 keto $PGF_{1\alpha}$ and TxB_2 are usually performed. An assay for mono-o-methyl TxB_2 as a more stable metabolite of thromboxane A_2 has been described (Granstrom 1980(a)) but this has not been widely used.

There has been some controversy as to the most appropriate metabolites to measure as a reflection of the parent compounds, as degradation to a number of metabolites may occur. A number of factors were taken into consideration in selecting the most appropriate assay method for the purposes of the present study.

Bioassay methods have the drawback of only assessing the capacity of the tissue or fluid to produce a particular prostaglandin but give no idea of the actual activity in vivo, as required in this study. GCMS was unavailable and it was therefore considered that radioimmunoassay of biological fluids would be the most appropriate method of measurement.

The development and characterisation of radioimmunoassay is however a specialised field in itself. Commercially available radioimmunoassay kits which have been subjected to careful quality control and validation testing are accepted as reliable and reproducible (Salmon 1983). As a limited study of a number of prostanoids was planned, the use of the commercially available radioimmunoassay kits was the logical and practical choice.

2.3.3.4 Problems associated with measurement of prostanoids

Prostaglandins and thromboxanes are tissue specific, the type being dictated by the enzyme specificity of the cell and are locally active. As the primary prostaglandins, prostacyclin and thromboxane A_2 are very unstable, measurements of metabolites has been advocated but the most appropriate metabolite to measure has been disputed.

Attempts have been made to assess the in vivo prostanoid status by the measurement of levels in a range of biological fluids. There is a marked lack of agreement however between results reported from different laboratories using the same method and this has led to confusion. Conflicting results are common in the literature and the reasons for this have been discussed at length by Granstrom (1978(a), Granstrom et al 1978(b)

and Keirse (1979). With the primary prostaglandins the discrepancies in reported levels are partly a reflection of their instability and rapid inactivation in the lungs, but other factors also affect assay reliability. Prostanoids can be released by the distortion of cells or platelet activation (Keirse 1979) and thus may be stimulated by endothelial trauma or cell disturbance during sampling particularly through a narrow gauge needle (Kelly 1985). The endogenous levels may then be buried under artefactual amounts of the prostanoid formed during sample collection.

The treatment of samples can also affect the radioimmunoassay results. This was investigated extensively by Morris et al (1981). It is logical to assume that the lowest result is the one least affected by artifactual producton of prostanoids. These workers found that blood samples were best obtained from an indwelling needle in a forearm vein kept open by a slow saline infusion with a saline flush before collection of the sample. Any difficulty in obtaining the sample was associated with elevated measurements particularly of TxB_2. Other variables which may affect the result are the choice of anticoagulant, the presence or absence of a prostaglandin inhibitor, centrifugation temperature, thawing the sample more than once, pH, protein concentration or high levels of crossreacting compounds (Morris et al 1981, Challis and Tulchinsky 1974, Salmon

1983).

As the prostanoids are rapidly metabolised there is doubt that circulating levels actually reflect endogenous concentrations of the parent compound and alternative approaches to their estimation have been taken. One approach is to measure the 13, 14, dihydro 15 keto metabolites of the primary prostaglandins which have a half life of 8 min., are present at higher concentrations than the parent prostaglandins and are not formed artefactually during collection of the sample (Granstrom 1978(a)). This method may therefore provide a more accurate reflection of endogenous levels. The 13-14 dihydro 15 keto $F_{2\alpha}$ metabolite (PGFM) has been used successfully as a measure of $PGF_{2\alpha}$ production (Norman et al 1982). Assays for the equivalent PGE metabolite have proven unreliable as PGEM is unstable in aqueous solution (Granstrom et al 1980 (b)). An assay for the more stable bicyclo-metabolite PGEM II (Mitchell et al 1982) or methyl-oximation to stabilise the PGE structure (Kelly and Abel 1983) have been reported. The prostaglandins E and F are metabolised in the liver to dinor and tetra compounds. The longer half life of the tetranor compounds means that measurement of these compounds in urine is sometimes preferable (Granstrom and Kindahl 1982). Measurement of plasma metabolites is useful for monitoring rapid changes (minutes or hours) whereas urine metabolites are preferable for changes of longer duration

(hours or days) (Green et al 1981).

Prostacyclin and thromboxane A2 are very unstable and measurement of the more stable metabolites 6 keto $PGF_{1\alpha}$ and TxB_2 is preferred.

Despite the controversies regarding the relevance of plasma levels of prostanoids and their metabolites, it is considered that meaningful results can be obtained if adequate precautions in the method of sampling and preparation of samples are undertaken (Norman et al 1982, Salmon 1983, Kelly 1985).

Another approach to the problem is the measurement of prostanoids in the tissue of interest, in the venous drainage of an organ or in a less metabolically active fluid such as amniotic fluid or urine. These methods have been used in pregnancy. Sampling the venous drainage of an organ has the advantage of localising the site of production but long catheters for sampling increase the chance of trauma to the vascular endothelium and blood (Dollery and Barrow 1985).

There is controversy in the literature regarding the need for extraction and chromatography of samples prior to assay. Extraction removes cross reacting compounds but may cause interference with subsequent RIA. Assay of unextracted plasma has produced inaccurate RIA values whereas extraction and chromatography increases the likelihood of error and may result in unacceptably low recovery of prostanoid, reducing it to the lower limit of the assay with consequent loss of sensitivity (Salmon 1978). The thin layer chromatography stage is particularly associated with poor recovery (Strickland et al 1982). Granstrom (1978(a)) reviewed the problem and concluded that extraction and chromatography may generate more problems than it solves.

Initial RIA measurements of prostanoid were unacceptably high but as a consequence of advances in techniques RIA now yields results comparable to GCMS. It is likely that most RIA techniques have cross reactivity for other metabolites of the substance under study. It therefore may be wrong to assume that the assay measures just for example 6 keto $PGF_{1}\alpha$. It is probably more precise to refer to "prostacyclin metabolite assay" as suggested by Belch et al (1983) or to prostacyclin immunoreactivity.

Since a number of metabolites of the parent prostanoid may be present in the sample, this cross reactivity may in fact be an advantage if one wishes to have a measure of biological activity of a particular prostanoid. Cross reactivity to other fatty acids can be reduced by the use of organic solvents in extraction (Hensby et al 1981). Both RIA and GCMS can produce reliable, reproducible results and are sensitive. RIA has the advantage of sample capacity, time and lower overall expenditure. The role of RIA probably lies more in comparative values than absolute concentrations, provided that standard conditions are used after adequate validation of each assay.

2.3.4 Biological Actions of Prostanoids

As a result of extensive investigations the biological effects of the prostanoids are known. These have been reviewed by Poyser (1984). The actions of prostanoids which may be important in the physiological changes in pregnancy are summarised in Table 2.I.

The prostanoids are locally produced moderators of responses to exogenous and endogenous stimulators and inhibitors. They tend to be tissue specific and are produced on demand. Although the prostaglandins have similar chemical structures their activities vary greatly and may differ according to the tissue affected. For example PGE_2 contracts the smooth muscle of the uterus but relaxes the smooth muscle of the arteriolar wall.

Prostanoids are potent vasoactive agents PGE_2 and prostacyclin being vasodilators while thromboxane and to a lesser extent $PGF_{2\alpha}$ are vasoconstrictors. These opposing effects may be important in control of blood pressure.

Initially it was believed that PGE_2 and $PGF_{2\alpha}$ were the main arachidonic acid products and that their known effects on vasodilatation and vasoconstriction were important in blood pressure control and organ perfusion. The subsequent discovery of the more potent prostacyclin

and thromboxane led to accumulation of evidence that these are probably more important physiologically (Moncada and Vane 1979). Thromboxane and prostacyclin have directly opposing pharmacological activity in many systems such as vascular smooth muscle and platelets. So it is postulated that interactions between these two prostanoids may be important in the regulation of blood pressure, blood flow to tissues and the activation of the coagulation system. than the primary prostaglandin.

The roles of prostanoids in pregnancy have now been evaluated for over 20 years yet the situation is still far from clear. As advances have been made in assay techniques together with a realisation of the problems of assay of prostanoids in biological fluids outlined above, doubt has been cast on much of the early work. Prior to the discovery of prostacyclin it is possible for example that studies purporting to measure PGE_2 may have measured mixtures of PGE_2, PGE_1 and prostacyclin (Terragno et al 1980). In addition failure to differentiate between chronic and gestational hypertension in pregnancy has also increased the confusion.

TABLE 2.I : BIOLOGICAL ACTIVITY OF THE PROSTANOIDS

(FROM POYSER 1984)

PGE_1	- weak inhibitor of platelet aggregation
PGE_2	- vasodilator
	- antagonist of angiotensin II
	- stimulates sodium excretion by inhibiting action of antidiuretic hormone on renal tubule
	- potentiates second wave of platelet aggregation
	- stimulates uterine contractions
$PGF_{2\alpha}$	- vasoconstrictor
	- stimulates uterine contractions
PROSTACYCLIN-	potent inhibitor of platelet aggregation
	- vasodilator
	- stimulates renin release
TxA_2	- potent stimulator of platelet aggregation
	- vasoconstrictor

2.4 THE ROLE OF PROSTANOIDS IN NORMAL PREGNANCY

2.4.1 Plasma Prostanoids in Normal Pregnancy

2.4.1.1 PGE and PGF

Prostaglandins E and F are produced by the mammalian blastocyst from early pregnancy (Shemesh 1983). PGE and PGF have been detected in uterine and intrauterine tissues during pregnancy and hence may play an important role in local regulation of blood flow. Terragno et al (1974) and Speroff et al (1976) described elevation of PGE levels in uterine veins of pregnant animals. Terragno found that the levels correlated with the uterine blood flow and both groups of workers demonstrated angiotensin II infusion stimulated PGE release and increased uterine blood flow.

Early workers suggested that local release of PGE may be the cause of the vasodilatation with a consequent drop in blood pressure and a mediator of the increased uteroplacental and renal blood flow in normal pregnancy (Speroff and Dorfman 1977).

There is considerable disagreement between authors regarding assays of PGE and PGF in maternal plasma both in respect of the circulating levels and the effect of gestational age. (Table 2.II)

TABLE 2.II : EFFECT OF GESTATIONAL AGE ON MATERNAL PLASMA PGE

ASSAY	FINDING	FIRST AUTHOR
BIOASSAY	Decrease with increasing gestational age	Van Orden 1975
RIA	Elevated in third trimester compared to non-pregnant	Ferris 1976
RIA	Peak a few weeks before term then declining	Coats 1977
RIA	Increased in third trimester	Whalen 1978
RIA (PGEM-II)	Increased in early pregnancy Decrease third trimester	Mitchell 1982
RIA (BICYCLO PGEM)	Unchanged in second and third trimester	Brennecke 1985
RIA (PGE_2)	Similar to non-pregnant until late pregnancy when increase observed	Ogino 1986(a)

Van Orden et al (1975) found plasma PGE levels to be reduced compared to non-pregnant whereas others (Ferris et al 1976, Speroff and Dorfman 1977, Bay and Ferris 1979) demonstrated increased levels. The reports on the effect of gestational age on maternal plasma PGE_2 are also conflicting. It was thus initially reported as decreasing with increasing gestational age (Van Orden et al 1975). The same workers however subsequently reported that levels were equivalent to non-pregnant in the first two trimesters but were increased in the third trimester (Whalen et al 1978) and a recent report (Ogino and Jimbo 1986(a)) is in agreement with this.

Because of the difficulties of measuring circulating PGE_2 levels Mitchell et al (1982) developed a radioimmunoassay for a stable metabolite 11 deoxy-13, 14 dihydro-15-keto 11, 16 cycloprostaglandin E_2 (PGEM-II) as a measure of PGE_2 activity. By this more reliable technique they demonstrated an increase in maternal plasma PGEM-II in early pregnancy but a decline in concentrations in the third trimester, and an increase again in labour. A later report by Brennecke et al (1985) using the same technique reported no change in bicyclo PGE_2 in human plasma during the second and third trimesters, or in labour.

Serum levels of $PGF_{2\alpha}$ and its more stable metabolite

13,14 dihydro 15 keto prostaglandin $F_{2\alpha}$ (PGFM) have been measured by many workers. The discrepancies between values obtained by different workers have been reviewed by Keirse (1979). Reported values range from 20.4 to 1850 pg/ml. It will be apparent from Table 2.III that most workers found plasma PGF unchanged in comparison to non-pregnant patients although Whalen et al (1978) found levels to be elevated.

TABLE 2.III : SERUM PGF IN PREGNANT COMPARED TO NON-PREGNAI

RIA	GESTATION	COMPARISON TO NON-PREGNANT	FIRST AUTHOI
$PGF_{2\alpha}$	3rd trimester	same	Brummer 197
$PGF_{2\alpha}$	-	same	Gutierrez-Cernosek 197
$PGF_{2\alpha}$	mean overall	same	Hennam 1974
	2nd trimester	lower	
PGF	3rd trimester	same	Challis 1974
PGF	all	same	Van Orden 19
PGF	all	elevated	Whalen 1978
PGFM	17 - 20 weeks	same	Ghodgaonkar
$PGF_{2\alpha}$	24 - 40 weeks	elevated	Ogino 1986(a

The effect of gestation on plasma PGF is also controversial. Some workers have found PGF to be lower in the second trimester than the first (Brummer 1973), to be lower in the second trimester than at any other time (Hennam et al 1974) or to be maximal in the second trimester (Guttierez-Cernosek et al 1972). Many workers have however found that PGFM levels in peripheral blood remain at a constant level throughout pregnancy but are elevated in labour. (Table 2.IV).

Keirse (1978) considered that the discrepancies between results of different workers are due to the fact that PGE and PGF are rapidly metabolised in the lung and that maternal plasma levels do not reflect synthesis in the feto-placental unit. The consistency however with which elevated levels of PGFM in plasma have been recorded in labour would imply that plasma levels do reflect increased synthesis or decreased catabolism at that time.

TABLE 2.IV : EFFECT OF GESTATIONAL AGE AND LABOUR ON MATERNAL PLASMA PGF

RIA	EFFECT OF GESTATIONAL AGE	EFFECT OF LABOUR	FIRST AUTHOR
$PGF_{2\alpha}$	Max. 2nd trimester	-	Gutierrez Cernosek 1972
$PGF_{2\alpha}$	Lower 2nd trimester	-	Brummer 1973
$PGF_{2\alpha}$	Lower 2nd trimester	Increased	Hennam 1974
$PGF_{2\alpha}$	-	No effect	Hillier 1974
$PGF_{2\alpha}$	None	Fluctuations	Johnson 1975
PGF	None	-	Van Orden 1975
$PGF_{2\alpha}$	Raised to 36 weeks then declined	-	Coats 1977
$PGF_{2\alpha}$	-	No effect	Kinoshita 1977
$PGF_{2\alpha}$	-	Increased correlated to stage of labour	Zuckerman 1978
PGF	-	No effect	Mitchell 1978(a)
PGFM	-	Increased	
$PGF_{2\alpha}$ PGFM	None	Increased at 7cms dilatation	Ghodgaonkar 1978
PGF	None	-	Whalen 1978
PGFM	None	Increased	Dubin 1981
PGFM	None	Increased	Mitchell 1981(a)
PGFM	None	-	Koullapis 1982
PGFM	None	-	Yamguchi 1984
$PGF_{2\alpha}$	None	-	Ogino 1986(a)

2.4.1.2 Prostacyclin

The discovery that vascular endothelium generates the vasodilator prostacyclin (Macintyre et al 1978) led to speculation that it may play an important role in the vasodilatation of normal pregnancy. The findings regarding maternal plasma prostacyclin levels in pregnancy however are contradictory. (Table 2.V). A preliminary study suggested that plasma 6 keto $PGF_{1\alpha}$ levels were higher at 14 - 18 weeks gestation than outside pregnancy but were lower after 26 weeks gestation (Mitchell 1978(b)) but the same group subsequently reported no effect of gestational age or labour on maternal 6 keto $PG_{1\alpha}$ levels (Turnbull et al 1979). Plasma 6 keto $PG_{1\alpha}$ levels have been reported to decrease (Bolton et al 1981, Spitz et al 1983) or increase (Lewis et al 1980, Barrow et al 1983) in late pregnancy or be unaffected by gestational age (Ylikorkala et al 1981(a)).

TABLE 2.V : MATERNAL BLOOD PROSTACYCLIN METABOLITE (6 KETO $PGF_{1\alpha}$) LEVELS IN NORMAL PREGNANCY

ASSAY	LEVELS COMPARED TO NON-PREGNANT	EFFECT OF GESTATIONAL AGE	FIRST AUTHOR
RIA PLASMA	Higher at 14-18 weeks Lower after 26 weeks	Peak 2nd trimester Decrease in 3rd trimester	Mitchell 1978(b)
RIA PLASMA	-	None	Turnbull 1979
GCMS PLASMA	Elevated 28 weeks	Peak 2nd trimester	Lewis 1980
RIA PLASMA	-	Peak 2nd trimester Decrease in 2nd half pregnancy	Bolton 1981
RIA PLASMA	-	None	Koullapis 1982
RIA PLASMA	Same	None	Ylikorkala 1981(a)
RIA PLASMA	-	None	Mitchell 1981(b)
RIA WHOLE BLOOD	-	Decrease after 33 weeks	Spitz 1983
RIA PLASMA	Low to unrecordable levels	-	Vermylen 1983
GC/NICIMS PLASMA	Same	Increase in late pregnancy	Barrow 1983
RIA PLASMA	-	None	Yamguchi 1984

There has also been considerable debate as to whether or not prostacyclin acts as a circulating hormone. It was postulated that not only is prostacyclin not metabolised in the lung (Dusting et al 1978, Gerkens et al 1978) but that it may be produced by the lung as levels of 6 keto $F_{1\alpha}$ measured by GCMS were found to be higher in the left ventricle than in the pulmonary artery (Hensby et al 1979). Generation of prostacyclin by the lung in animals has also been demonstrated. (Gryglewski et al 1978, Wharton et al 1978). Blair et al (1982) and Barrow et al (1983) measuring 6 keto $PGF_{1\alpha}$ by GCMS however concluded that the measured levels are too low to exert any physiological effect on blood pressure. Patrono et al (1982) and Christ Hazelhof and Nugteren (1981) also concluded that prostacyclin is not a circulating hormone. An editorial in the Lancet (1982) concluded that "whether or not prostacyclin behaves as a conventional circulating hormone may not in fact be crucial. A local vasodilator action exerted over a large enough area or powerful enough could influence the body's haemodynamics as a whole".

2.4.1.3 Thromboxane

Thromboxane A_2 production in vivo in pregnancy as assessed by measurement of maternal plasma TxB_2 levels has been studied by relatively few workers. Maternal plasma TxB_2 levels were found to be increased in pregnancy by Mitchell 1978(b) and Ylikorkala and Viinikka 1980 but not by Koullapis et al 1982. The effect of gestational age on circulating TxB_2 levels is also controversial with two groups of workers (Ylikorkala and Viinikka 1980, Mitchell et al 1978(c)) finding an increase and two (Koullapis et al 1982, Yamguchi and Mori 1984) no change with gestational age .

2.4.2 Urinary Prostanoids

2.4.2.1 Measurement of Urinary Prostanoids

Several workers have studied urinary prostaglandins because of the difficulties in obtaining reliable measurements of prostaglandin levels in the blood. There are several reasons for this. Firstly, the urine is less metabolically active and specimens can be obtained non invasively, thereby reducing the artefactual formation of prostaglandins in the sample. Secondly, circulating prostaglandin levels probably do not reflect uterine prostaglandin production but measure both local and total

body synthesis at the moment of sampling. (Keirse 1978). Measurement of urine metabolites will however give a measure of the overall synthesis of prostaglandins over a period of hours or days (Granstrom and Kindahl 1978(b)). A third consideration is that urinary prostaglandins particularly of the primary metabolites (Patrono and Dunn 1987) may reflect prostaglandin production in the kidneys which may be relevant to renal blood flow and renal function.

In pregnancy the increase in renal blood flow may be mediated by the increased local production of prostaglandins and urinary prostaglandins may reflect this physiological response. PGE_2 was initially considered the major renal prostaglandin (McGiff and Itskovitz 1973) although it subsequently appeared that $PGF_{2\alpha}$ is also produced and of importance (Nasjletti and Malik 1981). The roles of the various prostanoids in the kidney have been reviewed by McGiff and Wong (1979). PGE_2 is produced mainly in the medullary interstitial cells and enzymatic conversion to PGF_2 by a 9 keto reductase may occur. Prostacyclin is the principal product of the vascular tissues of the kidney where it stimulates the release of renin (Oates et al 1979). PgE_2 is half as potent as prostacyclin in stimulating renin release and its major role appears to be in the regulation of medullary blood flow and transport of salt and water together with a modulatory effect on the

vasculature (McGiff and Wong 1979). PGE_2 is released in response to reduced renal blood flow and prostacyclin is stimulated by an adrenergic agonist (Nadler et al 1983). It was originally thought that TxA_2 was only produced by damaged renal tissue (Morrison et al 1977) but it subsequently became apparent that TxA_2 is also synthesised in normal kidneys (Franco-Saenz et al 1980, Zipser and Martin 1982). The latter workers consider that extra renal sources also contribute to urinary prostanoid levels but that their contribution is related to the efficiency of renal function. Several authors have provided evidence that urinary 6 keto $PGF_{1\alpha}$ and PGE_2 reflect renal synthesis of prostacyclin and PGE_2 respectively (Patrono et al 1982, Nadler et al 1983).

2.4.2.2. Urinary Prostanoids in Normal Pregnancy

There have been relatively few studies of urinary prostaglandins in normal pregnancy but, in contrast to the findings in blood, the results are in reasonable agreement. Urinary PGE levels are higher in normal pregnancy than in non-pregnant controls (Bay and Ferris 1979, Pedersen et al 1983, Moutquin and Leblanc 1983, Kovatz et al 1981, Rathaus et al 1982). The first longitudinal study (Moutquin and Leblanc 1982) showed a **triphasic pattern with an initial increase at 15 - 18 weeks, a nadir at 24 - 26 weeks and a second increase at**

29 - 31 weeks followed by a gradual decline to term.

Urinary PGF values are also higher in pregnancy than in non-pregnant patients. (Kovatz et al 1981, Granstrom and Kindahl 1976, Pedersen 1983). Granstrom and Kindahl (1976) who measured a tetranor metabolite of PGFM in the urine throughout pregnancy in a single patient, demonstrated a 3-fold elevation over non pregnant values, increasing with gestational age and with a further marked increase in labour. Recently Bernheim et al (1986) reported a longitudinal study of 24 hour urinary excretion of PGE_2 and $PGF_{2\alpha}$ which showed that both progressively increased during pregnancy.

At the time of commencement of the study, urinary prostacyclin metabolites in normal pregnancy had been measured by only one group of workers. (Goodman et al 1982, Brash et al 1983). They measured the urinary excretion of dinor metabolites of prostacyclin by GCMS and reported that pregnant women had an increased excretion 5 times that of non pregnant women, and that excretion increased progressively with gestational age.

Subsequently using a radioimmunoassay technique to measure urinary excretion of 6 keto $PGF_{1\alpha}$ and another prostacyclin metabolite 2,3-dinor-6 keto $PGF_{1}\alpha$, Ylikorkala et al (1986(a)) were able to confirm these findings.

The same study provided the only available data of urinary TxB_2 excretion in pregnancy and reported no change in the excretion of TxB_2 during normal pregnancy. (Ylikorkala et al 1986(a)).

2.4.3. PROSTANOID PRODUCTION

Tissue homogenates of amnion, chorion, placenta and decidua are all capable of producing prostanoids in vitro (Mitchell 1981(a)). However, the different tissues preferentially produce different prostanoids and this may reflect their varying physiological roles in vivo. Thromboxane is the main product of the placenta although PGE and PGF are also produced in significant amounts (Makila et al 1984(b), Mitchell et al 1978(e), Dembele-Duchesne et al 1981, Robinson et al 1979). Makila et al (1984(b)) using bioassay coupled with RIA demonstrated that thromboxane production in vitro is 5 times that of 6 keto $PGF_1\alpha$ on both sides of the placenta. Other workers (Glance et al 1985) however found that on the maternal side of the isolated perfused human placental cotyledon, TxB_2 production exceeded that of $PGF_2\alpha$, PGE and 6 keto $PGF_1\alpha$, whereas on the fetal side the prostanoids were produced in roughly equal amounts. These differences in the relative amounts of prostanoids produced may be a reflection of differences in methodology.

The physiological importance of the large amounts of

thromboxane produced by the placenta are not known. It has been speculated that thromboxane may play a role in placental separation (Ylikorkala and Makila 1985) but it is quite possible that the relative amounts of the various prostanoids produced by the delivered placenta do not reflect their biological activity during pregnancy.

Most workers believe that PGE and PGF released by the fetal membranes and decidua may be involved in the initiation and maintenance of labour (Mitchell et al 1978(d)). Amniotic fluid levels of PGE and PGF are increased in spontaneous labour (Dray and Frydman 1976) and dispersed cells from fetal membranes and decidua also demonstrate greater production of PGE_2 and PGF2α after spontaneous labour than after elective caesarean section (Olson et al 1983).

Placental tissue in vitro shows antiaggegatory activity which has been attributed to prostacyclin-like activity by some workers (Myatt and Elder 1977) and anti-ADPase by others (Dembele-Duchesne et al 1982). However the placenta in vitro has recently been shown to produce prostacyclin (Keirse et al 1986) although in small amounts in comparison to other vascular tissues (Jeremy et al 1983, Elder et al 1983) and as a result its biological importance has been questioned. The in vitro production of prostacyclin may however be unrelated to its generation and activity in vivo. The biological

importance of prostacyclin production by the placenta is implied by the finding of reduced prostacyclin synthesis by placental tissue from pregnancies complicated by growth retardation (Remuzzi et al 1981(b), Jogee et al 1983). This could be the cause of the decreased uteroplacental blood flow which may occur in these pregnancies (Nylund et al 1983). These findings suggest that although the amount of prostacyclin produced by the placenta may not be great, due to its high biological activity even relatively low concentrations may have important vasodilator effects (Elder et al 1983).

There is good evidence that the prostanoids are important local regulators of intrauterine blood flows in pregnancy. It has been observed that PGE_2 has a vasodilator effect on the uterine circulation but acts as a vasoconstrictor in the fetal circulation (Rankin 1976, Abramovich et al 1984). This could be a mechanism for allowing independant control of the uteroplacental and fetal blood flows. In animals infusion of angiotensin II increases uterine blood flow by stimulating release of vasodilator prostaglandins (Franklin et al 1974, Speroff et al 1976). In vitro studies of human placentae have also demonstrated the release of PGE and the 6 keto $PGF_{1\alpha}$ metabolite of prostacyclin in response to infusion of angiotensin II into the fetal circulation (Glance et al 1985). These workers showed independent control of prostanoid release on the maternal and fetal sides of the

placenta and have subsequently shown (Glance et al 1986) that in vitro very little prostaglandin crosses the placenta. This suggests that prostanoids in the maternal and fetal circulations are kept separate by the presence of the trophoblast. The hypothesis of Symonds 1981 that uteroplacental prostanoid production may augment fetal perfusion during pregnancy is at variance with this theory. Further work is needed to ascertain if uteroplacental prostanoid production affects fetal blood flow. The production of vasodilator prostanoids in the decidua and on the maternal side of the placenta may however be important in the maintenance of uterine blood flow by modulation of the vasoconstrictor effect of angiotensin II (Symonds 1981).

The amnion also produces prostacyclin and the production increases in labour (Mitchell et al 1978(e)). Further evidence for the involvement of prostacyclin produced by the uteroplacental unit in parturition is found in the observations that amniotic fluid levels and maternal plasma and urine levels of 6 keto $PGF_{1\alpha}$ are increased after labour (Makarainen and Ylikorkala 1984, Ylikorkala et al 1981(b), Ylikorkala et al 1986(b)). Prostacyclin may play a role in maintenance of fetal blood flow and prevention of thrombosis during uterine contractions.

Although amniotic fluid thromboxane levels are higher in labour than before and are increased by amniotomy (Mitchell et al 1978(h)), placental production of thromboxane is not increased by labour (Mitchell et al 1978(c)).

2.4.4 Prostanoid Production by Umbilical Vessels

Karim (1967) first demonstrated the presence of prostaglandins in umbilical cords, identifying PGE, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ by biological assay. In 1973 Tuvemo and Wide demonstrated the active synthesis of a compound with prostaglandin immunoreactivity in umbilical arteries. Subsequent research indicated that the main arachidonic acid metabolite produced in vitro by the umbilical artery is prostacyclin (Hamburg et al 1979, Downing et al 1981). Mitchell et al (1980) using a superfusion technique coupled with RIA showed that both umbilical arteries and veins produced 6 keto $PGF_{1\alpha}$ at a high rate and also substantial amounts of PGE.

As PGE_2 has a vasoconstrictor effect on the umbilical cord (Tuvemo 1978) and as its umbilical artery production is increased after spontaneous compared to induced labour (Willman et al 1977), it has been suggested that closure of the cord after delivery may be mediated by this prostanoid.

The capacity of umbilical blood vessels to generate prostacyclin is much higher than blood vessels from normal adults (Remuzzi et al 1979). Umbilical arteries also produce substantially more prostacyclin in vitro than placental arteries (Kawano and Mori 1983). The production of prostacyclin by umbilical arteries does not seem to be related to gestational age (Stuart et al 1981) or to the mode of delivery (Stuart et al 1981, Sunderji and Stuart 1981). The production of prostacyclin by umbilical arteries in vitro has been reported to be greater than (Misiani et al 1981, Remuzzi et al 1979) less than (Kawano and Mori 1983) or the same (Makila et al 1982) as the production by umbilical veins.

It has been suggested that prostacyclin may be of particular importance in the regulation of fetoplacental blood flow. High levels of prostacyclin normally present in the fetus could play a role in the maintenance of the low peripheral resistance in the fetal circulation (Remuzzi et al 1979). The observation that production

of prostacyclin (Remuzzi et al 1979) is deficient in fetal blood vessels from pregnancies with impaired fetal growth, (Remuzzi et al 1980(a) Stuart et al 1981) support the theory that prostacyclin generated by the fetus may increase fetoplacental blood flow and that deficient production may lead to decreased umbilical blood flow and hence intrauterine growth retardation IUGR.

The release of thromboxane and PGF has been demonstrated from umbilical arteries but in very much smaller quantities than prostacyclin (Mitchell et al 1980, Makila et al 1982, Ritter et al 1982). Thromboxane and to a lesser extent $PGF_{2}\alpha$ constrict the umbilical artery in vitro (Tuvemo 1978).

The relationship between prostanoid production and fetal blood flow has been studied by two groups of workers. Ylikorkala et al (1983) showed no correlation of circulating maternal levels of the prostacyclin and thromboxane A_2 metabolites 6 keto $PGF_{1}\alpha$ and thromboxane B_2 to placental intervillous flow determined by the ^{133}Xe isotope method. They concluded that although blood flow is unrelated to circulating prostanoid levels, placental blood flow may nevertheless be controlled by local differences in prostanoid production. This was confirmed by reports that the umbilical artery generation of 6 keto $PGF_{1}\alpha$ but not thromboxane B_2 is related to the umbilical blood flow measured by Doppler ultrasound (Makila et al

1983(a)). Furthermore intervillous blood flow has been shown to be inversely related to the ratio of TxB_2 to 6 keto $PGF_{1\alpha}$ in placental tissue (Makila et al 1986).

Orchard et al (1983) have identified Meads acid (5,8,11 eicosatrienoic acid) in umbilical arteries. Meads acid is not normally found in adult blood vessels and is not a substrate for the cyclooxygenase enzyme. Its presence is usually regarded as an indicator of essential fatty acid deficiency. Ongari et al (1984) demonstrated an inverse correlation between the Meads-arachidonic acid ratio and prostacyclin production by the umbilical arteries and suggested that high levels of Meads acid would limit prostacyclin production by limiting the availability of the arachidonic acid substrate.

The presence of Meads acid in umbilical arteries therefore suggest that prostacyclin production may be decreased or prevented by a deficiency of the essential fatty acid precursors.

2.4.5 Fetal Prostanoids in Normal Pregnancy

There are several difficulties in extrapolating in vitro experiments to the situation in the fetus in vivo. Firstly, the measured tissue levels of prostanoids do not necessarily correlate with the measured production in vitro (Rakoczi et al 1983). Secondly, the capacity of an isolated tissue to generate prostanoids in vitro does not necessarily imply that it behaves the same in vivo, as the possible interactions with other local or circulating regulatory substances are excluded. In an attempt to elucidate the in vivo situation many workers have therefore turned to the measurement of prostaglandins in fetal blood. Attention to sampling technique and the treatment of samples is particularly important as fetal blood vessels have a high metabolic capacity for prostaglandin production.

The concentrations of PGE, PGF and PGFM in midtrimester umbilical blood specimens obtained by fetoscopy were compared to cord blood specimens at delivery by Mackenzie et al (1980). They demonstrated that PGF levels were higher in midpregnancy but that concentrations of PGE were similar at both stages of pregnancy. There is therefore evidence of a relative excess of PGE over PGF in fetal blood in late pregnancy.

Mackenzie et al (1980) also noted that 6 keto $PGF_{1\alpha}$ levels were consistently much higher in samples obtained at 16 - 19.5 weeks gestation than at term although the significance of this is uncertain (See below section 2.5.4).

The relative contributions of the fetus and the placenta to umbilical cord prostanoid levels are not known. No arteriovenous difference has been demonstrated in umbilical blood PGF and PGFM levels (Challis et al 1974(b), Bibby et al 1979, Mitchell et al 1978(g), Yamguchi and Mori 1984). Bibby et al (1979) and Mitchell et al (1978(g)) did find however venous PGE levels exceeded arterial and interpreted this as indicating a possible placental contribution to fetal PGE in late pregnancy. In animals injection of both PGE_2 and $PGF_{2\alpha}$ in utero into the umbilical circulation decrease umbilical placental blood flow (Berman et al 1978).

At term the fetal levels of the metabolites of thromboxane A_2 and prostacyclin are higher than maternal plasma levels, and do not show any arteriovenous difference. (Mitchell et al 1978(c), Ylikorkala et al 1981(b), Mitchell et al 1980). The ability of prostacyclin in vitro to relax umbilical arteries is greatest in a low oxygen environment (Tuvemo 1980) which suggests that prostacyclin may be important in maintaining umbilical flow in times of fetal stress.

Animal work has also suggested that prostaglandins probably play a role in local regulation of blood flows including umbilical flow in the resting fetus (Heymann and Rudolph 1976).

The effect of acute fetal distress on umbilical artery blood prostanoid levels was assessed in a recently published study by Ogino et al (1986(b)). They reported elevated levels of PGE_2, $PGF_{2\alpha}$, 6 keto $PGF_{1\alpha}$ and TxB_2 compared to normal controls, which may indicate an adaptive response to hypoxia or to forceps delivery. The most marked increases were seen in 6 keto $PGF_{1\alpha}$ and PGE_2 and although the authors considered this to be a vasodilator response to hypoxia, the evidence for the role of PGE_2 as an umbilical vasoconstrictor, at least in vitro (Berman et al 1978) suggests that these results are not entirely consistent with that theory.

The concentrations of PGE and F have been reported to be higher in fetal than maternal blood (Craft et al 1973, Challis et al 1974(b), Johnson et al 1975, Haning et al 1977, Mitchell et al 1978(f), Norman et al 1981) although one study (Yamguchi and Mori 1984) reported maternal PGFM levels to be higher than fetal. Higher levels of PGE, PGF and PGFM in umbilical venous blood were found after spontaneous vaginal delivery compared to samples taken after elective caesarean section (Bibby et al 1979). In this study and two others (Mitchell et al 1978(g), Norman et al

1981) the umbilical plasma levels of PGFM exceeded the levels of PGE which were in turn greater than the concentrations of PGF. The increase in umbilical prostaglandins after vaginal delivery may imply a role for prostaglandins in initiation and maintenance labour or a physiological response of the fetus to the stress of labour. Fetal umbilical blood levels of prostacyclin and thromboxane metabolites are unaffected by the mode of delivery (Mitchell 1978 et al(c), Ylikorkala et al 1981(b), Mitchell et al 1980).

Circulating levels of PGE at birth are very high dropping over the first few days of life (Mitchell et al 1978(f), Siegler et al 1977). It has been suggested that the tone of the ductus arteriosus is controlled by circulating PGE levels and that high levels in late pregnancy maintain the patency of the ductus with a subsequent fall in relation to closure of the ductus after birth (Coceani et al 1976). Research into the involvements of prostaglandins in patent ductus arteriosus has however indicated that other factors may be involved (Reviewed by Mitchell 1981(a)).

2.4.6 Amniotic Fluid Prostanoids in Normal Pregnancy

Determination of prostanoids in tissues and blood is difficult for the reasons already mentioned, and hence measurement in the amniotic fluid, which has little metabolic activity (Keirse 1978), is an attractive

alternative in the study of the fetoplacental production in vivo.

2.4.6.1 Effect of Gestational Age

The presence of prostaglandins in amniotic fluid was first demonstrated by Karim (1966) by a bioassay method. Most workers are in agreement that levels of the primary prostaglandins are higher in amniotic fluid in late pregnancy than midtrimester (Keirse and Turnbull 1973, Salmon and Amy 1973, Keirse et al 1974, Mitchell et al 1976, Dray and Frydman 1976, Norman et al 1981). Two longitudinal studies of prostaglandin F levels in amniotic fluid throughout pregnancy (Salmon and Amy 1973, Hibbard et al 1974) demonstrated stable but low levels until 36 weeks followed by a gradual increase to term. The other workers mentioned were unable to demonstrate this pattern of increase of PGF in samples taken near to term. Dray and Frydman (1976) demonstrated an increase in PGE levels at 35 - 36 weeks then remaining stable until term, but an increase of PGF prior to labour. Mitchell et al (1976) noted however that samples taken by amniotomy for induction of labour contained more PGF than samples obtained by abdominal amniocentesis. If all the samples were considered together a significant increase in PGF with gestational age was apparent but if the samples obtained by amniotomy and amniocentesis were taken separately no such association was seen. These workers (Mitchell et al 1976) also demonstrated a gradual increase of PGFM in amniotic fluid specimens

obtained between 34 and 42 weeks gestation. The increase with gestational age may therefore represent a stage of preparation for labour or it may be an artefact caused by local release of PGF during amniotomy. Prior to labour amniotic fluid PGE levels are higher than PGF (Norman et al 1981).

A progressive increase in amniotic fluid of prostacyclin like activity with gestational age was demonstrated by Wilcox et al (1983) using a bioassay based on the antiaggregatory activity of prostacyclin. Ylikorkala et al (1981(c)) described a similar relation of 6 keto $PGF_{1\alpha}$ in amniotic fluid to gestational age in normal pregnancy between 30 and 40 weeks gestation but Mitchell et al (1979) were unable to find any correlation with gestational age in patients studied after 35 weeks of pregnancy.

Thromboxane B_2 has also been measured in amniotic fluid in normal pregnancy by radioimmunoassay. Ylikorkala et al (1981(c)) noted a rise in TxB_2 with gestational age but 6 keto $PGF_{1\alpha}$ levels were five times higher than TxB_2. Mitchell et al (1978(h)) reported a correlation with gestational age in samples obtained by amniotomy before labour but not in the samples obtained by amniocentesis.

2.4.6.2 Effect of Labour

There is good agreement amongst workers in this field that amniotic fluid levels of PGF increase rapidly during labour

and this increase correlates well with the stage of labour. (Table 2.VI)

TABLE 2.VI : LEVELS OF PGE AND PGF IN AMNIOTIC FLUID BY RADIOIMMUNOASSAY

ASSAY	EFFECT OF GESTATIONAL AGE	LEVELS IN LABOUR	PG RELATED TO STAGE OF LABOUR	FIRST AUTHOR
PGE_1, PGE_2 $PGF_{2\alpha}$	Stable to 36/40. PGE at 36/40. PGF at term.	Increased	-	Dray 1976
PGE_2	-	Increased	Yes	Keirse 1973
$PGF_{2\alpha}$	Stable to 36/40. PGF from 36/40	Increased	Yes	Salmon 1973
PGF	Increase	Increased	Yes	Keirse 1974
PGF	-	Increased	Yes	Hillier 1974
$PGF_{2\alpha}$	Low levels before 36/40 then increased	Increased	-	Hibbard 1974
$PGF_{2\alpha}$	-	Increased	-	Johnson 1975
PGF PGFM	Increasing (See text)	-	-	Mitchell 1976
PGE_1 $PGF_{2\alpha}$	-	Same Increased	-	Kinoshita 1977
PGF	-	Increased	Yes	Tambyraja 1977
PGE PGF PGFM	-	All Increased	Yes	Reddi 1984

Two workers (Hillier et al 1974, Keirse et al 1974) noted that PGF levels were lower in patients in induced labour than spontaneous labour at the same stage of labour. Reddi et al (1984) demonstrated that amniotic fluid PGE, PGF and PGFM levels increased in normal labour with close correlation to the cervical dilatation but that patients with delayed labour (in the absence of cephalopelvic disproportion) failed to show the expected increase of PGF and PGFM. These observations add strength to the theory that PGF is important in the initiation and maintenance of labour.

Prostacyclin activity is increased in amniotic fluid during labour (Mitchell et al 1979, Makarainen and Ylikorkala 1984, Wilcox et al 1983) but the correlation to the stage of labour is less apparent than for the primary prostaglandins. Wilcox et al (1983) and Makarainen and Ylikorkala 1984) described a definite correlation of prostacyclin activity with cervical dilatation, but no such increase was noted by Reddi et al (1984). Prostacyclin levels in amniotic fluid are not increased by amniotomy (Mitchell et al 1979).

Levels of TxB_2 in amniotic fluid taken during labour have been reported as higher than before labour (Mitchell et al 1978(h)), Makarainen and Ylikorkala 1984) but whereas the latter study demonstrated a relationship to cervical dilatation, no such relationship could be demonstrated in

the former. Reddi et al (1984) were unable to demonstrate any elevation of TxB_2 in labour.

2.4.6.3 Origin of Amniotic Fluid Prostanoids

The origin of the prostanoids in amniotic fluid is unknown but fetal membranes, decidua and myometrium have all been suggested as potential sources (Keirse 1979). Amniotic fluid itself has little activity in synthesis or degradation of prostanoids (Keirse and Turnbull 1976). The fetal membranes have been shown to synthesise large amounts of prostanoids in vitro (Keirse and Turnbull 1976) and procedures such as 'sweeping' the membranes and amniotomy increase prostanoid production (Mitchell et al 1977, Mitchell et al 1976). Early workers (Keirse and Turnbull 1976) were able to demonstrate enzymatic activity in the chorion but not the amnion and suggested that the chorion may be a major contributor of amniotic fluid prostaglandins. Subsequently however, it has been shown by in vitro experiments by several workers that the amnion is a major source of prostanoid production and may be of particular importance in parturition (Nicolaides et al 1983, Skinner et al 1985, Olson et al 1983, Okazaki et al 1981). The uterine decidua, placenta and umbilical cord also produce prostaglandins in large amounts (Tuvemo et al 1976, Nicolaides et al 1983)

It is also possible that the fetus may contribute to amniotic fluid prostanoids via the urine. Casey et al (1983) found that the PGE_2 and $PGF_{2\alpha}$ content of the first voided urine of neonates related to the mode of delivery and mirrored the changes seen in amniotic fluid in normal labour, induced labour and elective caesarean section. Thus, amniotic fluid prostanoid levels may also reflect the synthesis by the fetal kidney.

2.4.6.7 Summary of Prostanoid Activities in Normal Pregnancy

Normal pregnancy is characterised therefore by stimulation of prostanoid synthesis and release but the vasodilator prostaglandins seem to predominate over the vasoconstrictor and hence could account for the generalised vasodilation seen in normal pregnancy. A summary of the prostanoid concentrations in normal pregnancy is shown in Table 2.VII.

The elevated maternal levels of vasodilators especially prostacyclin in normal pregnancy are thought to facilitate the increased cardiac output, decreased vascular resistance and increased renal and uterine blood flow.

TABLE 2.VII : SUMMARY OF PROSTAGLANDIN AND THROMBOXANE CONCENTRATIONS IN NORMAL PREGNANCY

SOURCE	PROSTANOIDS	
MATERNAL PLASMA	PGE	Increased. Effect of gestational age. Controversial.
	PGF	Increased. Then stable to term. Marked increase in labour.
	6 keto $PGF_{1\alpha}$	Increased. Possible decrease in late pregnancy.
	TxB_2	Increased. Correlated to gestational age.
MATERNAL URINE	PGE	Increased. Effect of gestational age. Controversial.
	PGF	Increased in first trimester. Increasing with gestational age.
	6 keto $PGF_{1\alpha}$	Increased in first trimester. Further increase with gestational age.
	TxB_2	No change.

PLACENTA AND MEMBRANES	PGE -	Main product of amnion. Increases with gestational age.
	PGF -	Produced by all tissues. Increases with gestational age.
	6 keto $PGF_{1\alpha}$ -	Produced in small amounts. Increases to term.
	TxB_2 -	Major product of placenta. 5 x greater than 6 keto $PGF_{1\alpha}$
UMBILICAL VESSELS	Mainly produce prostacyclin with smaller amounts of PGE and very little $PGF_{2\alpha}$ and thromboxane.	
FETAL BLOOD	PGE > Maternal.	Increasing to term.
	PGF > Maternal.	Peak second trimester then declining. Higher levels after labour compared to caesarean section.
	6 keto $PGF_{1\alpha}$ > Maternal.	Highest in second trimester then declining.
	TxB_2 > Maternal.	Unaffected by mode of delivery.
AMNIOTIC FLUID	PGE) Higher at term than midpregnancy. PGF)	
	6 keto $PGF_{1\alpha}$	Increasing over 3rd trimester.
	TxB_2	Increasing over 3rd trimester. 6 keto $PGF_{1\alpha}$ 5 x higher than TxB_2.

Alterations in platelet aggregability occur in pregnancy. The balance of proaggregatory thromboxane produced by platelets and antiaggregatory prostacyclin produced by the vascular endothelium may be important. The small increase in thromboxane may account for the increased platelet aggregability of normal pregnancy with the recognised propensity for venous thrombosis. The high levels of prostacyclin generally outweigh the effects of thromboxane and may be an adaptive response to a primary increase in platelet aggregability (Lewis et al 1980).

The decreased sensitivity of pregnant patients to angiotensin II may be mediated by high prostacyclin or PGE levels. It is likely that the prostaglandins interact with the angiotensin system in control of placental perfusion.

The human chorion has been shown to produce renin (Symonds et al 1968) and although a large percentage of this is inactive (Symonds 1983) it seems likely that the interaction of vasodilator prostaglandins and the renin angiotensin system may be important in the regulation of uterine blood flow at local levels.

The umbilical cord prostacyclin levels are high and this may be important in the maintenance of the fetal circulation. Umbilical cord PGE may be important in constriction of the cord at birth.

The significance of high fetal blood levels of prostacyclin in the second trimester is not known. Studies of normal placentation (Brosens et al 1967) have indicated that a secondary wave of trophoblast invasion of the spiral arterioles occurs at 16 weeks gestation. It is therefore possible that the vasodilator and antiaggregatory effects of prostacyclin at this time may be important in this process.

Vascular tone and platelet aggregation in pregnancy depend upon a delicate balance of the prostaglandins and thromboxane. A disturbance of this balance could therefore result in alterations in blood pressure, organ perfusion and the coagulation system. Raised blood pressure, decreased renal and uterine blood flows, coagulation abnormalities and increased sensitivity to angiotensin II in pre-eclampsia could be explained by a deficiency of the vasodilator antiaggregatory prostaglandins, an excess of the vasoconstrictor and proaggregatory thromboxane, or alterations in the ratio of one to the other.

2.5 PROSTANOIDS IN PREGNANCY COMPLICATED BY GESTATIONAL HYPERTENSION

2.5.1 Introduction

In the previous section the importance of high levels of vasodilator prostaglandins in pregnancy was discussed. Many workers have studied the role of prostanoids in pre-eclampsia in an attempt to elucidate its aetiology and in the hope of preventing its onset.

The term pre-eclampsia has been used in the past by many authors to encompass both the proteinuric and non proteinuric forms of gestational hypertension which may be different diseases or possibly different stages of progression of the same disease. It has already been noted (Part 2.2) that the pathological changes of pre-eclampsia are not uniform and that whereas some patients have severe hypertension with a contracted plasma volume, coagulation abnormalities and intrauterine growth retardation, many more have relatively mild disease with no evidence of decreased tissue perfusion or fetal compromise. In the context of the following discussion the term pre-eclampsia will be used as by the initial authors but the lack of differentiation in these studies between these different forms of the disease makes interpretation difficult.

By the mid 1970s several workers had provided evidence that prostaglandins may influence vascular responsiveness to vasoactive agents. It was already known that the renin angiotensin system is activated in normal pregnancy but with decreased sensitivity to its effects and that patients with pre-eclampsia demonstrate increased sensitivity to infused angiotensin II (See 2.2.1.4). It was demonstrated that the E series of prostaglandins are potent mediators of vascular reactivity in the kidney under a variety of conditions (McGiff and Itskovitz 1973, Lonigro et al 1973). Modulation of the vasoconstrictor activity of angiotensin by PGE was reported by McGiff and Itskovitz (1973). It was noted that uterine blood flow was positively correlated to uterine venous PgE levels in pregnant animals (Terragno et al 1974, Venuto et al 1975) and that infusion of angiotensin II increased these levels further (Terragno et al 1974, Franklin et al 1974).

This early work provided the first evidence that there are differences in prostaglandin production and/or catabolism in uteroplacental tissues in gestational hypertension and that prostaglandins may, by interactions with other vasoactive substances, be important modulators of blood flow.

2.5.2 PGE and PGF in Gestational Hypertension

Several early workers demonstrated evidence of decreased PGE activity in placentae from patients with pre-eclampsia compared to normal pregnancy (Ryan et al 1969, Alam et al 1973, Demers and Gabbe 1976). Demers and Gabbe (1976) also noted elevation of the vasoconstrictor PGF in placentae from pre-eclamptic patients, although the changes did not correlate with the severity of the disease. As PGE levels increase with gestational age some of the deficiency noted may however be accounted for by earlier delivery in patients with pre-eclampsia.

Robinson et al (1979) using RIA demonstrated differences in PGE, PGF and PGFM concentration in placental tissues and decidua from patients with pre-eclampsia. The concentrations of PGE in amnion, chorion, placenta and decidua were lower in patients with pre-eclampsia than normal pregnancy. PGF and PGFM concentrations were also lower in some of the tissues. The authors concluded that the effects may have been due to gestational age differences but the numbers were too small for valid statistical analysis. A later study of placental prostaglandins failed to show significant differences in tissue levels of PGE_2 and $PGF_{2\alpha}$ in placentae from normal and hypertensive patients (Hillier and Smith 1981).

Maternal urinary PGE levels have been reported to be reduced in pre-eclampsia compared to normal pregnancy but $PGF_{2\alpha}$ levels in urine are unchanged (Rathaus et al 1982, Pedersen et al 1983, Moutquin and Leblanc 1982).

Maternal venous concentrations of PGF do not differ between normal pregnancy and pregnancies complicated by gestational hypertension (Yamguchi and Mori 1985, Valenzuela et al 1983, Moodley et al 1984(b)). However Valenzuela et al (1983) reported elevated plasma PGF levels in radial arterial blood samples from patients with gestational hypertension. Peripheral venous blood levels of PGE also are unaltered in pregnancy complicated by gestational hypertension (Yamguchi and Mori 1985, Valenzuela et al 1983) but there has been one report of depressed central venous PGE levels in these patients (Moodley et al 1984(b)).

A single study of primary prostaglandins in amniotic fluid (Salmon and Amy 1973) was unable to demonstrate that $PGF_{2\alpha}$ levels differed significantly between pre-eclampsia and normal pregnancies.

The discrepancies between the different studies suggest that the primary prostaglandins are not the main aetiological factor in pre-eclampsia. The role of prostacyclin and thromboxane has been more extensively investigated and produced more consistent results.

2.5.3 Prostacyclin and Thromboxane in Gestational Hypertension

A deficiency of prostacyclin in pre-eclamptic pregnancies has been demonstrated by several workers. Umbilical arteries from patients with pre-eclampsia have been shown to have lower tissue levels and synthesise less prostacyclin in vitro than umbilical arteries from normal pregnancy (Remuzzi et al 1980(a) and (b), Downing et al 1980, Carreras et al 1981(a), Makila et al 1984(a), Dadak et al 1982). Bussolino et al (1980) made similar findings in a study of the ability of placental veins and maternal blood vessels to generate antiaggregatory activity which was attributed to prostacyclin. Umbilical artery serum was also reported to demonstrate prostacyclin deficiency in pre-eclampsia (Takagi and Den 1985). Kinetic analysis showed that there was less prostacyclin synthetase in umbilical arteries from pre-eclamptic pregnancies than normal (Downing et al 1982) which may explain the prostacyclin deficiency.

Amniotic fluid prostacyclin activity has also be found to be suppressed in pre-eclampsia (Bodzenta et al 1980, Ylikorkala et al 1981(c), Makarainen and Ylikorkala 1984) although Moodley et al (1984(a)) and Brown et al (1987) could demonstrate no difference from normal pregnancy.

Stuart et al (1981) demonstrated that the ability of the umbilical cord to generate prostacyclin was reduced not only

in pre-eclampsia but also in other conditions complicated by growth retardation. Thus umbilical prostacyclin deficiency may be an important factor in placental insufficiency. Makila et al (1984(a)) were unable however to confirm these findings and consider that prostacyclin deficiency is a specific feature of pre-eclampsia.

Variable levels of 6 keto $PGF_{1\alpha}$ in maternal plasma have been reported in pre-eclampsia. There have been several reports of decreased circulating 6 keto $PGF_{1\alpha}$ levels in severe disease (Lewis et al 1981, Greer et al 1983, Moodley et al 1984(b) Yamguchi and Mori 1985). However Ylikorkala et al (1981(a)) could not demonstrate any differences in 6 keto $PGF_{1\alpha}$ levels between normal pregnancy and a number of pregnancy complications including pre-eclampsia and two recent studies have confirmed this (Ogino et al 1986(b), Brown et al 1987). Koullapis et al (1982), in the largest study, even found elevated maternal plasma 6 keto $PGF_{1\alpha}$ levels in pre-eclamptic patients compared to normal. Hence the role of circulating prostacyclin in the pathogenesis of gestational hypertension is still in doubt but it seems likely that circulating prostacyclin metabolites are in the normal pregnant range in the majority of pre-eclamptics but may be reduced in severe disease.

Urinary 6 keto $PG_{1\alpha}$ metabolite levels were depressed in pre-eclampsia compared to normal pregnancy in most of the reported studies (Goodman et al 1982, Ylikorkala et al 1986(a), Fitzgerald et al 1987) and may precede the development of pre-eclampsia (Fitzgerald et al 1987).

The findings of investigations of prostacyclin in pre-eclampsia are summarised in Table 2.VIII.

TABLE 2.VIII : PROSTACYCLIN PRODUCTION IN PRE-ECLAMPSIA

SAMPLE	PRE-ECLAMPTIC PATIENTS (N)	CONTROL PATIENTS (N)	ASSAY	COMPARISON OF PRE-ECLAMPSIA TO NORMAL	FIRST AUTHO
UMBILICAL ARTERY PLACENTAL VEINS	5	9	Platelet Aggregation Inhibition	Reduced	Remuzzi 1980(a) and
UMBILICAL ARTERY	13	11	Bioassay	Reduced	Downing 198(
AMNIOTIC FLUID	5	15	Bioassay	Reduced	Bodzenta 1980
PLACENTAL VEINS	6	9	Bioassay	Reduced	Bussolino 1980
MATERNAL VESSELS	6	9	Bioassay	Reduced	
PLACENTAL VEINS	5	5	Bioassay	Same	Carreras 1981 (a)
UMBILICAL ARTERY	5	5	Bioassay	Reduced	
MATERNAL PLASMA	1	0	RIA of 6 keto $PGF_{1\alpha}$	Reduced	Lewis 1981

AMNIOTIC FLUID	27	27	RIA of 6 keto $PGF_{1\alpha}$	Reduced	Ylikorkala 1981(c)
UMBILICAL ARTERY	5	32	In vitro generation of 6 keto $PGF_{1\alpha}$	Reduced	Stuart 1981
MATERNAL PLASMA	22	22	RIA of 6 keto $PGF_{1\alpha}$	Same	Ylikorkala 1981 (a)
MATERNAL URINE	6	8	RIA dinor 6 keto $PGF_{1\alpha}$	Reduced	Goodman 1982
MATERNAL PLASMA	26	141	RIA of 6 keto $PGF_{1\alpha}$	Increased (but decreased 6ketoPGF$_{1\alpha}$: TxB_2 ratio)	Koullapis 1982
UMBILICAL ARTERY	10	8	Bioassay	Reduced	Dadak 1982
UMBILICAL ARTERY	12	11	Kinetic analysis of prostacyclin synthetase	Reduced	Downing 1982
UMBILICAL ARTERY	9	46	Superfusion coupled with RIA of 6 keto $PGF_{1\alpha}$	Reduced	Makila 1984(a)
PLACENTA	9	11		Same	Makila 1984(b)
CENTRAL VENOUS BLOOD	21	16	RIA 6 keto $PGF_{1\alpha}$	Reduced in Eclampsia	Moodley 1984(b)
AMNIOTIC FLUID	15	28	RIA 6 keto $PGF_{1\alpha}$	Reduced	Makarainen 1984
AMNIOTIC FLUID	19	44	RIA 6 keto $PGF_{1\alpha}$	Same	Moodley 1984(a)
MATERNAL PLASMA	15	23	RIA 6 keto $PGF_{1\alpha}$	Reduced	Yamguchi 1985

UMBILICAL ARTERIAL BLOOD	Not stated		RIA 6 keto $PGF_{1\alpha}$	Reduced	Tagaki 1985
PLACENTA	10 12	11 12	Bioassay and 6 keto $PGF_{1\alpha}$	Reduced	Walsh 1985 (a) and
MATERNAL PLASMA	14	20	RIA 6 keto $PGF_{1\alpha}$	Same	Ogino 1986 (c)
URINE	17	16	RIA 6 keto $PGF_{1\alpha}$	Reduced	Ylikorkala 1986 (a)
URINE	23	16	RIA 6 keto $PGF_{1\alpha}$	Same	Fievet 1986
MATERNAL PLASMA			RIA 6 keto $PGF_{1\alpha}$	Same	Brown 1987
AMNIOTIC FLUID	12	24		Same	
URINE			RIA 2-3 dinor 6 keto $PGF_{1\alpha}$	Reduced	Fitzgerald 1987

In addition to prostacyclin deficiency thromboxane excess could tip the balance towards platelet aggregation, a feature of pre-eclampsia.

There have been fewer investigations of thromboxane activity in pregnancies complicated by pre-eclampsia but most of them find that thromboxane levels are comparable to normal pregnancy. This would suggest that prostacyclin deficiency is the major feature of pre-eclampsia which in the presence of normal thromboxane levels leads to relative overactivity

of the vasoconstrictor, proaggregatory thromboxane.

Most studies have found that maternal plasma thromboxane levels in pre-eclamptic patients are not significantly different to normal (Mitchell et al 1978(c), Yamguchi and Mori 1985). Koullapis et al (1982) however, demonstrated an elevation of both 6 keto $PGF_{1\alpha}$ and thromboxane B_2 in maternal plasma from patients with pregnancy induced hypertension but noted that thromboxane levels were more elevated resulting in a drop in the 6 keto $PGF_{1\alpha}$ to thromboxane B_2 ratio. Thus the vasoconstrictor element was increased. In another study maternal plasma thromboxane levels were noted to be elevated only if there was evidence of platelet consumption (Greer et al 1983). This suggests that the abnormalities may be demonstrable only in severe pre-eclampsia. The inclusion of large numbers of mild pre-eclamptics with no demonstrable impairment of the organ blood flow or coagulation system may obscure changes associated with severe disease.

An increase in the reactivity of the platelet thromboxane pathway in maternal plasma in pregnancies complicated by fetal growth retardation has been described (Wallenburg and Rotmans 1982(a)). Platelet reactivity in hypertensive pregnant patients with normal fetal growth was found to be slightly elevated but not significantly different from normal on statistical analysis.

Maternal urinary TxB_2 excretion was found to be unaltered in pre-eclampsia by Ylikorkala et al (1986(a)) whereas Fievet et al (1986) described elevation of urinary TxB_2 levels with normal 6 keto $PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ excretion in labile pregnancy induced hypertension. As Ylikorkala et al 1986(a) described supression of 6 keto PGF_1 excretion in pre-eclampsia these studies both demonstrate a relative vasodilator deficiency in some pre-eclamptic patients.

Intrauterine thromboxane levels in hypertensive pregnancy have been measured. Ylikorkala et al (1981(c)) and Moodley et al (1984(a)) comparing amniotic fluid TxB_2 between normal pregnancy and pregnancy with pre-eclampsia were unable to detect any differences. Early studies were unable to demonstrate any differences in thromboxane levels in placenta and membranes in pre-eclampsia compared to normal pregnancy (Robinson et al 1979). Makila et al (1984(b)) however in a superfusion experiment combined with RIA, demonstrated that thromboxane production was increased on the fetal side of the placenta in pregnancies complicated by pre-eclampsia and chronic hypertension. 6 keto $PGF_{1\alpha}$ production was normal in the latter study and neither the thromboxane or 6 keto $PGF_{1\alpha}$ levels were related to placental weight. Increased placental production of thromboxane in pre-eclampsia was also found by Walsh et al (1985(a)) but they found prostacyclin production to be decreased (Walsh et al 1985(b)).

The regulation of the prostacyclin and thromboxane pathways are unclear. Endogenous inhibitors and stimulators of prostanoid synthesis have been described and are present in maternal plasma, placental tissue and amniotic fluid (Brennecke et al 1984, Remuzzi et al 1981, Saeed and Mitchell 1983, Rehnstrom et al 1983, Mitchell et al 1985). Whilst abnormalities of these controlling systems are an attractive hypothesis for the defect underlying the prostanoid imbalance in pre-eclampsia the available data is not consistent. "Lupus" anticoagulant, an inhibitor of prostacyclin synthesis, is associated with an increase in intrauterine growth retardation and intrauterine death (Carreras et al 1981(b), 1981(c)) but other endogenous inhibitors of prostaglandin synthesis do not seem to play a role in the regulation of prostaglandins in pregnancy (Brennecke et al 1984). 12 HPETE (12 hydroperoxy-eicosatetranoic acid) is a potent inhibitor of prostacyclin synthesis and its product 12 HETE has been demonstrated in placental tissue (Saeed and Mitchell 1983) but whether it plays a role in prostacyclin suppression in pre-eclampsia is not known. Measurements of plasma stimulators of prostacyclin synthesis have however been shown to be elevated in pre-eclampsia (Remuzzi et al 1981) which makes it unlikely that these endogenous regulators of prostanoid synthesis are major factors in the pathogenesis of pre-eclampsia.

2.5.4 Evidence for Prostanoid Imbalance in Pre-Eclampsia

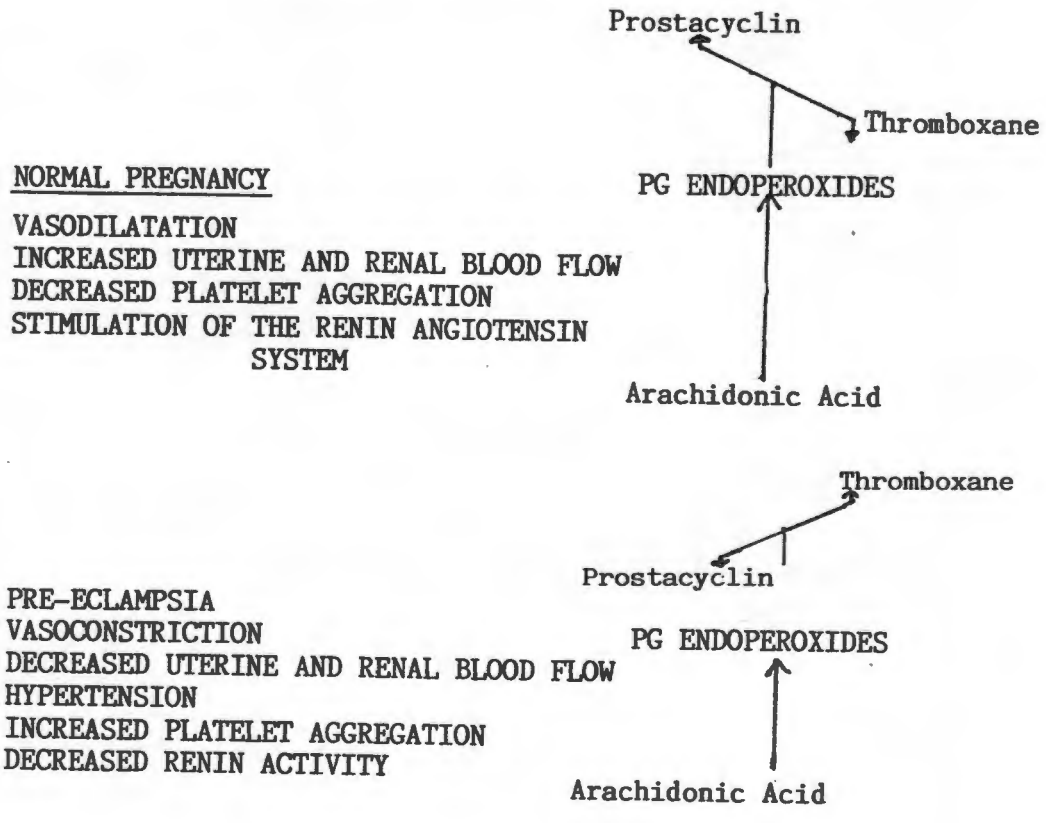
Normal pregnancy is characterised by stimulation of the prostaglandins and increased prostacyclin activity in normal pregnancy may account for the generalised vasodilatation and elevated plasma renin activity. (Editorial, Lancet 1982). Thromboxane modulates the antiaggregatory activity of prostacyclin by attenuating its stimulatory activity on platelet adenyl cyclase (Whittle and Moncada 1983). Thromboxane and prostacyclin probably also interact in regulation of renin release (Patrono et al 1982).

In normal pregnancy a second wave of intravascular cytotrophoblastic invasion down the spiral arteries occurs at about 16 weeks gestation with consequent dilatation of the spiral arteries as the muscular coat is replaced by fibrous tissue. High prostacyclin levels at this time may be mediators of this activity (Mackenzie et al 1980). Hence the observation that this transformation is incomplete in patients with pre-eclampsia (Brosens et al 1970) strengthens the argument for prostacyclin deficiency as the primary abnormality.

Most of the evidence for prostacyclin deficiency as a cause of pre-eclampsia comes from fetal tissues and it is difficult to see how changes in fetal prostacyclin thromboxane balance could explain the maternal changes particularly in view of recent evidence that prostaglandins

cross the placenta only in small amounts (Glance 1986). It is possible that a reduced uteroplacental blood flow is the primary abnormality leading to a secondary rise in blood pressure to maintain perfusion. A hypothesis for the possible mechanism of prostanoid abnormalities in the generation of the maternal changes in pre-eclampsia is illustrated in Figure 2.2. The role of the other prostanoids in this vasoconstrictor/ vasodilator balance is uncertain as few investigators have measured the activity of all the major prostanoids in the same subject.

FIGURE 2.2 : THE PROPOSED HYPOTHESIS FOR THE BALANCE OF BIOLOGICAL ACTIVITY OF PROSTACYCLIN AND THROMBOXANE IN NORMAL PREGNANCY AND IN PRE-ECLAMPSIA



The evidence for prostacyclin deficiency as a major factor in pre-eclampsia has led to attempts to treat it with prostacyclin infusion (Fidler et al 1980, Lewis et al 1981) or thromboxane synthetase inhibitors (Van Assche et al 1984). Prostacyclin infused intravenously reverses some of the features of pre-eclampsia by reducing blood pressure, particularly the diastolic, secondary to its vasodilator effect and stimulating renin release (Patrono et al 1982, Ylikorkala and Viinikka 1981(d)). Prostacyclin infusion was associated with fetal distress in one of the cases described (Fidler et al 1980) and intrauterine fetal death in another (Lewis et al 1981) and whilst this may have been because the cases were treated too late, it is also possible that reduced placental perfusion pressure in an already compromised fetus was a contributory factor. In the four cases treated by Van Assche et al (1984 and personal communication) with a thromboxane synthetase inhibitor, there were two intrauterine deaths. So any attempt to favourably influence the prostacyclin/thromboxane ratio should be made at any early stage of pregnancy. It has been suggested that small doses of aspirin may reduce the incidence of pre-eclampsia. (Goodlin et al 1978, Crandon and Isherwood 1979, Hoogendijk and Tencate 1980). Two recent studies have confirmed this (Beaufils et al 1985, Wallenburg et al 1986) and it is possible that this effect may be by favourable alteration of the prostacyclin-thromboxane balance. The role of aspirin and dipyridamole in prevention of pre-eclampsia is considered in detail in Part 7.

2.6 PROSTANOIDS IN CHRONIC HYPERTENSION IN PREGNANCY

A large proportion of the patients managed in the Hypertension Clinic at Groote Schuur Hospital have underlying chronic essential hypertension and these patients are known to have an increased tendency for later development of pre-eclampsia (Walters 1966, MacGillivray 1961, Gallery et al 1977). Very little work has been done on the role of prostanoids in pregnancy complicated by essential hypertension.

Non pregnant chronic hypertensives have been found to have lower levels of urinary PGE than normotensives and there is evidence of a correlation between urinary PGE levels and plasma renin activity in these patients (Tan et al 1978, Zawada 1982). In pregnancy urinary PGE excretion has been found to be increased (Moutquin and Leblanc 1982), but this increase was less in women with chronic hypertension. Although in another study (Kovatz et al 1981) the urinary PGE_2 was found to be increased in pregnant patients with essential hypertension, the $PGF_{2\alpha}$ excretion had a greater increase and a reduction of the PGE_2 to $PGF_{2\alpha}$ ratio compared to normal pregnancy was demonstrated. The increase in prostaglandin excretion may represent the early response to hypertension to try to prevent glomerular damage (Rathaus et al 1982, Zawada 1982) but it would appear that a relative

deficiency of renal PGE_2 is a feature of chronic hypertension in pregnancy.

Prostaglandins E and F were unaltered in placentae from chronic hypertensive patients (Hillier and Smith 1981) but Makila et al 1984(b) described increased thromboxane production with normal prostacyclin on the fetal side of the placenta in vitro. Umbilical arteries from pregnancies associated with chronic placental insufficiency including chronic hypertension have been shown to have defective prostacyclin production (Stuart et al 1981) although Makila et al (1984(a)) could not confirm this.

Maternal levels of prostacyclin in chronic hypertension have not been extensively investigated.

Investigations of prostacyclin and thromboxane in non-pregnant chronic hypertensives have shown as many discrepant results as investigations of their roles in pre-eclampsia. In atherosclerosis, a disease frequently associated with chronic hypertension, there is evidence of an arterial deficiency of prostacyclin and attempts have been made to treat atherosclerosis with prostacyclin infusion (Fitzgerald et al 1979, Gryglewski and Szczeklik 1981). Circulating prostacyclin metabolites in non-pregnant chronic hypertensives were however found to be slightly but not significantly higher than normal controls (Friedman et al 1981) and a role for prostacyclin in chronic hypertension is

unproven (Dollery 1981).

Plasma levels of prostacyclin and thromboxane metabolites have not been reported in pregnant chronic hypertensives but a recent study of urinary prostacyclin metabolites demonstrated that chronic hypertensive pregnant patients had comparable excretion to normal pregnancy in contrast to reduced excretion in pre-eclampsia (Fitzgerald et al 1987).

In a prospective study of pregnant chronic hypertensives, Redman et al (1978) using rising plasma urate levels as an index of pre-eclampsia described a drop in platelet count in those women who later developed pre-eclampsia. Raised serum urate levels may not be diagnostic of pre-eclampsia (Davey 1985) as some patients with chronic hypertension already have raised serum urate levels (Messerli et al 1980). Nevertheless rising uric acid levels suggests impairment of renal function, which coupled with activation of the platelet coagulation system and pathological changes characteristic of pre-eclampsia suggest that pre-eclampsia superimposed on chronic hypertension is the same condition as pre-eclampsia arising de novo. What is less clear, however, is the reason for the increased propensity of chronic hypertensives to develop pre-eclampsia and the existing studies of prostanoids in pregnant patients with chronic hypertension have not addressed this point.

2.7 PROSTANOIDS IN PREGNANCIES COMPLICATED BY INTRAUTERINE GROWTH RETARDATION

Prostanoids particularly prostacyclin and thromboxane may be important regulators of fetoplacental blood flow. (Rankin 1976, Myatt and Elder 1977, Tuvemo 1980). The primary prostaglandins $PGF_{2\alpha}$ and PGE_2 contract the human umbilical artery in vitro whereas PGE_1 relaxes it at low concentrations and contracts it at high concentrations (Tuvemo 1978). Prostacyclin is the dominant prostanoid produced by the umbilical cord and thromboxane by the placenta (see 2.4.4 and 2.4.3) in vitro. It has been postulated that in vivo the vasodilator activity of prostacyclin is important in maintaining fetal blood flow and infusion of prostacyclin has been shown to increase the uterine blood flow in pregnant animals (Clark et al 1982).

Uteroplacental blood may decrease in pregnancies complicated by intrauterine growth retardation (IUGR) (Nylund et al 1983) and the available evidence suggest that this may be mediated by vasoactive prostanoids (Makila et al 1986).

The first proof that prostanoids may be involved in IUGR came from the work of Stuart et al (1981) who demonstrated diminished prostacyclin production in umbilical arteries from pregnancies with chronic placental insufficiency. In this study placental insufficiency was not defined and prostacyclin depression occurred equally in pregnancies with growth retarded infants and in chronic hypertension or pre-

eclampsia without growth retardation.

Similarly Dadak et al (1982) demonstrated depressed umbilical artery prostacyclin in pregnancies complicated by conditions known to be associated with placental ischaemia and intrauterine death, but all the infants were in fact well grown. Thus factors other than umbilical prostacyclin production may be involved in the causation of poor intrauterine growth.

It has been found that spontaneous labour increases the amounts of umbilical 6 keto $PGF_{1\alpha}$ released compared to elective caesarean section or induced labour (Makila et al 1984(a)). Therefore some of the depression of prostacyclin production noted in the studies of Stuart et al (1981) and Dadak et al (1982) may have been related to an increased incidence of caesarean section or induced labour in complicated pregnancy. The study of Matsumoto et al (1985) reported decreased release of a prostacyclin-like substance from umbilical vessels in patients delivered vaginally of growth retarded babies. However these patients also had severe pre-eclampsia so it is difficult to ascertain which pathology caused the changes or if the changes were caused by their combined effect. Adjusting for mode of delivery and diagnosis Makila et al (1984(a)) reported that prostacyclin deficiency in vitro is a specific feature of umbilical arteries from pre-eclamptic pregnancies.

Umbilical artery prostacyclin production in vitro is dependant upon the availability of free fatty acids (Ongari et al 1984). It may also be suppressed by nicotine (Stoel et al 1982) which would explain the occurrence of IUGR in the offspring of smoking mothers.

Other possible factors in the aetiology of growth retardation could include excess thromboxane activity or prostacyclin deficiency in the uteroplacental arterial bed leading to thrombotic occlusion and placental infarction. Malondialdehyde production, a measure of the platelet thromboxane pathway, was found to be increased in pregnancies with inadequate fetal growth (Wallenburg and Rotmans 1982(a)) and decreased prostacyclin production by placental cells has been demonstrated in placentae of growth retarded infants (Jogee et al 1983).

PGE, PGF and PGFM in umbilical venous blood and amniotic fluid were reported to be normal in pregnancies complicated by poor fetal growth (Norman et al 1981). Amniotic fluid 6 keto $PGF_{1\alpha}$ and TxB_2 have also been shown to be comparable between normal pregnancy and those with IUGR (Ylikorkala et al 1981(c)). Thus although there is convincing evidence of a reduced capacity of the placenta and umbilical vessels to produce vasodilator prostanoids, these changes do not seem to be reflected in measured fetal levels in vivo. Maternal plasma 6 keto $PGF_{1\alpha}$ and TxB_2 levels were also not different to normal in the presence of IUGR and were not related to

uteroplacental blood flow or umbilical vein blood flow (Ylikorkala et al 1981(a), 1983, 1984).

Thus at present, it is not clear if the prostanoid abnormality which may underlie IUGR is in the umbilical artery, the placenta or both. Studies of fetal circulating prostacyclin and thromboxane metabolites may provide better evidence to the true situation in vivo and to my knowledge there have been no reports of umbilical blood levels of prostanoids in patients with isolated IUGR.

2.8 OESTROGEN PROGESTERONE AND HUMAN PLACENTAL LACTOGEN IN HYPERTENSIVE PREGNANCIES AND INTERRELATIONSHIPS WITH PROSTANOIDS

The placenta produces large amounts of oestrogens and progesterones in pregnancy. Oestrogen production requires fetomaternal integration as the C19 precursors supplied by the fetus are converted by the placenta to oestrogens.

Plasma and urinary oestrogens have been variously reported as increased, decreased or unchanged in association with pre-eclampsia. There is some evidence of an increase in oestrogens prior to the development of pre-eclampsia. Gant et al (1971) reported increased metabolic clearance of the oestradiol precursor dehydroisoandrosterone sulphate (DS) prior to the development of pre-eclampsia with a subsequent fall some weeks before the development of the disease. Plasma oestriol levels were also higher in the midtrimester in women destined to be pre-eclamptic (Suidan et al 1984).

In established pre-eclampsia decreased urinary oestriol excretion has been demonstrated (Rosing and Carolstrom 1984, Hytten and Lind 1973, Klopper 1969) but low oestriol excretion seems to be more strongly related to intrauterine growth retardation and impending fetal death than pre-eclampsia per se (Beischer and Brown 1982).

Blood oestrogen levels in pre-eclampsia show no consistent changes. Unconjugated plasma oestrogens have been reported as unchanged (Rosing and Carlstrom 1984) increased (Hughes et al 1980) or decreased (Ranta et al 1980) in pre-eclamptic patients. There is however evidence of decreased conjugated oestrogens in pre-eclampsia (Rosing and Carlstrom 1984) which may reflect a general decrease in fetoplacental oestrogen production with possibly a disturbance of the maternal hepatic conjugation.

It has been suggested that oestradiol may play a role in the control of prostaglandin production by the fetoplacental unit as oestradiol has been shown in vitro to stimulate prostaglandin release (Speroff and Dorfman 1977, Lytton and Poyser 1982). Uterine blood flow may be controlled by the stimulatory effect of oestradiol on vasodilator prostaglandin production (Ryan et al 1974, Speroff and Dorfman 1977). A role for oestradiol in controlling the onset of labour is also possible, as 17- oestradiol stimulates PGE_2 production in decidual cells (Olson et al 1983). 17- oestradiol was shown to stimulate the production of the prostacyclin metabolite 6 keto $PGF_{1\alpha}$ from umbilical vessels (Makila et al 1982), an effect which was suppressed by progesterone. As prostacyclin seems to act as a vasodilator in the umbilical cord this is a possible regulatory mechanism of fetal blood flow. The association of prostaglandins and oestrogens is also implied by the reported correlation of urinary oestrogen excretion with

placental concentrations of PGE (Robinson et al 1979) and with amniotic fluid PGF and PGFM (Turnbull et al 1977).

Serum progesterone levels were reported to be unaltered in pre-eclampsia (Rosing and Carlstrom 1984, Sammour et al 1982) although progesterone catabolism may be reduced (Diaz-Zagoya and Arias 1981).

Nevertheless it is possible that the oestrogen-progesterone balance is disturbed in at least some pre-eclamptic patients. As progesterone opposes the stimulatory effect of oestradiol on vasodilator prostaglandin production (Lytton and Poyser 1982, Makila et al 1982), reduced uterine and uteroplacental flow could be mediated by a relative excess of progesterone.

Serum hPL is a useful measure of placental function and has been widely used as a predictor of impending fetal demise (Spellacy et al 1975) and intrauterine growth retardation. Low hPL values have been reported in pre-eclampsia but are probably a reflection of poor fetal growth and not the pre-eclamptic condition itself (Obiekwe et al 1984). In fact in their study Obiekwe et al (1984) demonstrated elevated hPL levels in primigravid patients with pre-eclampsia. The consensus of opinion seems to be that the serum hPL does not show marked trends in relation to pre-eclampsia and that its interpretation as a predictor of fetal pathology is similar to that in other pregnancies (Axelsson and Malmstrom 1983,

Sagen et al 1984).

2.9 PLASMA VOLUME EXPANDERS IN PRE-ECLAMPSIA

Plasma volume increases throughout normal pregnancy (Pirani et al 1973) and it has been suggested that this may be mediated by vasodilator prostanoids (Lewis et al 1980). In pregnancy complicated by hypertension there is a relative hypovolaemia (Cloeren and Lippert 1972, Assali and Vaughan 1978) which may be related to vasoconstriction (Gallery et al 1979, Arias 1975). Reduced plasma volume occurs particularly in those pregnancies additionally complicated by growth retardation (MacGillivray and Campbell 1980, Hays et al 1985) or with evidence of uteroplacental insufficiency (Soffronoff et al 1977). The evidence that this vasoconstriction may be secondary to a vasodilator prostanoid deficiency, particularly of prostacyclin has been presented in Part 2.5.

Artificial expansion of the plasma volume has been attempted in hypertensive pregnant patients and has been shown to temporarily reduce blood pressure (Gallery et al 1981), decrease systemic vascular resistance (Groenendijk et al 1984) and improve renal function (Cloeren and Lippert 1972). The mechanism by which plasma volume expansion brings about these changes is unclear. In non-pregnant normotensive patients a short lived drop in blood pressure after rapid infusion of colloid volume substitutes has been described in

a minority of patients (Bland et al 1973, Ring et al 1977). The incidence is however very low (0.033% in the study of Ring et al 1977) and it has been attributed to an anaphylactic reaction to an unidentified component of the infusion fluid (Bland et al 1973). This mechanism would therefore not explain the consistent drop in blood pressure in pregnant hypertensives after plasma volume expansion.

The most consistent haemodynamic changes with plasma volume expansion in hypertensive pregnant patients are a decreased peripheral resistance, decreased haematocrit, increased plasma volume and an increased urinary output (Groenendijk et al 1984, Seghal and Hitt 1980, Gallery et al 1981, Gallery et al 1984(a)), which are evidence of vasodilatation. The study of Groenendijk et al (1984) used Swan-Ganz thermodilution catheters for monitoring during infusion of 1000 to 3500ml of Haemacel and significant falls in blood pressure and systemic vascular resistance were only induced by further vasodilatation with a dihydralazine infusion. These workers concluded that the ability of the pre-eclamptic patient to vasodilate is impaired and that this may be related to elevated vasopressor sensitivity. They observed insignificant blood pressure changes with volume expansion alone although Gallery et al (1981) observed a fall in both systolic and diastolic pressures in response to 500ml of plasma substitute. The latter workers considered that the findings indicate the role of volume contraction in maintenance of elevated blood pressure in

pre-eclampsia.

The role of prostaglandins as mediators of the vasodilator response to plasma volume expansion in patients with gestational hypertension was evaluated by Gallery et al (1984(b)). The plasma levels of PGFM and 6 keto $PGF_{1\alpha}$ were significantly suppressed by the infusion. In a similar study of pregnant chronic hypertensives reported by the same authors (1984(a)) the effects of plasma volume expansion on plasma PGFM and 6 keto $PGF_{1\alpha}$ were less apparent.

The antihypertensive effect of plasma volume expanders in hypertensive pregnant patients seems to be mediated by factors other than simple volume expansion. It is possible that the effect may be brought about by release of an endogenous vasodilator or a suppression of an endogenous vasoconstrictor. Although the causative mechanism is undetermined it is possible that prostanoids are involved at local tissue level or that alterations in the balance of vasoconstrictor to vasodilator prostanoids are more important than absolute levels.

PART 3

HYPOTHESIS, RATIONALE AND OUTLINE OF STUDY

3.1 HYPOTHESIS FOR THE ROLE OF PROSTANOIDS IN NORMAL PREGNANCY AND PREGNANCY COMPLICATED BY HYPERTENSION AND GROWTH RETARDATION

In Part 2 the studies implicating prostanoids in the pathogenesis of pre-eclampsia and intra-uterine growth retardation (IUGR) have been reviewed. The results of these investigations can be summarised as follows:

1. Prostanoids are produced by the trophoblast and placenta. A placental factor is likely to be intimately involved in the aetiology and pathogenesis of pre-eclampsia as removal of the placenta usually results in rapid resolution of the disease.

2. Prostanoid levels are increased in normal pregnancy with an excess of vasodilator prostanoids which could explain some of the physiological changes characteristic of normal pregnancy.

3. Fetal umbilical blood prostacyclin metabolite levels are elevated at 16 weeks gestation. This may be a requirement of the second wave of trophoblastic invasion of the uterine spiral arteries which occurs at this time in normal

pregnancy. A deficiency in the production of prostacyclin at this stage of pregnancy could be responsible for the failure of the second wave of trophoblastic invasion which is a feature of pre-eclampsia.

Alternatively the abnormalities of placentation and prostacyclin deficiency in pre-eclampsia may be secondary to a defect in the trophoblast or in the maternal immunological response.

4. Women with pre-eclampsia show an increased sensitivity to infusion of angiotensin II which could be explained on the basis of their relative prostacyclin deficiency.

5. There is consistent evidence of a deficiency of prostacyclin in fetal and placental tissues in pregnancies complicated by pre-eclampsia and growth retardation. The production of prostacyclin by the umbilical vessels may be an important factor in maintaining vasodilation and low pressures in the fetal circulation. If the fetus is unable to respond in this way, because of the inability of the cord to produce adequate amounts of vasodilator prostanoids, its blood supply may be compromised.

One could propose that the prostanoid production by the umbilical cord and placenta may vary according to the severity of the disease. The initial response to maternal hypertension may be an increase in vasodilator prostanoids by the umbilical vessels to counteract maternal vasoconstrictors. As the hypertensive disease progresses, or if the infant is destined to be growth retarded, that response may be inadequate. Thus whether or not the fetus becomes growth retarded may depend on its ability to produce vasodilators to counteract a vasoconstrictor tendency in the mother. Hence low prostacyclin producing capability of the cord may be more closely related to IUGR than to maternal hypertension.

The prostacyclin production in pre-eclampsia probably represents a range from normal to severely depressed depending upon the incidence of IUGR. If all grades of severity are considered together this would lower the mean of the group as a whole compared to normal because of the higher incidence of growth retardation in pregnancies complicated by pre-eclampsia.

Early and late onset pre-eclampsia may be different diseases or may represent two ends of the same disease spectrum. However we do not understand why some patients with gestational or chronic hypertension develop IUGR and others

have normally grown babies. It is also unclear why there is an increased incidence of pre-eclampsia in chronic hypertension but some patients with chronic hypertension develop pre-eclampsia and others do not. It is possible that the time of onset of the prostacyclin deficiency dictates the course of events. It could be postulated that early prostacyclin deficiency results in severe pre-eclampsia associated with IUGR whereas a later deficiency gives rise to the milder form of the disease.

3.2 RATIONALE FOR STUDY

Although prostanoid imbalance in the uterus or its contents could explain the vasoconstriction and decreased blood flow in gestational hypertension, the effects on the blood pressure and coagulation systems imply that the vasodilator deficiency is not confined to the uterus but is generalised. Hence one would anticipate if a vasodilator prostanoid deficiency is the cause of elevation of blood pressure, coagulation defect and increased vasopressor sensitivity, it should be demonstrable in peripheral blood. As prostanoids are generated and metabolised locally, it could be postulated that small variations in production in individual blood vessels may occur which are not reflected by changes in circulating blood levels and this could account for the inconsistencies in reported studies. The combined effect of

even small alterations in prostacyclin production in the vessel endothelium however if it was widespread, could account for the physiological changes in pre-eclampsia.

There have been few reports of prostacyclin and thromboxane levels in maternal blood in pre-eclampsia and growth retardation and even fewer in chronic hypertensives. Furthermore, the studies that have been published are contradictory.

However, improvements in the RIA technique for prostanoids together with the knowledge of factors which can cause erroneous results, make it worthwhile to reassess maternal blood prostanoid levels in the hypertensive disorder of pregnancy. In addition the interrelationships of the four major prostanoids in blood and urine in pre-eclampsia have not previously been studied. These interactions may be more important than the levels of individual prostanoids.

One consistent finding of previous workers is that prostanoid levels in umbilical cord plasma are higher than in maternal plasma. This has two possible implications. Firstly, that plasma samples do reflect prostanoid activity or alternatively that fetal vessels exhibit a greater response to the trauma of sampling.

The measurement of the prostanoids in maternal urine eliminates some of the problems of blood sampling. Urine is less metabolically active than blood and samples are collected without trauma so artefactual release is less likely. Urine levels, particularly of PGE, are likely to reflect local prostanoid production in the kidney and may relate to renal blood flow and function. Urinary prostanoids are also likely to reflect overall synthesis in the body so may be a more reliable way of detecting the summation of a number of small alterations. It would also be of interest to assess if there is any relationship between blood and urine values.

Amniotic fluid at term also has the advantage of limited metabolic activity and as it consists mainly of fetal urine prostanoid levels are likely to be a good reflection of the fetal response to its environment. If fetal growth retardation is due to the impaired ability of the placenta and umbilical vessels to generate prostacyclin, it is hoped that measurement of 6 keto $PGF_{1\alpha}$ and TxB_2 in umbilical arterial blood, may reflect this response. A study of the amniotic fluid and umbilical arterial blood prostanoids may be of value in finding the cause of IUGR.

If abnormalities of prostaglandins and thromboxanes are the underlying cause of gestational hypertension and IUGR, it is possible that intervention may restore the balance. Plasma volume expansion has been shown to cause vasodilatation and a fall in blood pressure in pre-eclampsia. Rapid expansion of the plasma volume may stimulate prostacyclin release by distension of the vessel walls and may therefore mediate the vasodilator response. Part of the study tested this hypothesis.

Prevention of pre-eclampsia is likely to be more effective than treatment. The onset of prostanoid imbalance may originate early in the pregnancy and preventative therapy in the form of low dose aspirin and dipyridamole has been claimed to reduce the incidence of pre-eclampsia but its mode of action is unknown. It is proposed that this favourable outcome may be achieved by manipulation of the prostacyclin/thromboxane balance, but this has not previously been fully investigated.

3.3 OUTLINE OF THE STUDY

The study was performed in 4 parts over the three years from 1984 to 1987.

Study A (Part 4)

A single blood sample and a 4 hour urine collection were obtained from patients with normal pregnancy and pregnancies complicated by gestational and chronic hypertension. Samples were assayed for PGE, PGFM, 6 keto $PGF_{1\alpha}$ and TxB_2 by radioimmunoassay as measures of PGE, $PGF_{2\alpha}$, prostacyclin and thromboxane A_2 activity respectively. Renal function tests, assays of placental hormones and blood count were also performed. The relationships of these variable to each other and to hypertension and fetal growth were analysed.

Study B (Part 5)

Amniotic fluid samples obtained by amniocentesis and umbilical arterial blood sampled by direct aspiration of the cord at the time of elective caesarean section were assayed for 6 keto $PGF_{1\alpha}$ and TxB_2, in normal and hypertensive patients.

Study C (Part 6)

Maternal plasma 6 keto $PGF_{1\alpha}$ and TxB_2 levels were measured before and after plasma volume expansion with 500ml of stabilised human serum in a group of hypertensive pregnant patients.

Study D (Part 7)

A group of pregnant patients with elevated midtrimester blood pressures were selected as high risk for development of pre-eclampsia. In a randomised controlled study they were allocated to no treatment or therapy with either low dose aspirin or a combination of low dose aspirin and dipyridamole.

Maternal plasma 6 keto $PGF_{1\alpha}$ and TxB_2 were measured before treatment and after 1 week and 4 weeks of therapy, to assess the effects of these drugs on circulating levels.

PART 4

STUDY A - MATERNAL PROSTANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCY

4.1 INTRODUCTION

In part 2 the evidence regarding the involvement of prostanoids in the physiological changes of normal pregnancy and in the pathogenesis of pre-eclampsia is presented. There is convincing evidence of a deficiency of vasodilator prostanoids in fetal and placental tissues in pre-eclampsia and intrauterine growth retardation. The evidence regarding possible changes in maternal prostanoid levels in pre-eclampsia however is contradictory.

It was suggested in Part 3 that if there are major alterations of prostanoids in the mother in pre-eclampsia then, with careful attention to sampling technique and treatment of specimens, it should be possible to detect changes in maternal peripheral blood. It was also suggested that urinary prostanoid excretion may be a better measure of generalised vasoconstriction as well as local production in

the kidney. Estimates of urinary prostanoids may provide a measure of the role of these substances in the impairment of renal blood flow and function which is characteristic of pre-eclampsia as well as the underlying aetiology of the disease.

An intrauterine deficiency of prostacyclin has also been demonstrated in pregnancies complicated by IUGR and has appeal on a physiological basis. (See Part 2.7). Current evidence suggests this may not be reflected in maternal blood and urine samples.

4.2 OBJECTIVES OF THE STUDY

The aims of the study were as follows:

(1) Determine the extent of changes in maternal plasma and urine prostanoids in normal pregnancy and in pregnancy complicated by hypertension and IUGR.

(2) Assess the interrelationships of the different vasoconstrictor and vasodilator prostanoids and the relationship of the levels of individual prostanoids to blood pressure, placental function, pregnancy complications and gestational age.

(3) Study the changes in prostanoid levels in relation to the duration of gestational hypertension to assess if the changes were progressive.

(4) Evaluate the clinical value of measurements of maternal prostanoid levels in the prediction of pregnancy complications.

(5) Determine if there are any differences in prostanoid levels between the different hypertensive diseases in pregnancy, particularly between those more frequently associated with IUGR (early onset pre-eclampsia and chronic hypertension) and late onset pre eclampsia where fetal outcome is usually good.

4.3 PATIENTS AND METHODS

4.3.1 Patient Selection

A study was undertaken on 3 groups of pregnant patients between 24 and 38 weeks gestation.

Group 1. Normal Pregnancy

Normal pregnant patients were selected from a routine antenatal clinic. All patients with a normal singleton pregnancy in the second or third trimester and with a DBP

not exceeding 80mmHg were selected for study. Women with a history of hypertension or other medical disorders were excluded. None of the patients had a known complication of pregnancy at the time of the study. Patients with a DBP exceeding 80mmHg at any time later in pregnancy or the puerperium were excluded from analysis.

Hypertensive patients (not on treatment) were selected from the antenatal ward at Groote Schuur Hospital if the average DBP of 4 readings taken after 24 hours rest in hospital was 90mmHg or more.

Group 2. Chronic Hypertension

The criteria for the diagnosis of chronic hypertension are summarised in Appendix 1B. In the majority of cases it was possible to confirm continued elevation of the blood pressure or maintenance of normotension only by antihypertensive therapy, at the six week postnatal visit.

Patients with chronic hypertension who subsequently developed proteinuria (i.e. superimposed pre-eclampsia) were reclassified as pre-eclampsia for the purposes of the study. The justification for this is that the clinical course and complications are similar to other patients with pre-eclampsia (Redman 1984) and they demonstrate the characteristic renal glomerular lesions (Pollack and Nettles 1960).

Group 3. Gestational Hypertension

This group of patients was selected by the definition of gestational hypertension in Appendix 1A. The presence or absence of proteinuria (> 300mg/24 hours) was noted.

Exclusion Criteria

To clarify analysis, patients with any of the following were excluded from study

(a) Unclassified hypertension
(b) Known or suspected chronic renal disease
(c) Co-existent medical disorder
(d) Multiple pregnancy
(e) Obstetric complications of pregnancy excepting intra-uterine growth retardation (IUGR).
(f) Antiprostaglandin therapy in the previous two weeks.
(g) Antihypertensive therapy
(h) Gestation > 38 weeks or uterine contractions
(i) Vaginal examination in the previous 24 hours.

Informed consent was obtained from each patient (Appendix 4).

4.3.2 Outline of Study

A single blood specimen and a 4 hour urine collection were obtained from each patient. The method of sampling and of analysis for prostanoids are described below. All prostanoid measurements were obtained using a commercial radioimmunoassay kit (Seragen, Boston). Blood samples were assayed as follows

(a) Plasma prostaglandin E_2 (PGE_2)

(b) Plasma 13,14 dihydro 15 keto prostaglandin $F_{2\alpha}$ (PGFM)

(c) Plasma 6 keto prostaglandin $F_{1\alpha}$

(d) Plasma thromboxane B_2

(e) Serum total oestradiol (E_2) using the in house assay of the Gynaecological Endocrine Laboratory (tritiated label anti sera extraction method)

(f) Serum total progesterone using ^{125}I Diagnostic Products kit

(g) Human placental lactogen (hPL) using IRMA kit ^{125}I (Amersham Laboratories)

(h) Serum urea, urate and creatinine by routine hospital laboratory assay (Sequential Multiple Analysis plus computer (SMAC))

(i) Full blood count by routine hospital laboratory assay (Coulter counter)

A 6ml aliquot of the urine collection was obtained and samples assayed for

(a) Prostaglandin E_2

(b) 13, 14, dihydro 15 keto prostaglandin $F_{2\alpha}$ (PGFM)

(c) 6 keto prostaglandin $F_{1\alpha}$

(d) thromboxane B_2

(e) Protein. Testing was initially performed by multi reagent strip (Multistix SG Ames). If this was positive the presence of proteinuria was confirmed by sulphosalicyclic acid "cold" test. If the four hour specimen was positive for protein by this method, a 24 hour sample was collected for protein quantitation. For the purposes of analysis significant proteinuria was defined as > 300mg per 24 hours (as per ward protocol).

The following information was recorded at the time of sampling

1. Age
2. Gravida
3. Race
4. Known duration of hypertension
5. Presence or absence of oedema
6. Gestational age
7. Blood pressure

The blood pressure at the time of sampling was measured in each patient by the same research sister using standard technique (Appendix 3). Diastolic blood pressure was recorded as Korotkoff phase 4.

8. Presence or absence of significant proteinuria.

An example of the charts used for collection of data is included in Appendix 5.

4.3.3 Methods and Validation of Radioimmunoassay for Prostanoid Metabolites

A number of factors are known to influence the measurement of prostanoid metabolites in biological fluids by radioimmunoassay and these variables may include the technique of sample collection, the treatment of samples, extraction procedure and the assay of plasma samples (Part 2.3.3.4). These differences in technique of sampling, extraction and assay may account for some of the differences in the results reported by different workers. A number of aspects of the method were investigated to determine the most reliable method of sampling and extraction as well as the validity of the radioimmunoassay.

4.3.3.1 Method of Sampling

A 16G indwelling cannula was inserted into a large forearm vein with minimal venous occlusion. Blood samples were taken without constriction into plastic syringes and immediately dispensed into chilled polypropylene tubes containing 40µl of an EDTA/theophylline solution (10g% ethylenediamene tetraacetic acid (EDTA) and 540mg% theophylline in distilled water at pH 7.0) and 8µl of acetyl salicilic acid solution (12g% in methanol) per ml of sample. The two solutions were freshly prepared and mixed just prior to blood sampling. The tubes were mixed gently by inversion and kept on ice for not more than 1 hour and then centrifuged at 3000g at 4°C for 15 minutes in a Sorvall refrigerated centrifuge. The plasma was transferred to another polypropylene tube care being taken not to disturb the plasma/red cell interface and samples were stored at -20°C for not more than 48 hours prior to extraction. After extraction (see below) the evaporated samples were stored at -20°C for later analysis by radioimmunoassay. The radioimmunoassay was performed according to the manufacturers protocol (see Appendix 6).

The urine samples were collected over a 4 hour period on the

same morning as blood samples were obtained. A 4 hour collection period was chosen to allow estimation of urinary prostanoid excretion but to avoid the compliance problems of collection over a longer period of time. The total urine volume was noted and an aliquot of the measured 4 hour sample was transferred into tubes containing the same solutions as for blood sampling, and the samples were spun to remove sediment. Extraction and storage were then performed as for blood samples.

The method of blood sampling was similar to that recommended by Morris et al (1981), with the indwelling needle connected to a slow running saline infusion and a saline flush prior to sampling. Samples were discarded if any difficulty was encountered in obtaining the blood sample such as slow withdrawal of blood or the need to manipulate the indwelling cannula. Plasma was used for assay, as allowing the blood to clot has been reported to cause elevation of prostanoid levels (Morris et al 1981, Challis and Tulchinsky 1974(a)). Other variables known to affect the results of the radioimmunoassay such as centrifugation temperature and pH were rigorously controlled and all samples were thawed once only (Morris et al 1981).

The effect of the timing of sampling after insertion of the

needle was investigated by comparison of the prostanoid level obtained in blood withdrawn immediately after placement of the needle (time 0) with that found in blood obtained 1 hour later through the same cannula. Although the values found in samples at time 0 and 1 hour frequently differed, these differences did not achieve statistical significance ($p > 0.05$) and statistical analysis by the Sign test that the differences showed no trends with increasing duration of placement of the cannula (p 0.27 - 0.58). The two values correlated in all cases ($p < 0.05$) but the Spearman rank correlation coefficients were sometimes low (r values ranging from 0.32 to 0.61). These findings suggest that many factors affect the RIA result in addition to the timing of the sampling in relation to placement of the needle. Blood samples were subsequently obtained at 1 hour after placement of the cannula.

4.3.3.2 Extraction of Prostanoids

Extraction of the prostanoids from the samples was undertaken in order to reduce interference from other substances. Three extraction methods were investigated for reliability and reproducibility and recoveries were measured by addition of triated PGE_2, $PGF_{2\alpha}$, 6 keto $PGF_{1\alpha}$ or TxB_2.

For each prostanoid three 1ml samples were extracted with each method and each extract was then assayed in duplicate.

The extraction methods for each 1ml sample were as follows:

Method A

Acidification to pH < 3 with 0.5ml 1N HCl, then extraction with 6ml diethyl-ether once only (Mitchell 1978(c)).

Method B

Acidification with 200µl glacial acetic acid to pH < 3 then one extraction with 6ml ethyl acetate (Seragen RIA kit instruction booklet).

Method C

Addition of 3ml petroleum ether each to 1ml sample to remove neutral lipids. The organic layer was then discarded and extraction performed with 3ml of a 3:3:1 solution of ethyl acetate : isopropanol : 0.2N HCl. After mixing, 2ml of ethyl acetate and 3ml of water were added (Jaffe et al 1973, Jaffe and Berhman 1978).

For each method the fractions were separated by freezing the aqueous layer. The organic phase was then transferred to a clean polypropylene tube and evaporated to dryness under N_2 at 37°C.

Before assay samples were reconstituted in 1ml of assay buffer with a final pH 7.0. Method C produced unpredictable recovery rates. Methods A and B yielded consistent recovery rates in each assay with mean recovery rates of 81.8 (SD 2.2)% and 56.2 (SD 2.0)% respectively. Extraction method A was therefore subsequently used for all assays. The recovery was monitored in each assay batch by the addition of the tritiated prostanoid to several blank samples. The extraction recoveries during the study varied between 65 and 76%.

4.3.3.3 Evaluation of the Radioimmunoassay

The principles of assessment of a radioimmunoassay are evaluation of sensitivity, specificity, precision, accuracy and reproducibility (Midgley et al1969). These factors were appraised for each prostanoid metabolite assay.

Sensitivity

The mean of the least detectable mass resulting in a response of two standard deviations from the zero concentration was:

$PGF_{1\alpha}$	7.4 pg/ml
TxB_2	9.4 pg/ml
PGFM	7.1 pg/ml
PGE	6.0 pg/ml

Specificity

The cross reactivities of the antibodies used in each radioimmunoassay had been evaluated by the manufacturers (Seragen) and are listed in detail in Appendix 6 Tables I - IV.

Precision

The intraassay coefficient of variation was 7.5% for 6 keto $PGF_{1\alpha}$, 6.1% for TxB_2 and 9.0% for both PGFM and PGE. This was calculated from control samples inserted randomly into each assay. All the samples in each part of the study were analysed in a single assay run using assay kits from a single manufacturing batch to eliminate interassay variations. As the 4 studies described in this thesis took place at different times over a 3 year period, it was not possible to calculate interassay variability.

The validation of the RIA and analysis of the samples in Studies A and D were performed by the same technologist. The extractions and RIA of samples in Studies B and C were performed by the author, with the guidance of the technologist.

Accuracy

The best measure of the accuracy of an assay method is by comparison with an established reference method, in this case, GCMS. GCMS was not available and an assessment of the reproducibility was made by comparing the results of the assay duplicates.

Reproducibility

The Spearman rank correlation was calculated for the duplicates of the radioimmunoassay counts obtained in each assay to evaluate analytical errors. The correlation coefficients (r) were PGE in plasma Ø.95 and in urine Ø.98, PGFM plasma Ø.94, urine Ø.95, 6 keto $PGF_{1\alpha}$ plasma Ø.88, urine Ø.95 and TxB_2 plasma Ø.97, urine Ø.9Ø. (Figures 4.1 - 4.8 show these relationships and the 95% confidence limits). An assessment of the error in reading the values from the standard curve was made by comparison of the results obtained from the assay duplicates. These also showed good correlations with correlation coefficients ranging from Ø.9Ø to Ø.98).

FIG. 4.1: REPRODUCIBILITY OF DUPLICATE RADIOIMMUNOASSAY COUNTS FOR PLASMA 6 KETO PGF1α

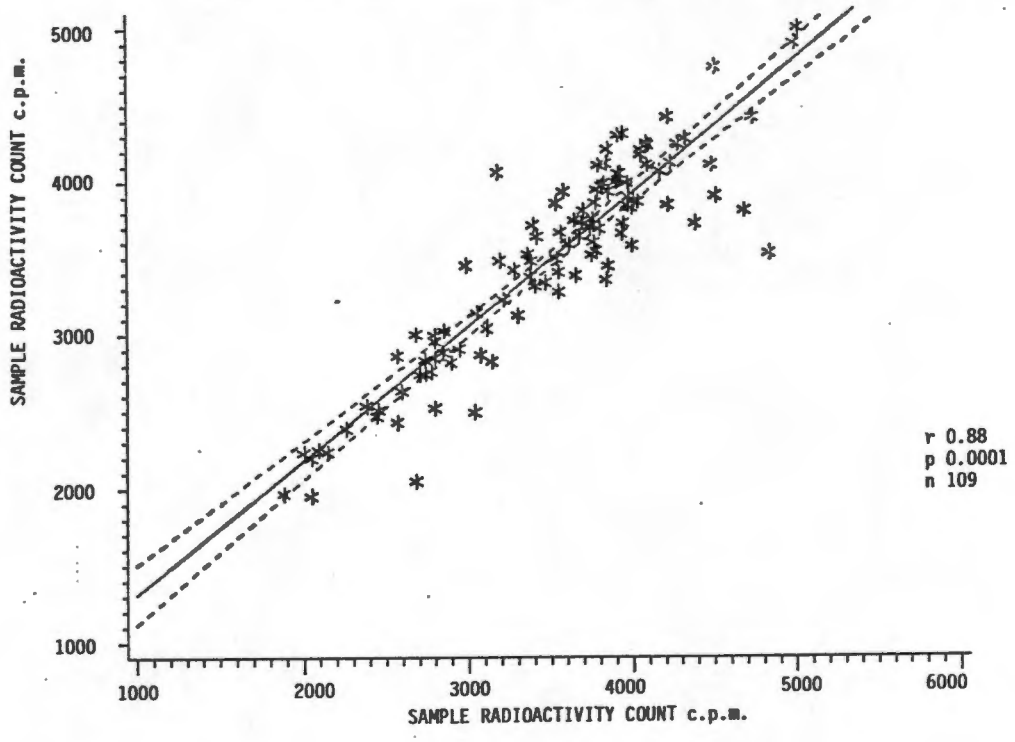


FIG. 4.2 : REPRODUCIBILITY OF DUPLICATE RADIOIMMUNOASSAY COUNTS FOR PLASMA TxB_2

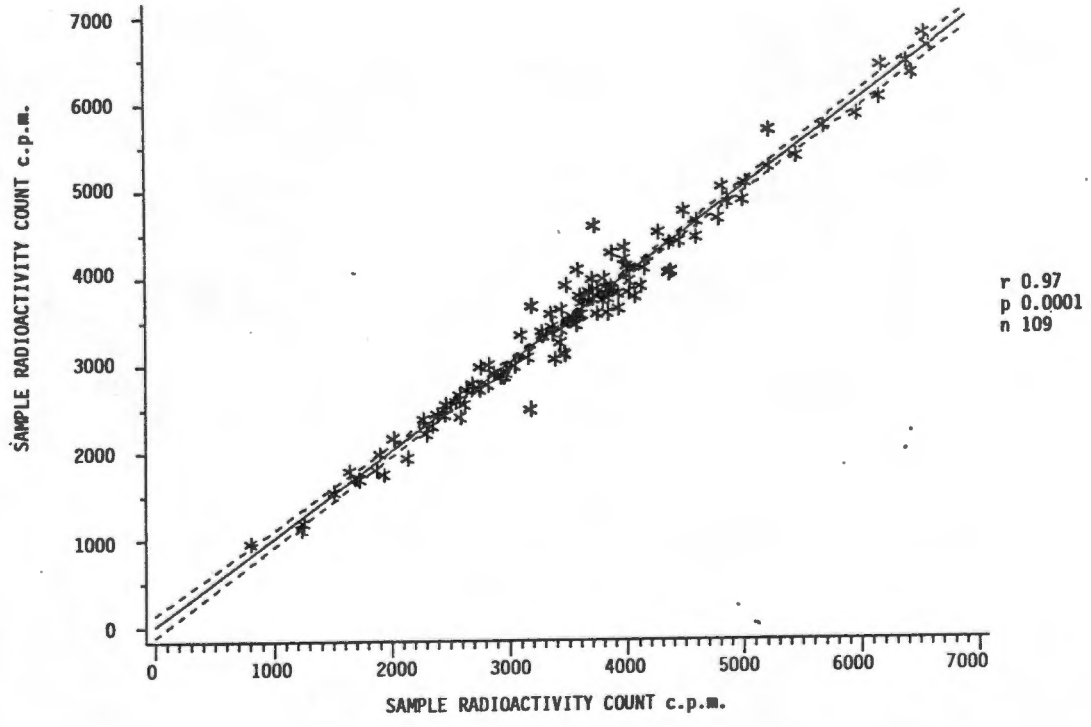


FIG. 4.3: REPRODUCIBILITY OF DUPLICATE RADIOIMMUNOASSAY COUNTS FOR PLASMA PGE

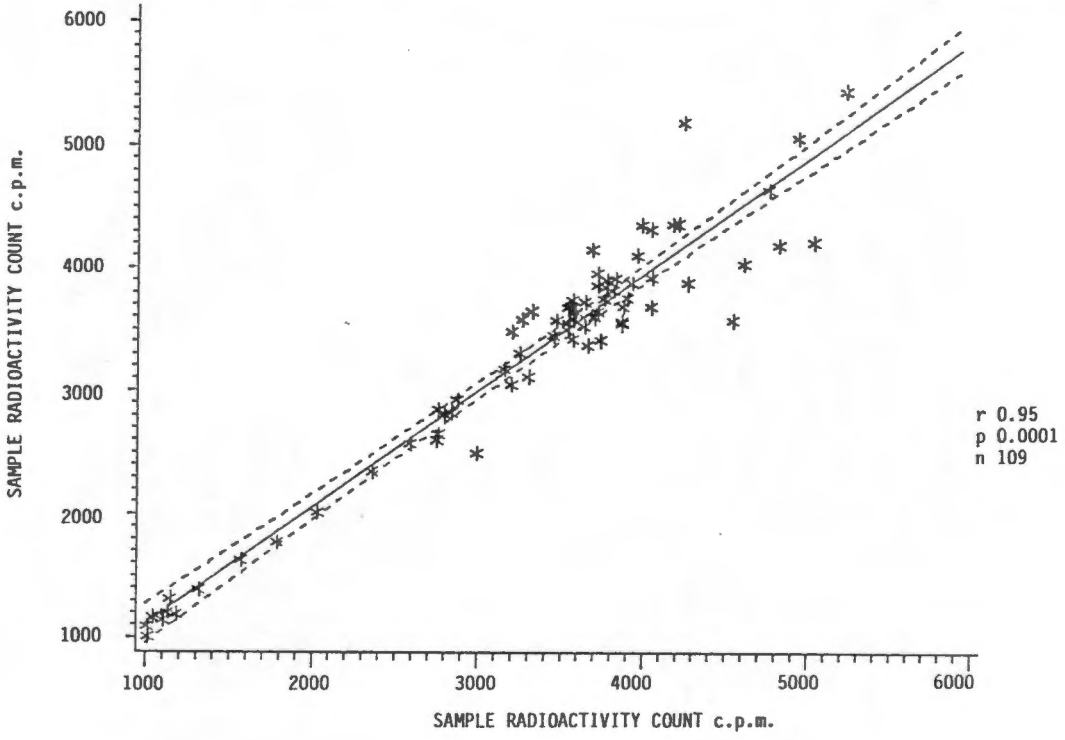


FIG. 4.4: REPRODUCIBILITY OF DUPLICATE RADIOIMMUNOASSAY COUNTS FOR PLASMA PGFM

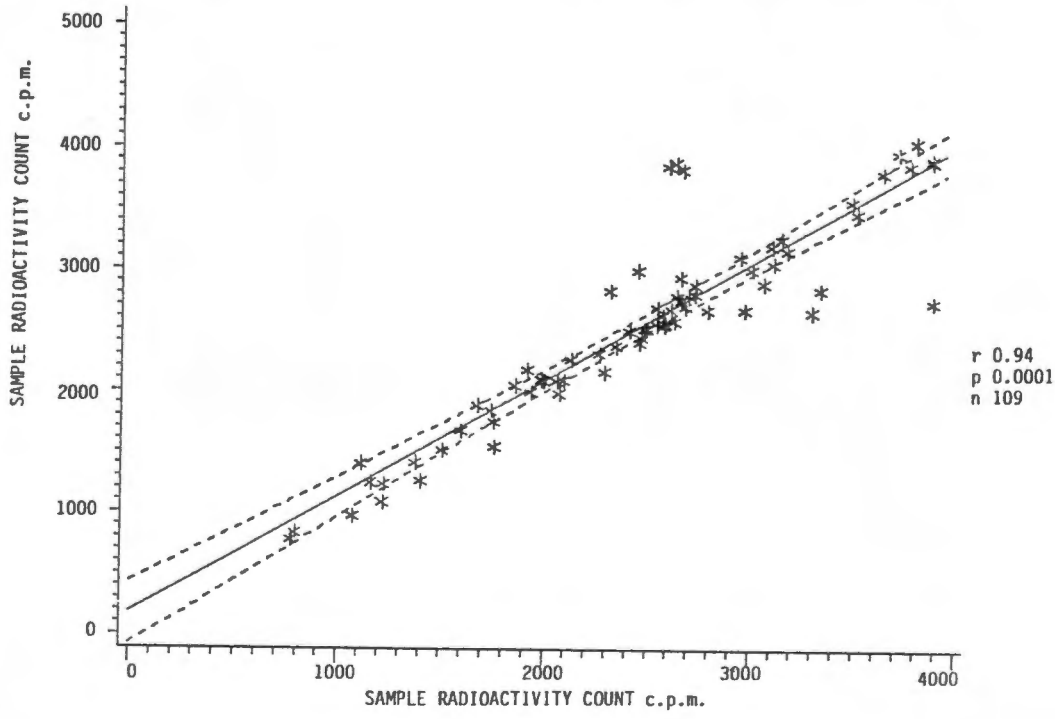


FIG. 4.5: REPRODUCIBILITY OF DUPLICATE RADIOIMMUNOASSAY COUNTS FOR URINE 6 KETO PGF1α

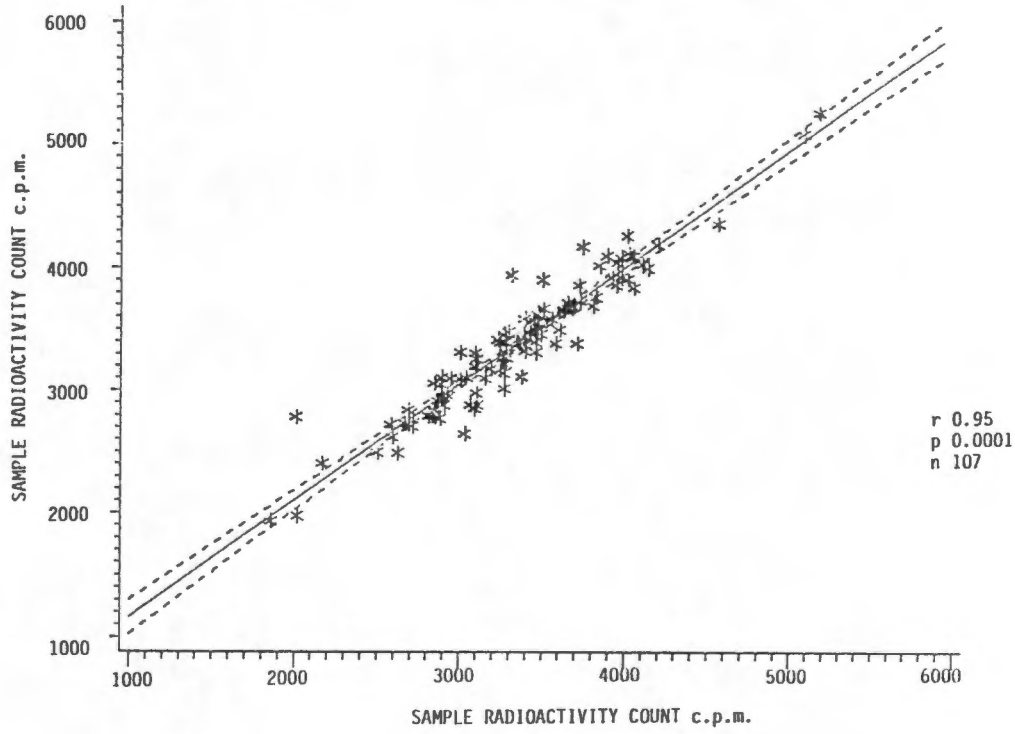


FIG. 4.6: REPRODUCIBILITY OF DUPLICATE RADIOIMMUNOASSAY COUNTS FOR URINE TxB_2

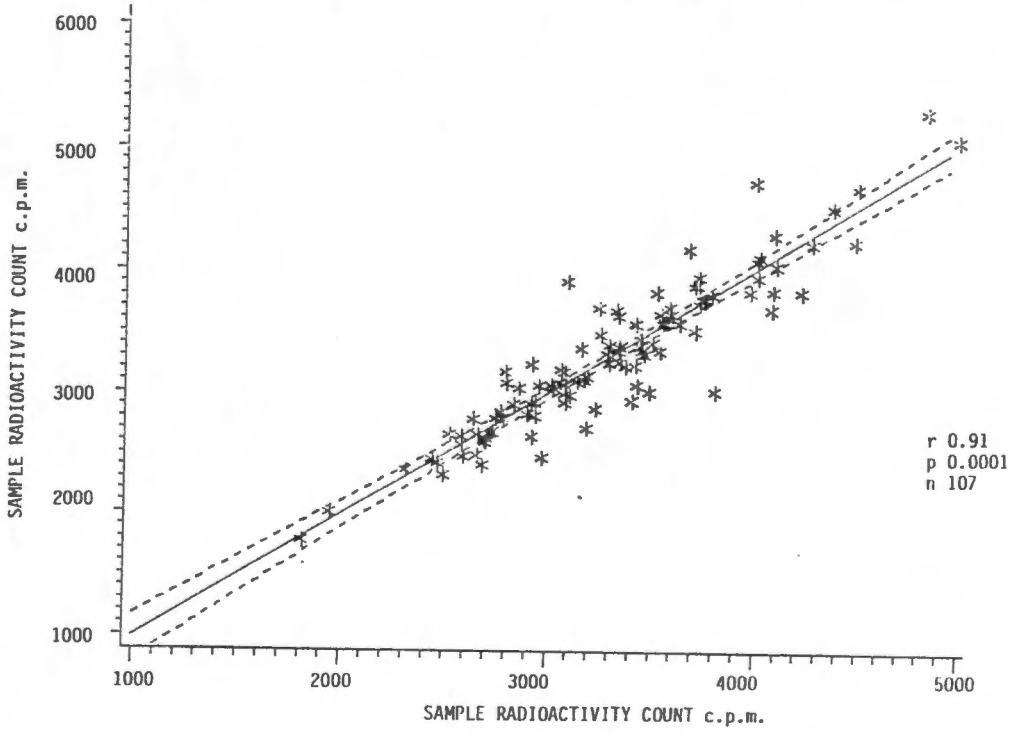


FIG. 4.7: REPRODUCIBILITY OF DUPLICATE RADIOIMMUNOASSAY COUNTS FOR URINE PGE

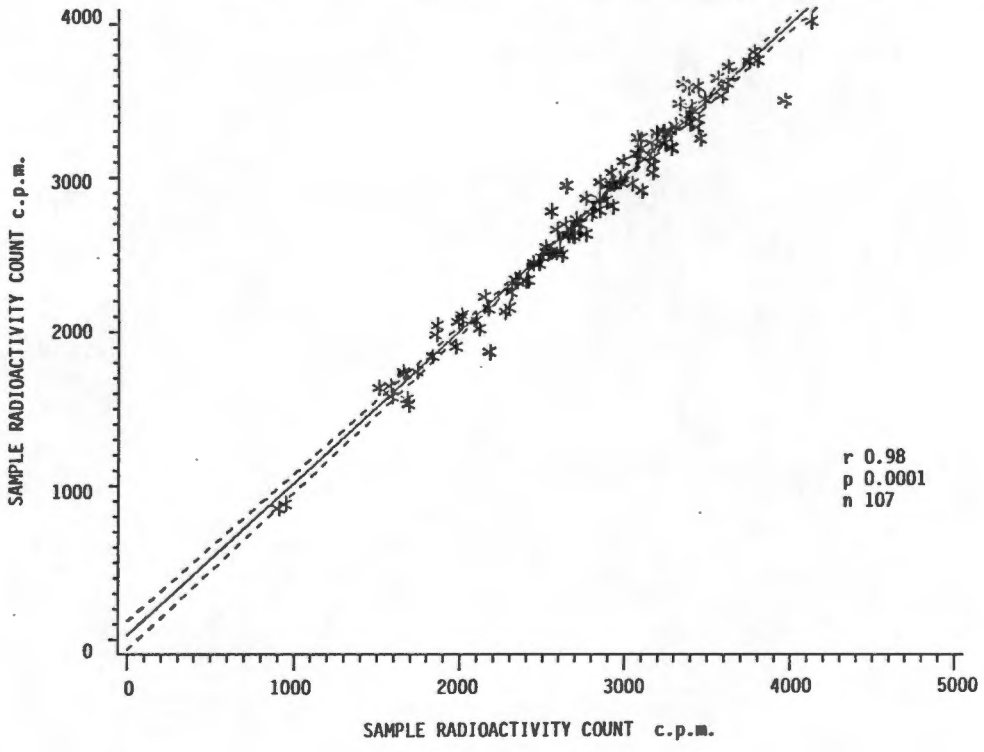
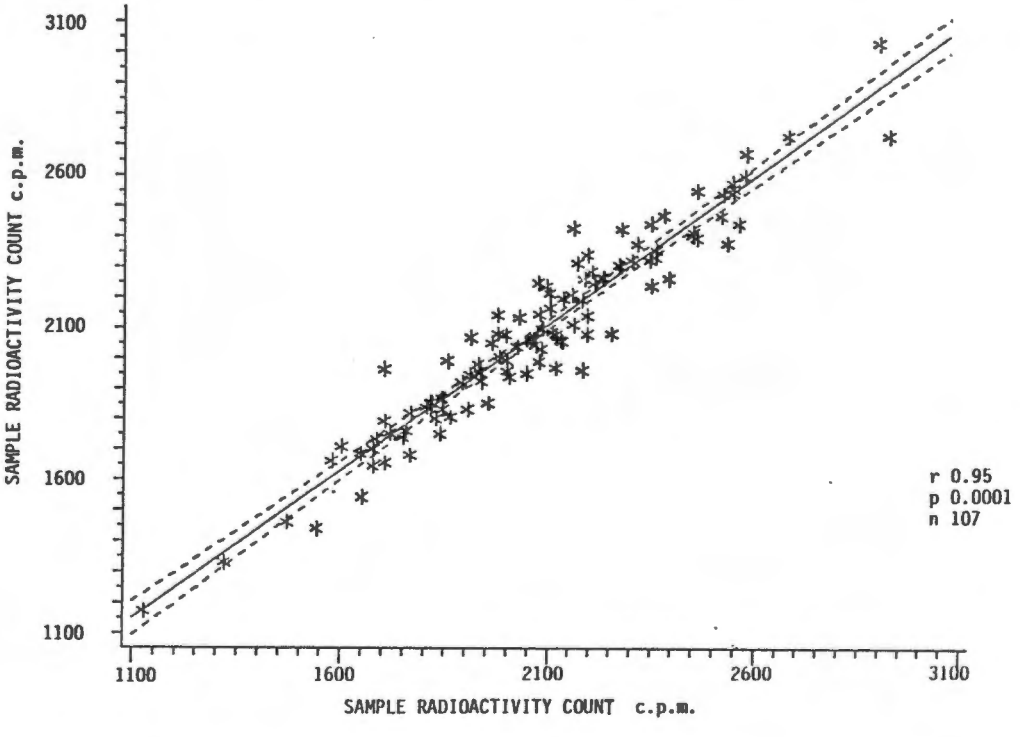


FIG. 4.8: REPRODUCIBILITY OF DUPLICATE RADIOIMMUNOASSAY COUNTS FOR URINE PGFM



The validity of the assay of the prostacyclin metabolite 6 keto $PGF_{1\alpha}$ was also tested by infusion of prostacyclin according to the method described by Ylikorkala et al (1981(d)). Synthetic prostacyclin dissolved in glycine buffer at pH 10.5 was infused into two pregnant patients, using incremental doses of 1, 2, 4 and 8 ng/kg/min for 20 min. each. Blood samples were obtained before commencing the infusion, at the end of each 20 min. infusion period and again 1 hour after completion of the infusion. The concentrations of 6 keto $PGF_{1\alpha}$ rose with increasing doses of prostacyclin (from 190 to 452 pg/ml in one patient and 137 to 298 pg/ml in the other) and decreased markedly 1 hour after completion of the infusion (to 33 and 185 pg/ml respectively). The circulating level of 6 keto $PGF_{1\alpha}$ correlated to the infusion rate in each patient (r 0.90 and r 0.68 respectively). Although a vasodilator response (as evidenced by headache, facial flushing and fall in blood pressure) occurred at different infusion rates in the two patients, in each patient the response was seen at measured circulating 6 keto $PGF_{1\alpha}$ levels of approximately 220 pg/ml in keeping with previous reports that levels around 200 pg/ml are required for physiological effect (Myatt 1987).

4.3.4 Statistical Methods

Statistical analysis was performed by the Institute of Biostatistics at the South African Medical Research Council, using the SAS software system for data analysis (SAS 1985) on an IBM mainframe computer.

The information was recorded on specially designed collection sheets (Appendix 5) and then computerised. The data was then checked against the originals to detect transcription inaccuracies.

Initially the distribution of each variable was studied to assess the most appropriate method of statistical analysis. The measure of central tendency is expressed as the arithmetic mean for data with a Gaussian distribution and as the median for data with a skewed distribution. The measures of variation were expressed as the standard deviation (SD) and interquartile range (Q_3-Q_1) respectively. The interquartile range is the distance between the 75th (Q_3) and 25th (Q_1) percentiles. The data are visually presented in a summary distribution display, the box plot (Tukey 1977 and Fig. 4.9). The upper and lower quartiles of the data are portrayed by the top and bottom edges of the rectangle. The sample median is represented by the dashed line within the body of the rectangle (*----*) and the mean

by a centred plus sign (+). Central vertical lines (whiskers) extend from the box as far as the data extends, to at most a distance of 1.5 interquartile ranges. Any value more extreme than this is marked as a zero (o) if it is within 3 interquartile ranges of the box and with an asterisk (*) if it is still more extreme. Thus the box plot gives a comprehensive impression of distribution of the data showing the central location, the range and the number and values of extreme observations.

The association between each of the variables was analysed by least squares linear regression and relationships are described by the Spearman rank correlation coefficient (r) (Kendall 1955). Rank correlations were used because of uncertainty of the distribution of the data. Scatter diagrams of the data are presented and the regression line describing the relationship has been drawn. Where appropriate (Figures 4.1 - 4.8), the 95% confidence limits of the regression line are also indicated. Spearman rank correlations were used to assess the relationships of the prostanoid levels with each other and with the placental hormones. They were also used to assess the effect of variables such as maternal or gestational age, parity and

renal function tests on prostanoid levels in the normotensive patients. The aim of this was to detect factors which may influence prostanoid levels and which may therefore need to be taken into account in more detailed analysis of the effect of hypertension.

Differences in continuous variables which could be assumed to have a normal distribution were evaluated by analysis of variance followed by multiple comparisons using the t-test (Armitage 1971). An overall significance level of $p = 0.05$ was used. Thus when 3 pairwise comparisons were performed (between the 3 study groups) the significance level for a comparison of any 2 groups was $p = 0.016$. Some of the continuous variables eg. Na, creatinine, were found to have a skew distribution and were analysed using the non-parametric Kruskal-Wallis test followed by pairwise comparisons by the Mann-Whitney test (Kruskal and Wallis 1952, Mann and Wallis 1947). Discrete variables were analysed by Chi-Square test (X^2).

Initial graphic representation of the prostanoid levels by scatter diagrams, box plots and normal probability plots showed that the data were not normally distributed. Non parametric tests were used to evaluate differences between

groups and a logarithmic transformation (natural logarithm - Log n) of the data which were then approximately normally distributed allowed more detailed statistical analysis by methods which are based on a theoretical assumption of a Gaussian distribution.

The transformed prostanoid data were initially tested by one way analysis of covariance to assess the effect of gestational age. Analysis of variance followed by pairwise comparison by t-tests was then performed to evaluate if there were differences between diagnostic groups. The source of these differences was then further assessed by analysis of covariance with diagnosis, intrauterine growth retardation and the interaction of these factors as covariables.

As the placental hormones showed positive correlations to gestational age covariance analysis was used, after adjustment for gestational age, to study the effects of diagnosis and intrauterine growth retardation.

4.4 RESULTS

4.4.1 Patient Characteristics

176 pregnant women between 15 and 43 years of age were studied. Sixty-four patients were initially selected as

having a normal pregnancy but 8 were later excluded from analysis (6 developed pre-eclampsia and 2 were subsequently found to be expecting twins). Thus 168 patients were available for study of consisting of 56 with normal pregnancies (Group 1), 35 patients with chronic hypertension (Group 2) and 77 patients with gestational hypertension (Group 3).

Some characteristics of the different groups of patients are summarised in Table 4.I. Analysis of variance was performed initially to detect if there were any differences between the groups. Multiple comparisons using the t-test were then performed to detect the differences between means, using an overall significance level of $p = 0.05$. The patients with chronic hypertension were, as expected, significantly older than the patients in group 1 and 3 (Figure 4.9). Patients with gestational hypertension were delivered at a significantly earlier stage of gestation than patients with normal pregnancy or chronic hypertension and the mean birthweight of the infants was correspondingly less. (Table 4.I)

THE BOX PLOT

Range (Upper)

75th percentile (Q_3)

Median (*.....*)

Mean (+)

25th percentile (Q_1)

Range (Lower)

FIG. 4.9: AGE DISTRIBUTION OF PATIENTS

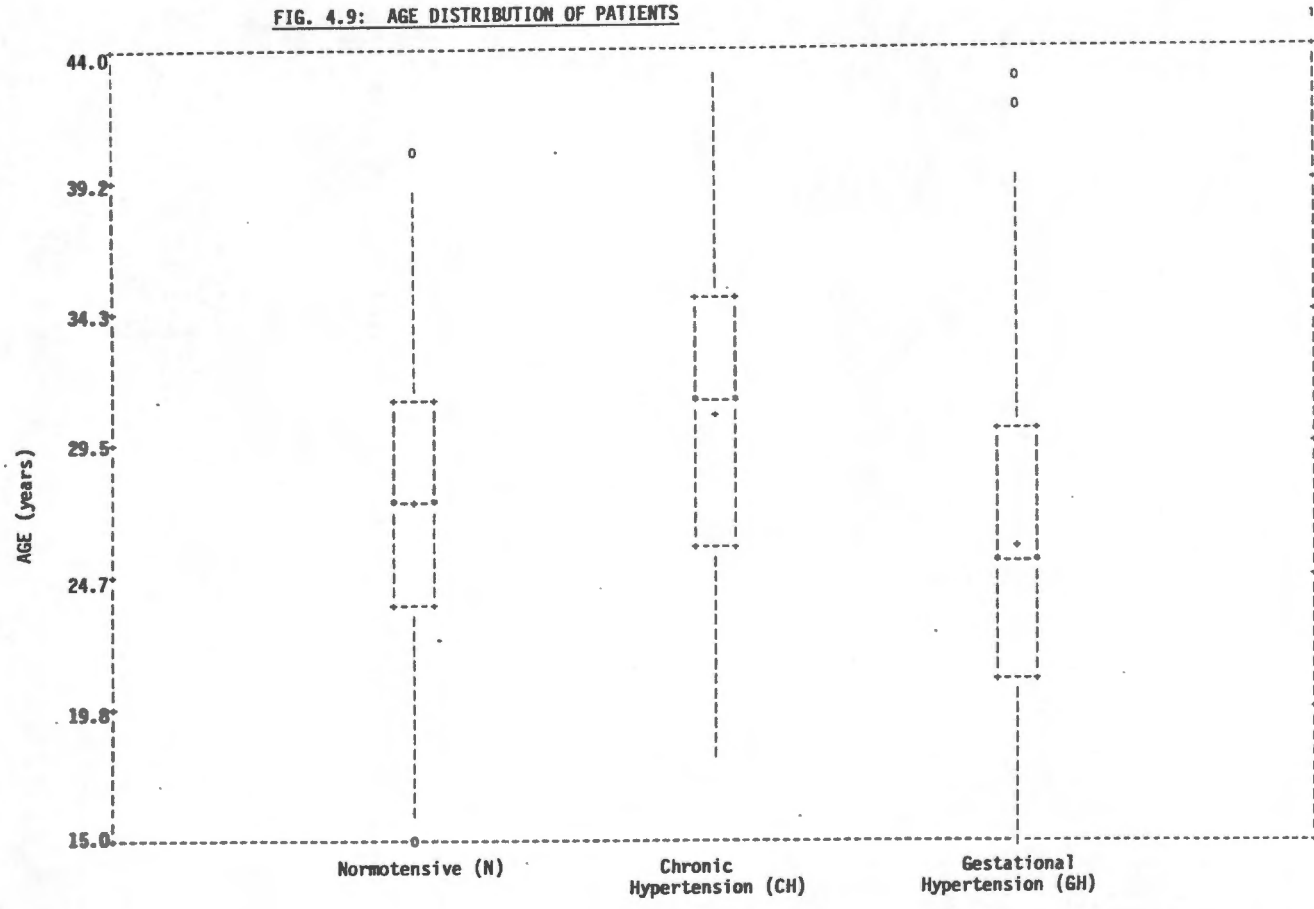


TABLE 4.I : CHARACTERISTICS OF THE STUDY POPULATION

TYPE OF PREGNANCY	N	RACE COLOURED/ AFRICAN	N (%) NULLI-PAROUS	AGE MEAN (SD) YEARS	BP AT SAMPLING MEAN (SD) mmHg	GESTATION AT DELIVERY MEAN (SD) WEEKS	BIRTH-WEIGHT MEAN (SD) gm
NORMAL	56	48/8	16 (28.5%)	27.5 (5.9)	115 (12) / 73 (8)	37.9 (2.3)	3008 (616)
CHRONIC HYPERTENSION	35	31/4	3 (8.6%)	30.7 (6.0)*	141 (16) / 95 (9)	37.9 (2.6)	2870 (593)
GESTATIONAL HYPERTENSION	77	60/17	37 (48.1%)	25.8 (6.7)	145 (17)* / 97 (11)	35.1* (3.3)	2290 (763)
STATISTICAL ANALYSIS		X^2 2.43 p 0.29	X^2 19.18 p 0.004	Anova p 0.0008	Anova p 0.0001	Anova p 0.0003	Anova p 0.0001

*p 0.05 vs NORMAL

Anova = analysis of variance

Birthweight

The distribution of birthweight percentiles adjusted for sex and gestational age are shown in Table 4.II.

Table 4.II

BIRTH PERCENTILE BY DIAGNOSIS

Birthweight Percentile	< 10		10 - 25		26 - 100	
	n	%	n	%	n	%
NORMAL (1)	8	14.3	6	10.7	42	75.0
CHRONIC HYPERTENSION(2)	3	8.6	10	28.6	22	62.8
GESTATIONAL HYPERTENSION(3)	16	21.0	17	22.4	43	56.6
TOTAL	27	16.1	33	19.8	107	64.1

In the control group 25% of birthweights were below the 25th percentile and 58.5% below the 50th percentile, indicating that the charts of Lubchenco (1963) are reasonably applicable to the study population and can be used to compare the birthweight distribution in the different patient groups. Twenty one % of the patients with gestational hypertension had infants with birthweight below the 10th percentile for gestational age. There were however no statistically significant differences in birthweight percentiles between the diagnostic groups. (Chi square analysis was performed

using 6 birthweight percentile groups p = 0.40, X^2 = 10.45; df = 10). The findings nevertheless suggest that some women with gestational hypertension give birth to infants who are growth retarded which may be dependant upon the time of onset and severity of the disease.

Race

139 patients were Cape Coloured and 29 were African (Black) There were no white patients in the study. There were no statistically significant differences in the racial distribution between groups (X^2 2.43; df 2; p 0.29).

Parity

The parity distribution in the normal group did not differ from either of the hypertensive groups (versus Group 2 X^2 6.39; df 3; p 0.094, versus Group 3 X^2 5.44; df 3; p 0.142). There were however significantly fewer nullipara amongst the chronic hypertensive patients compared to gestational hypertensive patients (X^2 16.81; df 3; p 0.001). This is compatible with the general experience that chronic hypertension is common in older multiparous women and gestational hypertension is commoner among younger nulliparous women.

Proteinuria

Thirty-four (44.7%) of gestational hypertensives had significant proteinuria (> 300mg/24 hours). No patient in the normal or chronic hypertensive groups had proteinuria, as patients with suspected renal disease were excluded from the study.

Blood pressure in midpregnancy

Blood pressure recordings were available for 88 patients at 20 +/- 2 weeks gestation and are shown in Table 4.III.

The blood pressure readings were significantly different between groups (p 0.0001). The patients destined to develop gestational hypertension had significantly higher midtrimester systolic and diastolic blood pressures than those who remained normotensive (p 0.001).

TABLE 4.III: MIDPREGNANCY BLOOD PRESSURE READINGS

MEAN (SD)

	n	SBPmmHg	DBPmmHg
NORMAL	35	112 (13)	65 (10)
CHRONIC HYPERTENSION	18	141 (16)	94 (11)
GESTATIONAL HYPERTENSION	35	123 (18)	75 (14)

4.4.2 Renal function tests

Renal function tests were performed on 96 patients, 35 with normal pregnancy (Group 1), 23 with chronic hypertension (Group 2) and 38 with gestational hypertension (Group 3).

Variables with a Gaussian distribution were evaluated statistically by analysis of variance with pairwise comparisons by t-test and results are expressed as mean (SD). Data with a skewed distribution were analysed using the non-parametric Kruskal Wallis test followed by multiple comparisons using the Mann Whitney test and results are expressed as median (interquartile range Q_3 - Q_1). The results are shown in Table 4.IV.

The serum urea and K did not differ between diagnostic groups (p Ø.71 and p Ø.31 respectively) but the urate values did differ between groups (F value 5.58; df 2; p Ø.ØØ51). Serum urate levels were higher in gestational hypertensives (299(91)µmol/l) than the normal patients (234 (51µmol/l), p Ø.ØØ17). These findings are compatible with the modest decrement of renal function which is known to occur in gestational hypertension with reduced renal clearance of uric acid. Overall however the mean serum urate values were not significantly different between the 3 groups (p Ø.47).

TABLE 4.IV : BLOOD CHEMISTRY

DIAGNOSTIC GROUP	UREA MMOL/L MEAN (SD)	CREATININE μMOL/L MEDIAN (Q_1-Q_3)	Na MMOL/L MEDIAN (Q_1-Q_3)	K MMOL/L MEAN (SD)	URATE μMOL/L MEAN (SD)
NORMAL n = 35	2.74 (0.63)	55 (12)	139 (3)	3.85 (0.25)	234 (51)
CHRONIC HYPERTENSION n = 23	2.73 (0.64)	56 (20)	137* (4)	3.83 (0.33)	249 (86)
GESTATIONAL HYPERTENSION n = 38	2.86 (0.84)	59 (15)	138 (4)	3.94 (0.33)	293* (91)
ALL n = 96	2.79 (0.72)	59 (23)	138 (4)	3.89 (0.3)	261

*p 0.05 vs NORMAL

(See Part 4.4.2)

Serum sodium was slightly lower in chronic hypertensives (137(4)mmol/l) than in normal patients (139(3) mmol/l) and this difference was statistically significant (p Ø.Ø16). There was no significant difference between the median creatinine values of the 3 groups.

4.4.3 Haemoglobin and Platelet Count

The mean haemoglobin and platelet counts were not significantly different between diagnostic groups (Table 4.V).

TABLE 4.V : HAEMOGLOBIN AND PLATELET COUNTS IN NORMAL AND HYPERTENSIVE PREGNANCIES

	Hb gm/dl Mean (SD)	Platelets 10^9/l Mean (SD)
NORMAL	11.5 (1.4)	244 (61)
CHRONIC HYPERTENSION	12.1 (1.1)	26Ø (67)
GESTATIONAL HYPERTENSION	11.9 (1.4)	234 (71)
KRUSKAL WALLIS TEST VALUE	p Ø.16 3.69	p Ø.25 2.75

4.4.4 Plasma Oestradiol, Progesterone and hPL

Plasma oestradiol, progesterone and hPL increased with gestational age (p Ø.ØØØ1) although the results showed a wide variation at each gestation. Scatter plots showing the relationship of the values to gestational age are shown in Figures 4.1Ø, 4.11 and 4.12. Linear regression analysis was performed and the regression line that describes the relationship has been drawn. The correlation coefficients were not high but the relationship with gestational age for each parameter was statistically significant for all the diagnostic groups combined and each considered separately at p < Ø.Ø16 (using the significance level for these multiple comparisons).

FIG. 4.10: RELATIONSHIP OF SERUM OESTRADIOL TO GESTATIONAL AGE

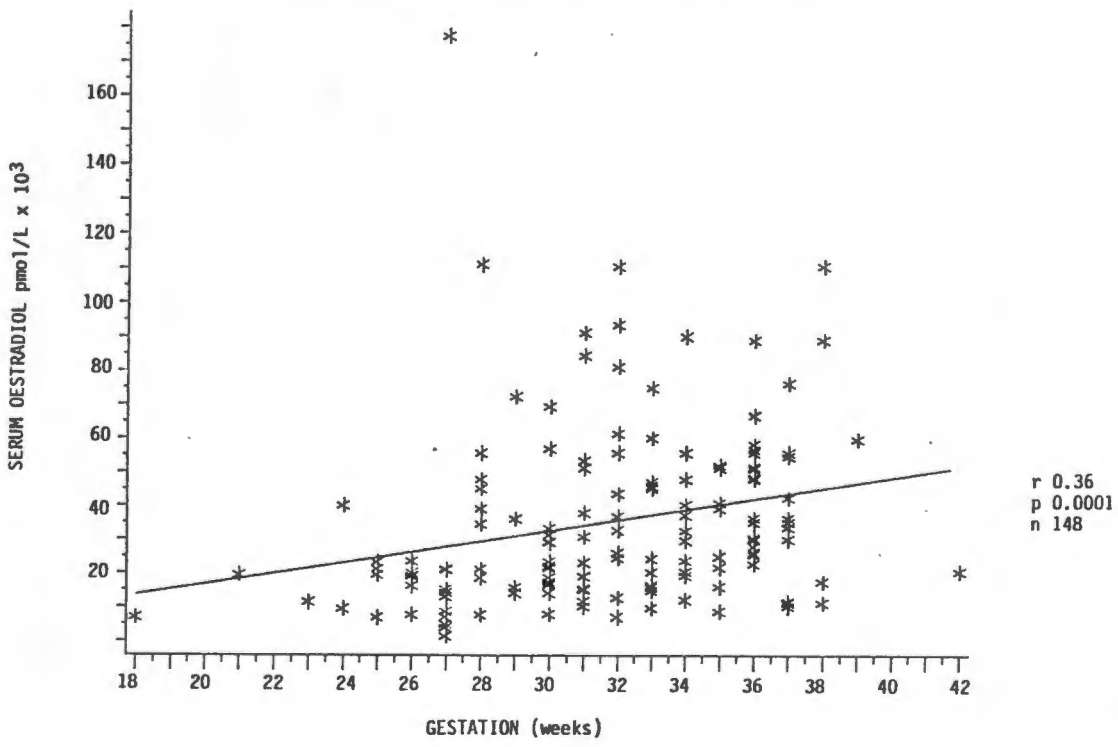


FIG. 4.11: RELATIONSHIP OF SERUM PROGESTERONE TO GESTATIONAL AGE

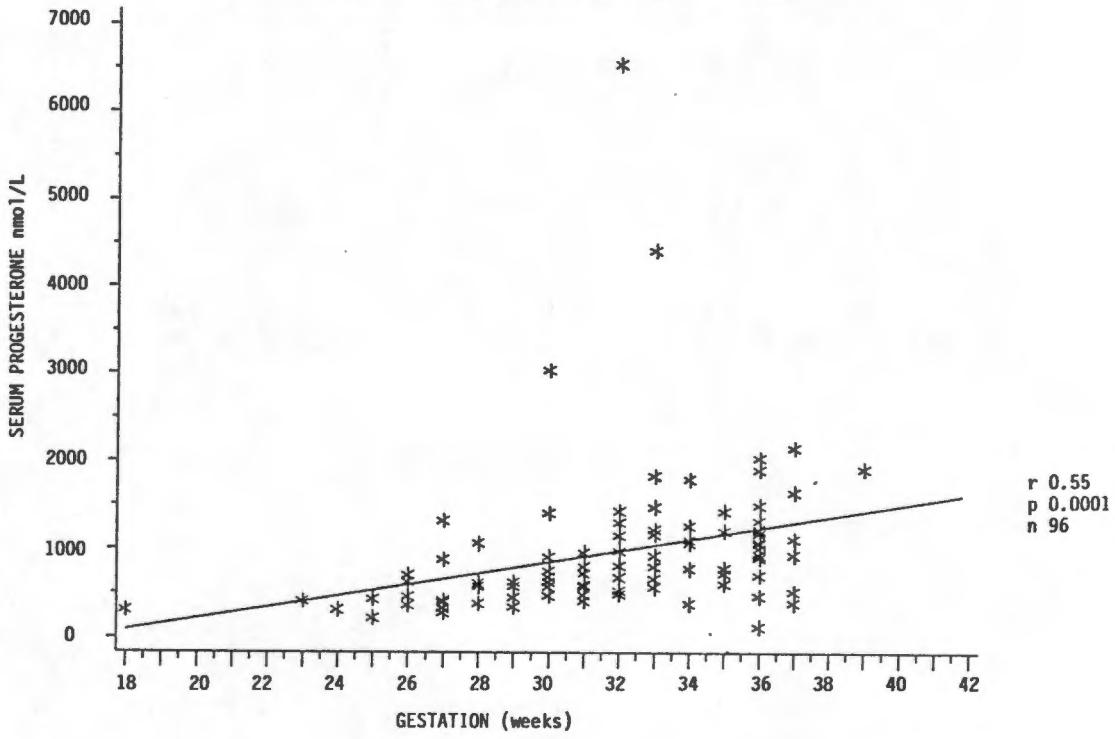


FIG. 4.12: RELATIONSHIP OF SERUM hPL TO GESTATIONAL AGE

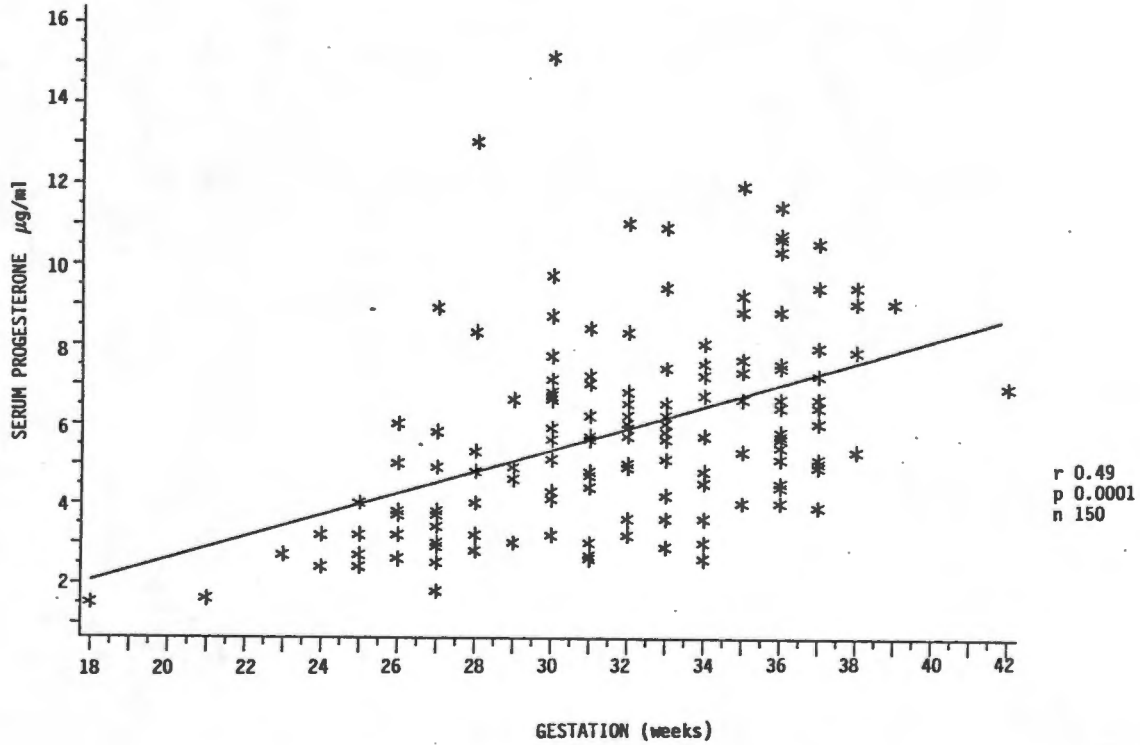
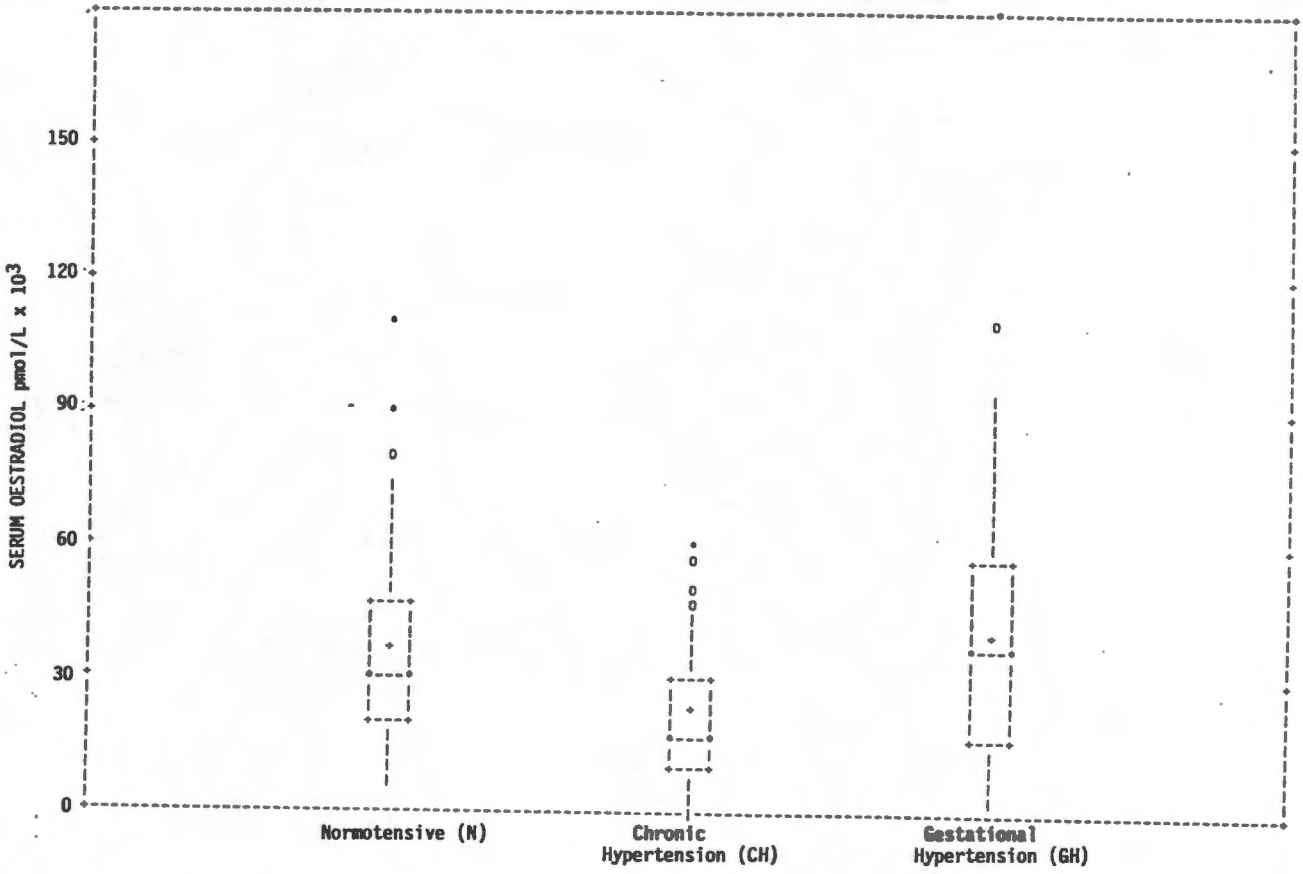


FIG. 4.13: SERUM OESTRADIOL IN NORMOTENSIVE AND HYPERTENSIVE PATIENTS



The relationship of placental hormones to diagnosis and birthweight percentile was evaluated by analysis of covariance (Table 4.VI) after adjustment for gestational age. Oestradiol values were lower in chronic hypertensives than both the other groups, (p Ø.Ø14) Figure 4.13. This was not related to the eventual birthweight percentile (p > Ø.Ø5).

Serum progesterone and hPL did not differ between diagnostic groups but mean hPL was significantly lower in mothers of infants where birthweight was < 10th percentile (p 0.0016 F = 10.4). Oestradiol, progesterone and hPL were positively correlated with each other which is probably a reflection of their common relation to gestational age and placental function. (Table 4.VII)

Oestradiol to progesterone ratios were unaffected by gestational age (p 0.63) and did not differ between the groups.

Linear regression analysis showed no relationship of placental hormone levels to diastolic or systolic blood pressure.

TABLE 4.VI : SERUM OESTRADIOL, PROGESTERONE AND hPL IN NORMAL AND HYPERTENSIVE PREGNANCY

(Mean (SEM) adjusted for gestational age)

	n	OESTRADIOL (pmol/1)	n	PROGESTERONE (nmol/1)	n	hPL (ug/ml)
NORMOTENSIVE	43	36,100 (3800)	35	1039 (135)	43	6.3 (0.3)
CHRONIC HYPERTENSION	34	24,100 (4300)	23	700 (167)	34	5.1 (0.3)
GESTATIONAL HYPERTENSION	71	39,900 (2900)	38	1044 (129)	73	5.8 (0.2)
		p 0.014		p 0.1		p 0.08

TABLE 4.VII

INTERRELATIONSHIPS OF PLACENTAL HORMONES n = 96

(Spearman rank correlation coefficients (r))

	Progesterone r	hPL r
Oestradiol	Ø.465*	Ø.333*
Progesterone	-	Ø.6Ø5*

* p = Ø.ØØØ1

4.4.5 Maternal Prostanoids

In the initial part of the study from 1 March to 31 October 1984, blood and urine samples were obtained from 96 patients for assay of four prostanoid metabolites (PGE, PGFM, 6 keto $PGF_{1\alpha}$, TxB_2), renal function and full blood count. In the latter part of the study from 1 January to 31 October 1985, it was necessary to make concessions to the escalating costs of radioimmunoassay kits and analysis of 6 keto $PGF_{1\alpha}$ and TxB_2 only was undertaken. During this time an additional 47 maternal blood samples were obtained.

4.4.5.1 Prostanoid Metabolite Immunoreactivity

The measured levels of prostanoid immunoreactivity in maternal plasma and urine are summarised in Table 4.VIII. Results are expressed as the median value with

the interquartile distance ($Q_3 - Q_1$) as a measure of variation.

TABLE 4.VIII : CONCENTRATIONS (pg/ml) OF PROSTANOIDS IN MATERNAL PLASMA AND URINE

Median (Interquartile range $Q_3 - Q_1$)

	n	Plasma	n	Urine
PGE	94	1914 (3677)	95	2919 (4131)
PGFM	94	246 (727)	95	9 (46)
6 KETO $PGF_{1\alpha}$	142	408 (379)	95	553 (297)
TxB_2	142	422 (787)	95	19 (178)

The values are high and ranges wide, particularly for PGE. Urine concentrations of PGFM and TxB_2 in many patients were below the sensitivity of the assays which were 7.1 pg/ml and 9.4 pg/ml respectively (See 4.3.3.3). To allow numerical analysis of the data, these values were recorded as this lowest readable value.

As there were differences in some parameters between the diagnostic groups (see 4.4.1 and 4.4.2) linear regression analysis was performed to assess the effect of these factors on prostanoid levels. An assessment

was therefore made to assess if the age of the mother, the levels of diastolic and systolic blood pressure in mid pregnancy or the urea, urate and creatinine had any effect on the prostanoid levels in the group of normotensive patients. There were no statistically significant relationships between plasma or urine prostanoid levels and maternal age, midpregnancy blood pressure or renal function tests. These factors were therefore considered unlikely to be confounding variables in the analysis of differences between normotensive and hypertensive pregnancies. Plasma PGFM and 6 keto $PGF_{1\alpha}$ immunoreactivity however had a positive correlation to parity (Spearman rank correlations r = Ø.44 p Ø.ØØ8 and r = Ø.38 p Ø.Ø125 respectively).

4.4.5.2 Effect of gestational age

Plasma prostaglandin and thromboxane levels were not related to gestational age, with correlation coefficients ranging from -Ø.ØØ7 to Ø.144.

Urine PGE and PGFM excretion were also unrelated to gestational age (r -Ø.Ø5 p Ø.62 and r -Ø.18 p Ø.Ø8 respectively). Urinary 6 keto $PGF_{1\alpha}$ and TxB_2 had a weak but statistically significant negative correlation to gestational age (r -Ø.28 p Ø.ØØ7 and r -Ø.29 p Ø.ØØ4 respectively).

4.4.5.3 Prostanoid interrelationships

The most consistent finding was a positive correlation of each prostanoid in the plasma with each of the others (p Ø.ØØØ1). Urinary prostanoid excretions were also positvely correlated to each other. Scatter plots of these relationships are shown in Figures 4.14 - 4.25. They demonstrate that the relationship is most striking if the values are extremely high. Plasma prostanoid levels were not however related to excretion of the corresponding metabolite in the urine with correlation coefficients ranging from Ø.ØØØ to Ø.1Ø7.

FIG. 4.14: RELATION BETWEEN PLASMA 6 KETO PGF1α AND PLASMA PGFM

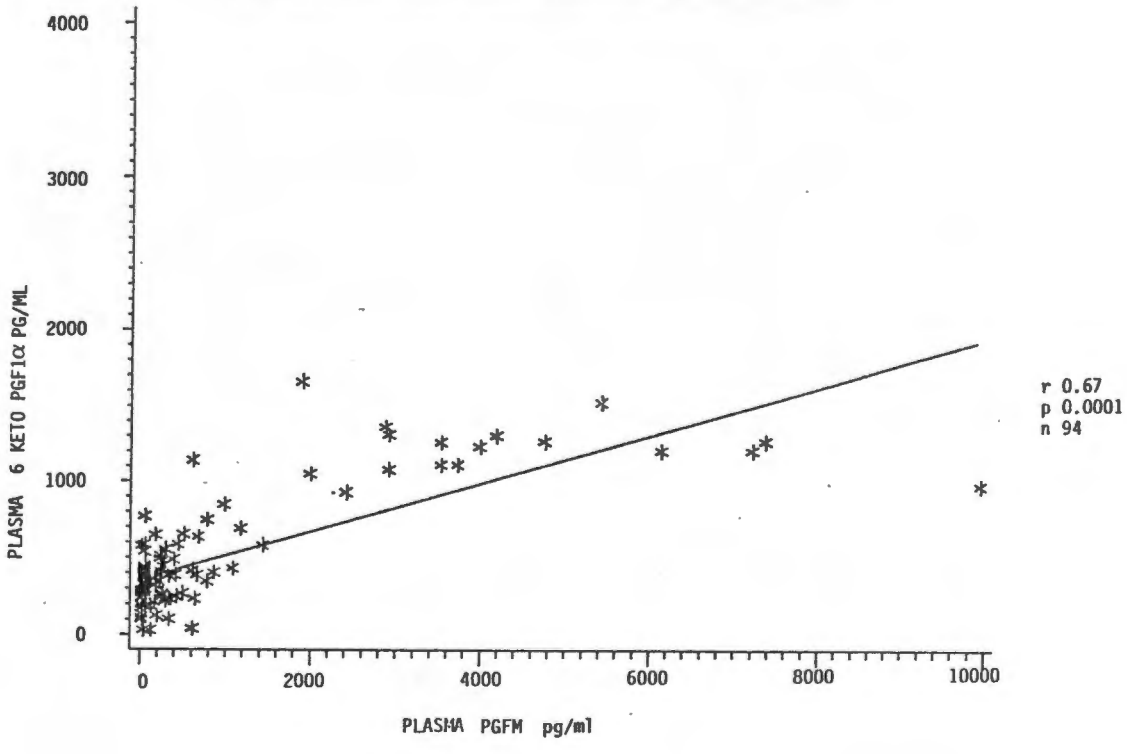


FIG. 4.15: RELATION BETWEEN PLASMA 6 KETO PGF1α AND PLASMA PGE

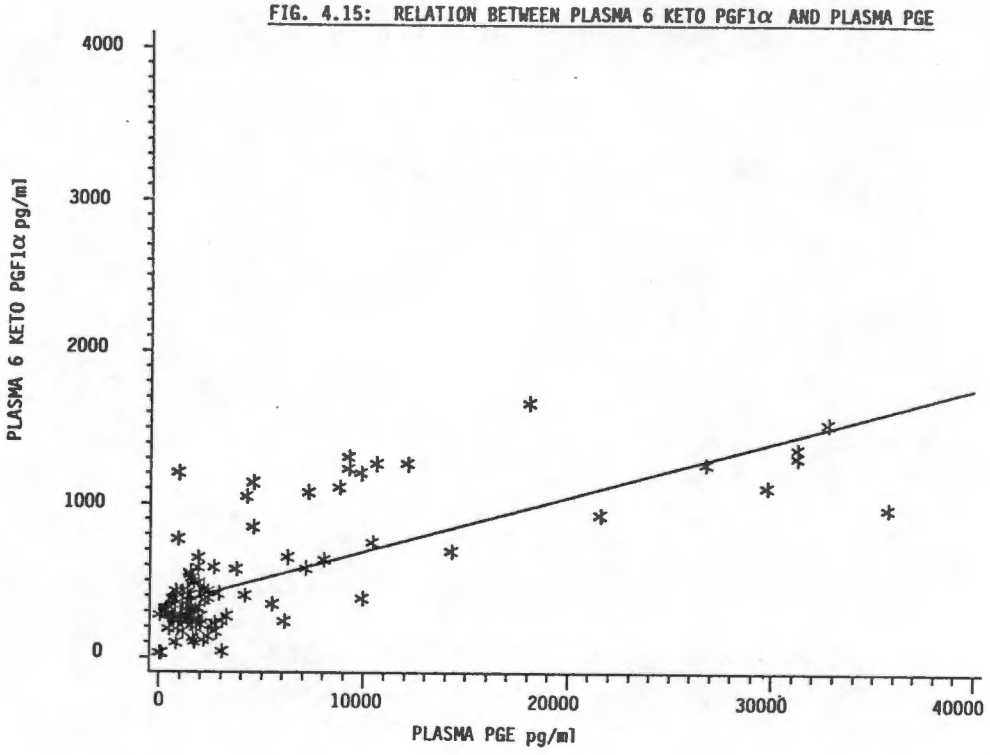


FIG. 4.16: RELATION BETWEEN PLASMA 6 KETO PGF1α AND PLASMA TxB_2

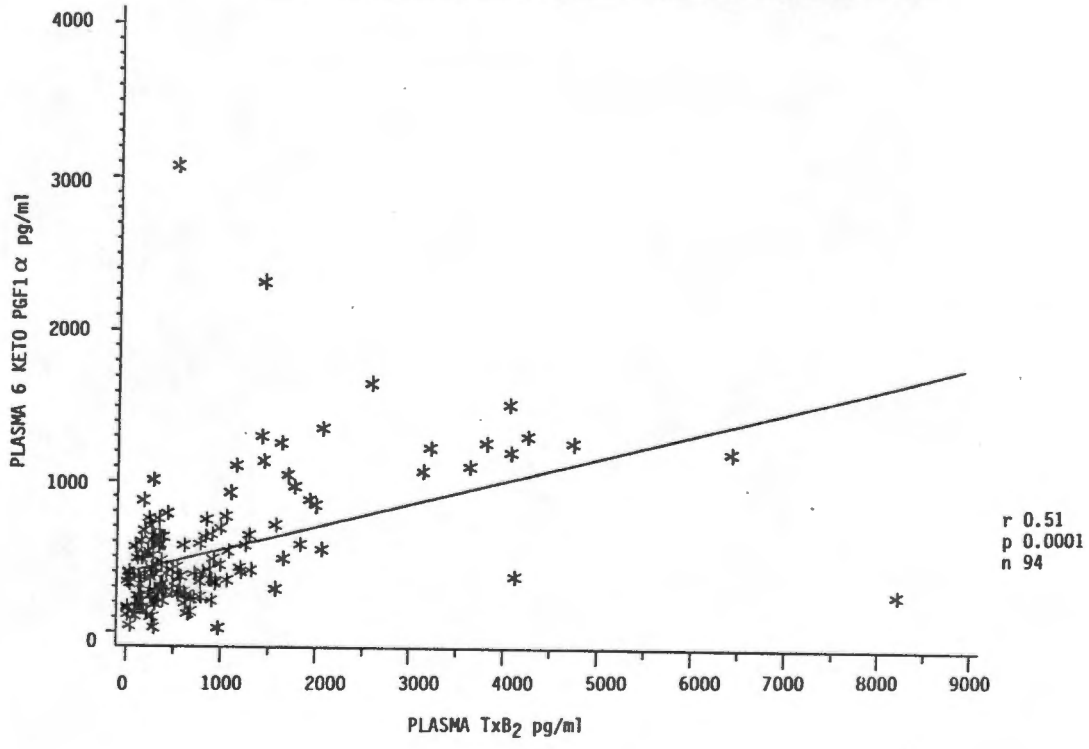


FIG. 4.17: RELATION BETWEEN PLASMA PGFM AND PLASMA PGE

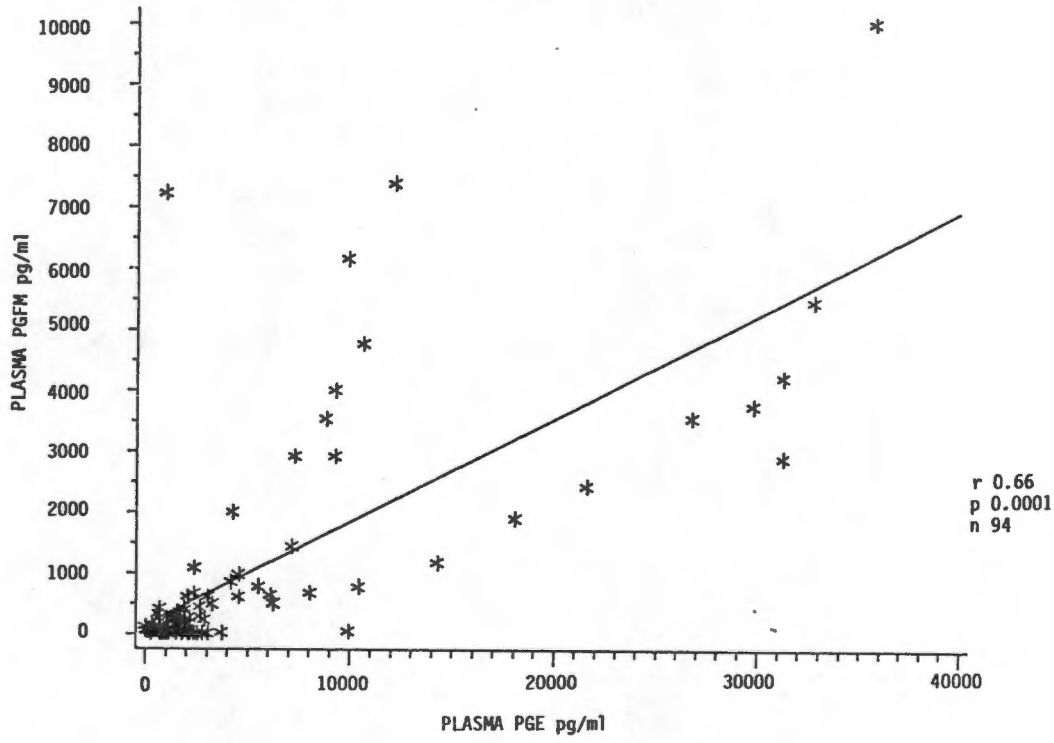


FIG. 4.18: RELATION BETWEEN PLASMA PGFM AND PLASMA TxB_2

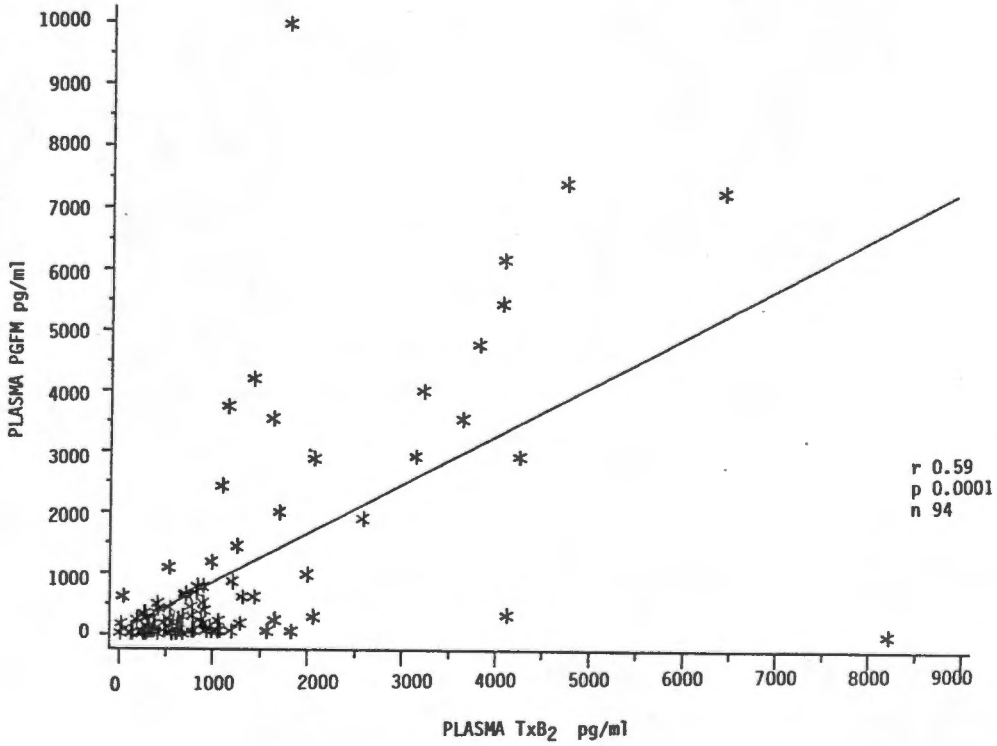


FIG. 4.19: RELATION BETWEEN PLASMA PGE AND PLASMA TxB_2

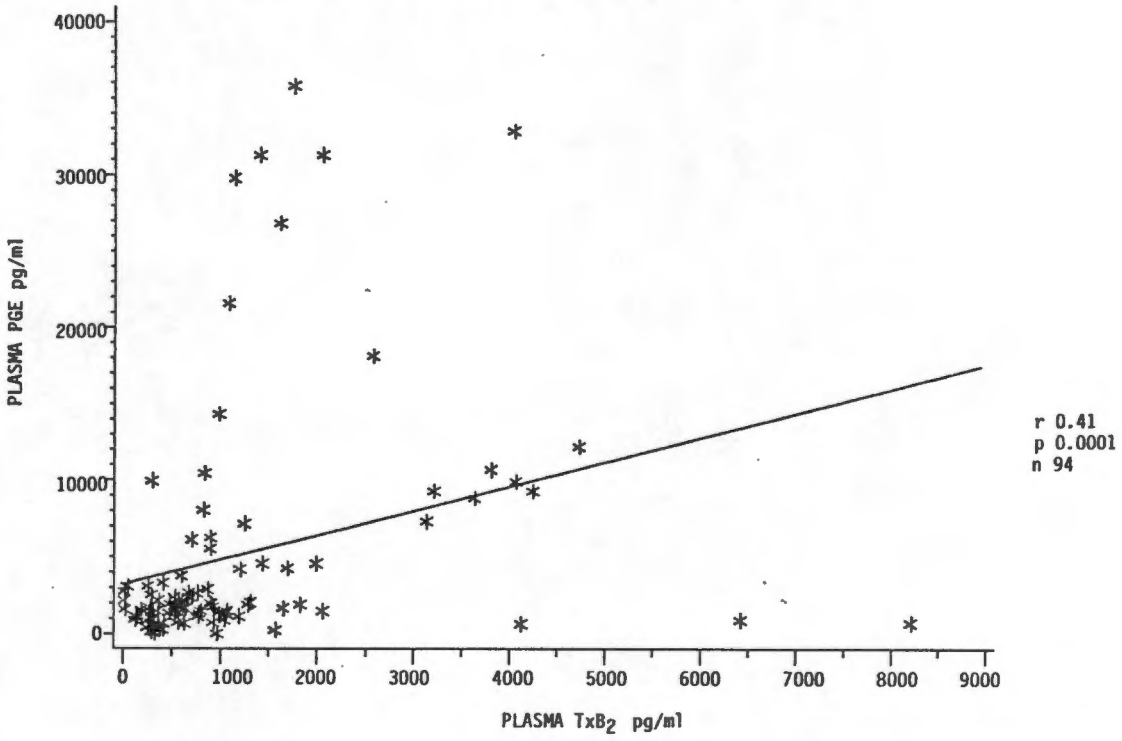


FIG. 4.20: RELATION BETWEEN TOTAL 4 HOUR URINARY EXCRETION OF 6 KETO PGF1α AND TxB_2

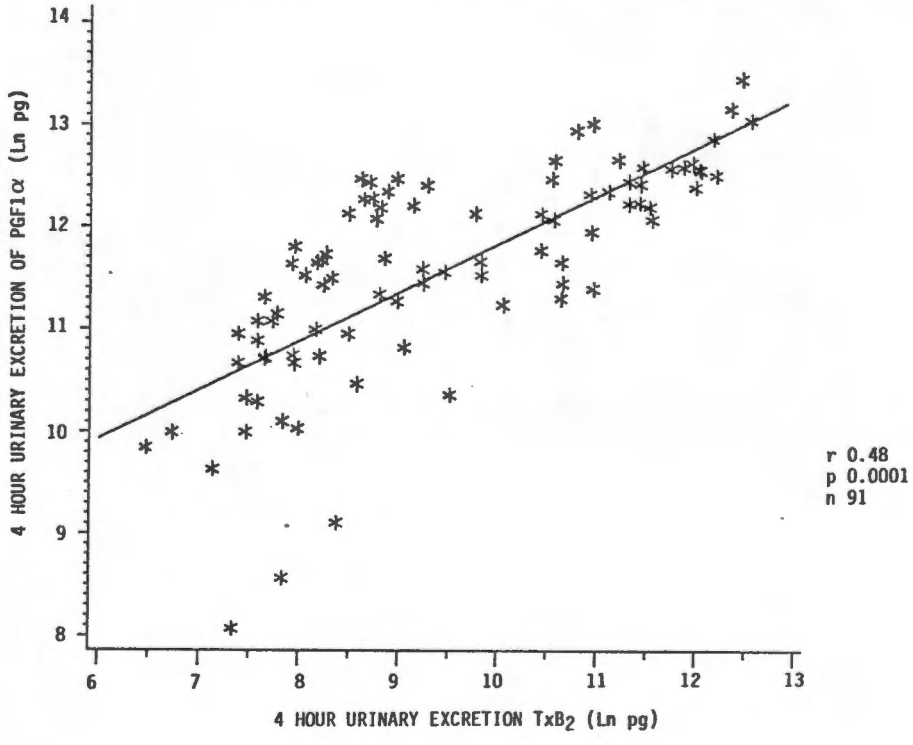


FIG 4.21: RELATION BETWEEN TOTAL 4 HOUR URINARY EXCRETION OF PGE AND 6 KETO PGF1α

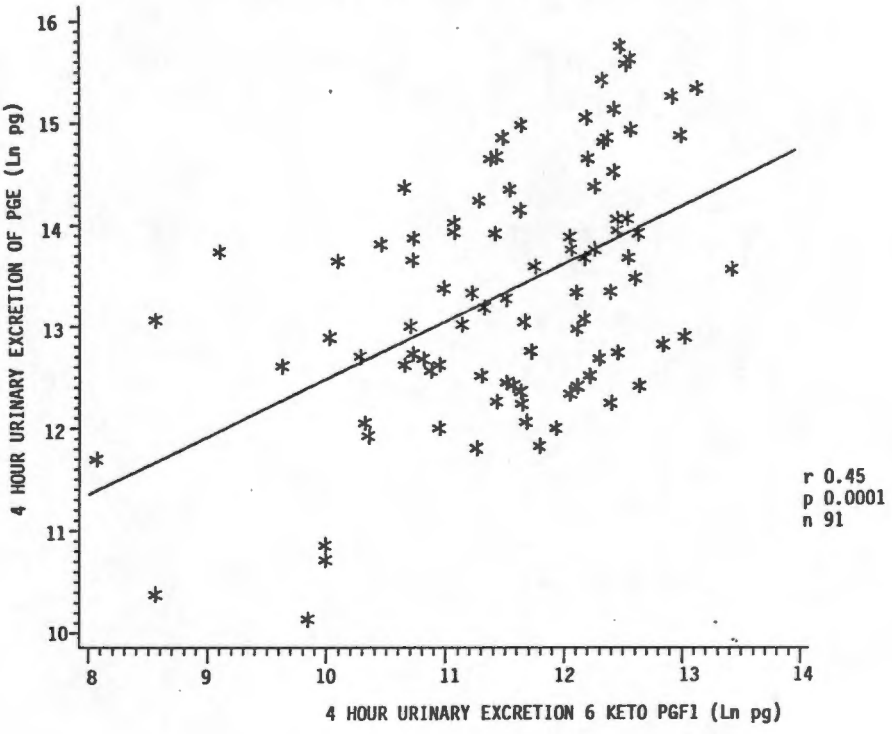


FIG. 4.22: RELATION BETWEEN TOTAL 4 HOUR URINARY EXCRETION OF 6 KETO PGF1α AND PGFM

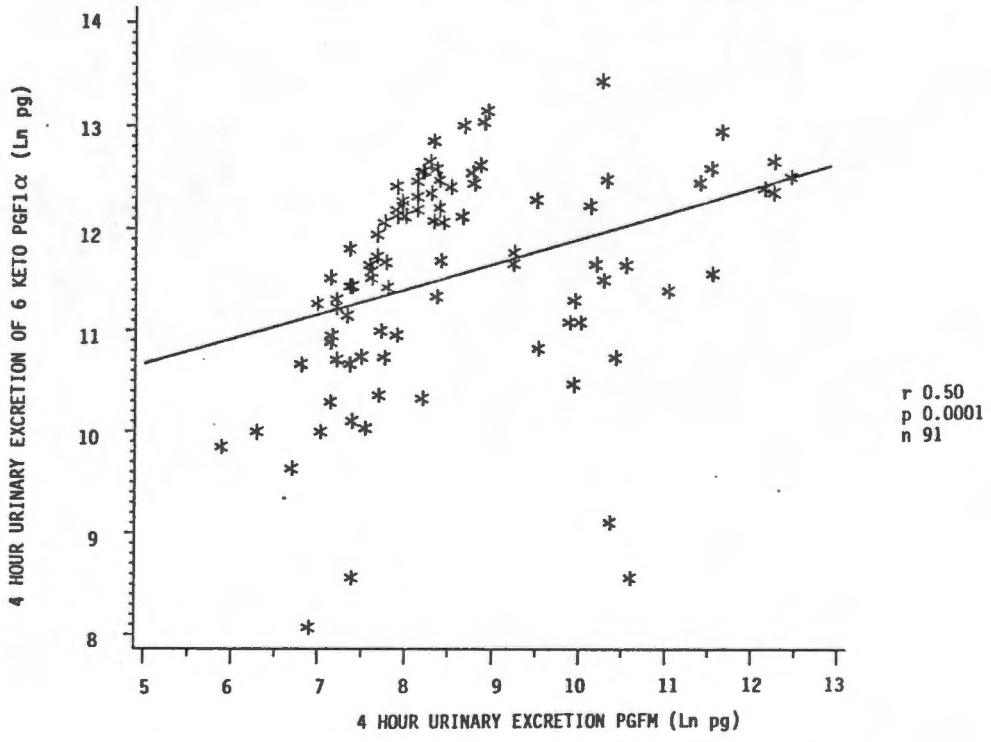


FIG. 4.23: RELATION BETWEEN TOTAL 4 HOUR URINARY EXCRETION OF PGE AND TxB_2

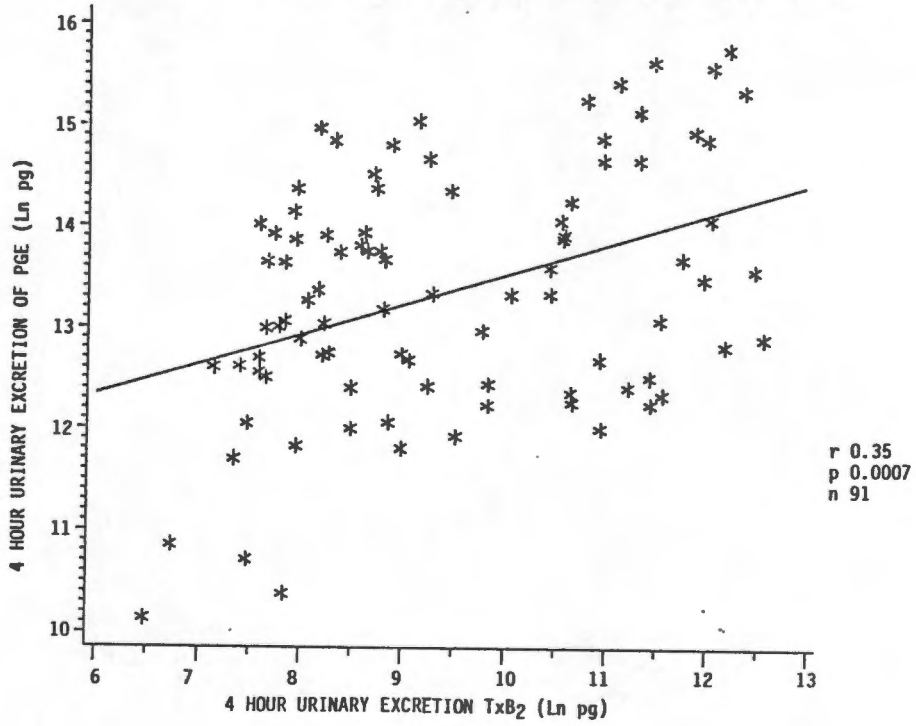


FIG. 4.24: RELATION BETWEEN TOTAL 4 HOUR URINARY EXCRETION OF PGFM AND TxB_2

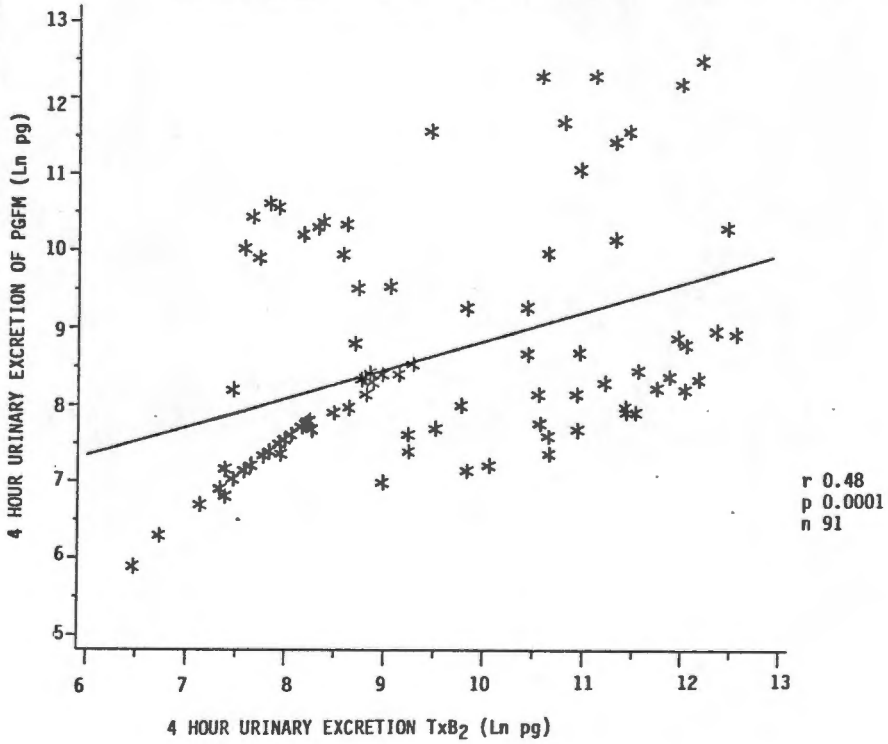
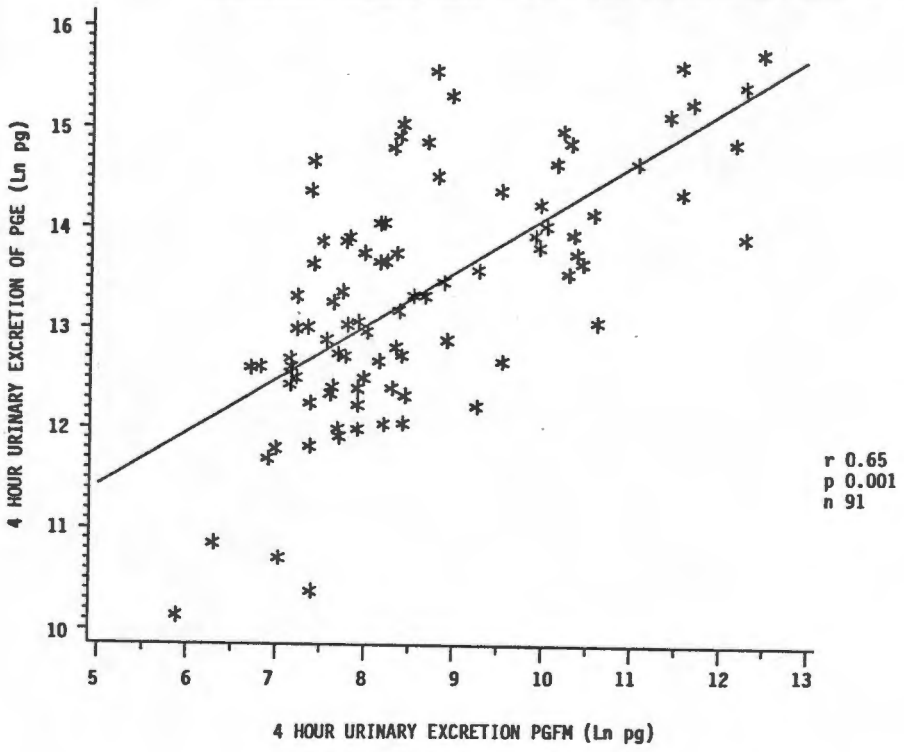


FIG. 4.25: RELATION BETWEEN TOTAL 4 HOUR URINARY EXCRETION OF PGE AND PGFM



As the prostanoid values were not normally distributed logarithmic transformation (Log n) of the data was performed to improve the normality of the data and allow more detailed statistical analysis of the effects of hypertension and fetal growth (Appendix 9 Tables I and II). The effectiveness of the transformation in creating a Gaussian distribution can be judged from the distribution displays in Figures 4.26 - 4.33 which show the logarithms of the levels of plasma prostanoids and

the urinary prostanoid excretion in each diagnostic group. Plasma prostanoid concentration and the total urinary prostanoid excretion over the 4 hour collection period were studied by analysis of variance and Spearman rank correlation. Where appropriate, analysis of covariance was used to consider the effects of gestational age and parity. An overall significance level of p = Ø.Ø5 was used.

4.4.5.4 Comparison of Plasma Prostanoids between Normal and Hypertensive Pregnancies

The actual values of the plasma prostanoids are shown in Table 4.IX and the logarithmic values in Appendix 9 Table I.

TABLE 4.IX: MATERNAL PLASMA CONCENTRATIONS (pg/ml) OF PROSTANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCIES

MEDIAN (Q_3-Q_1)

	NORMOTENSIVE		CHRONIC HYPERTENSION		GESTATIONAL HYPERTENSION	
	n		n		n	
PGE	38	1488(3788)	22	2158(6346)	35	2119(3241)
PGFM	35	262(727)	21	242(1Ø27)	38	191(754)
6KETOPGF$_{1\alpha}$	43	415(2ØØ)	31	387(361)	68	361(416)
TxB$_2$	43	781(27Ø)	31	319(168)	68	365(184)

There were significant differences in plasma TxB_2 levels between the diagnostic groups. Plasma TxB_2 immunoreactivity was lower in both the chronic hypertensives (p Ø.Ø154) and gestational hypertensives (p Ø.Ø172) than the normotensive women. (Figure 4.26).

In the plasma the median values of PGE were higher in hypertensive than normotensive patients, but due to the wide range of values, analysis of variance of the logarithmic values demonstrated no differences among the three groups (p Ø.35). (Figure 4.27).

Median values of PGFM were similar between normotensive pregnancies and those complicated by hypertension (p Ø.84). Figure 4.28.

Plasma 6 keto $PGF_{1\alpha}$ levels were lower in hypertensives than normotensives, the lowest median value 361 (Q_3–Q_1 416) pg/ml being measured in gestational hypertensives but the analysis of variance showed that these differences were not statistically significant (p Ø.28). Figure 4.29.

FIG. 4.26: PLASMA TxB_2 LEVELS IN NORMAL AND HYPERTENSIVE PREGNANCIES

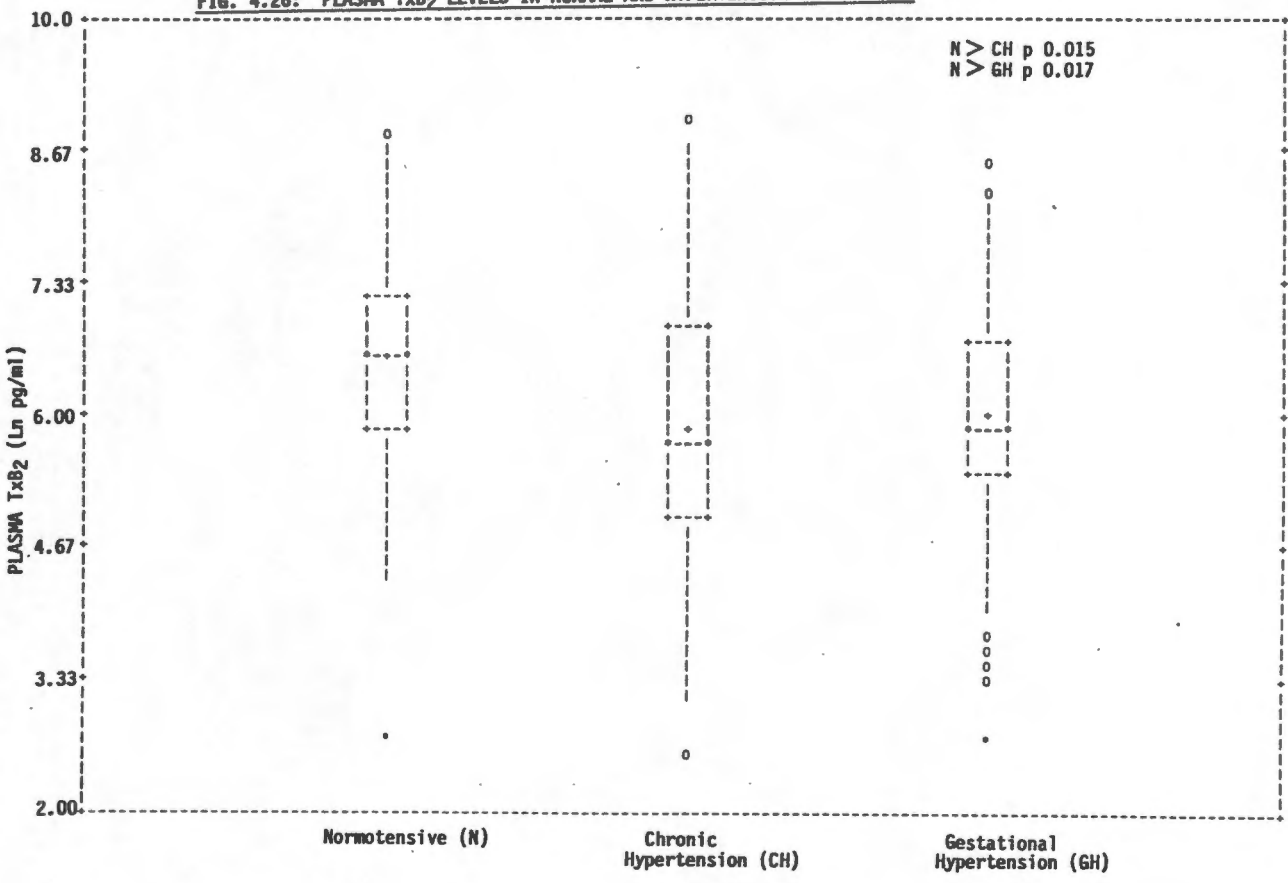


FIG. 4.27: PLASMA PGE LEVELS IN NORMAL AND HYPERTENSIVE PREGNANCIES

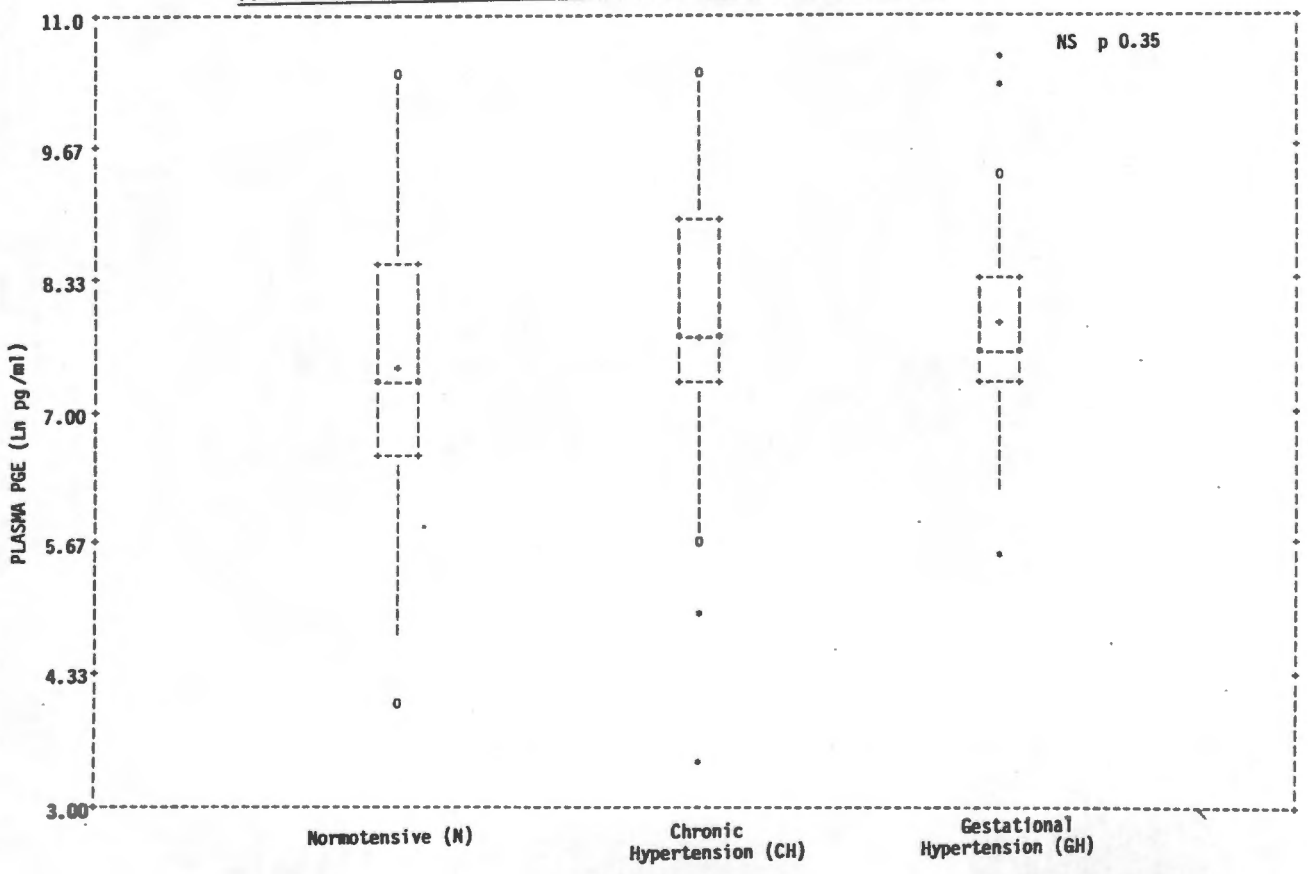


FIG. 4.28: PLASMA PGFM LEVELS IN NORMAL AND HYPERTENSIVE PREGNANCIES

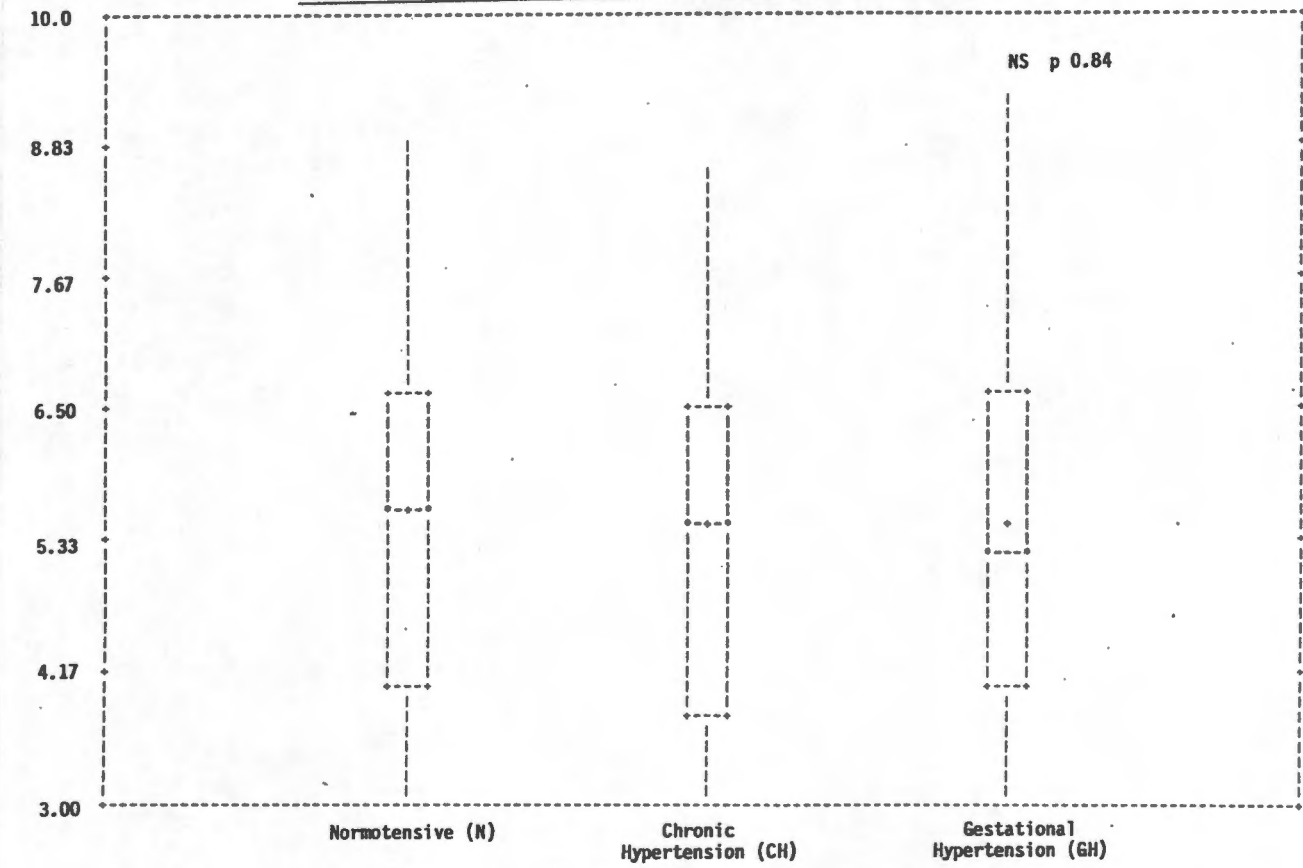
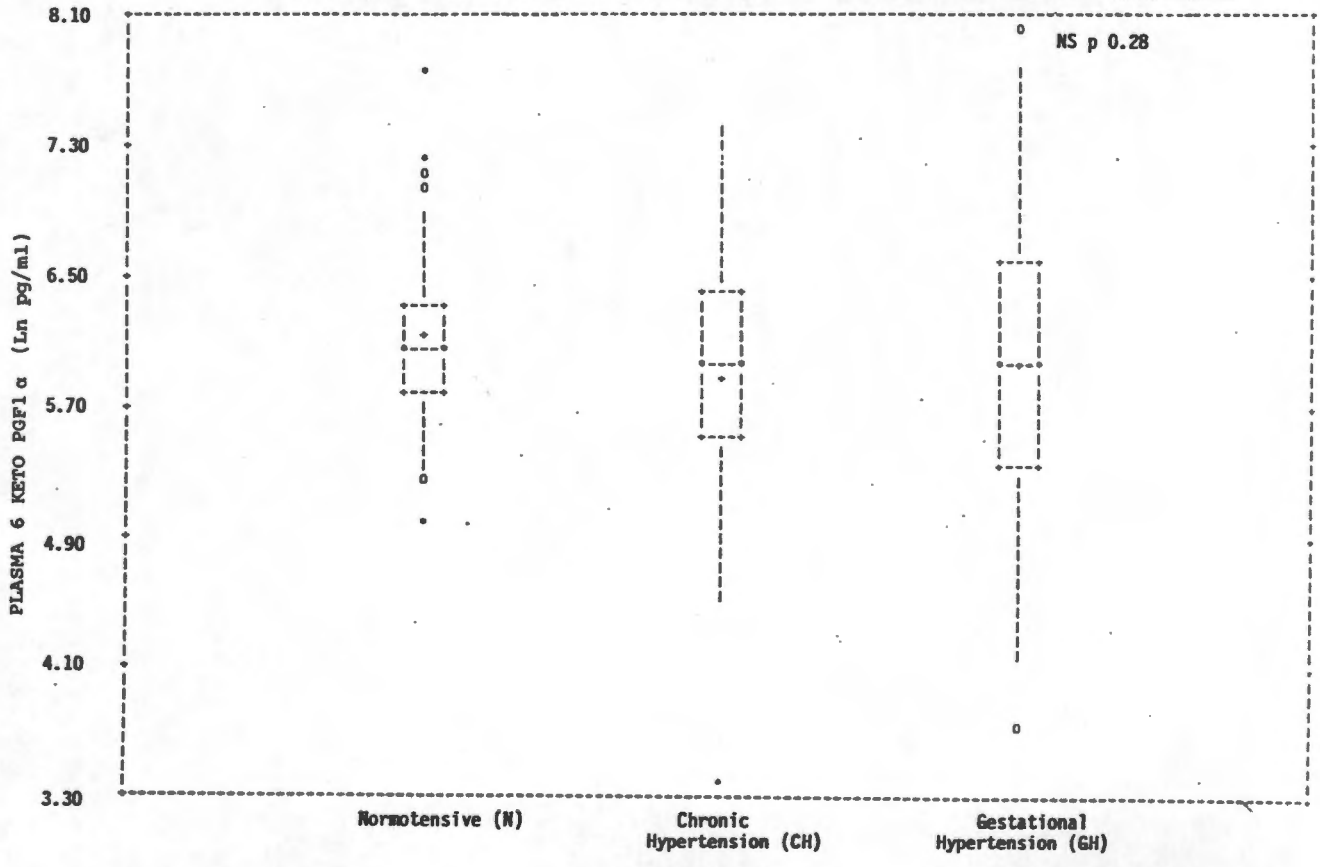


FIG: 4.29: PLASMA 6 KETO PGF1 α LEVELS IN NORMAL AND HYPERTENSIVE PREGNANCIES



4.4.5.5 Comparison of Urinary Prostanoids between Normal and Hypertensive Pregnancies

Urinary prostanoids were studied by analysis of variance for PGE and PGF and analysis of covariance (to take account of gestational age) for 6 keto $PGF_{1\alpha}$ and TxB_2. The actual values of the urinary prostanoid concentration and the 4 hour urinary excretion are shown in Table 4.X and 4.XI.

TABLE 4.X: MATERNAL URINE CONCENTRATIONS (pg/ml) OF PROSTANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCIES

MEDIAN (Q_3-Q_1)

	NORMOTENSIVE (n 35)	CHRONIC HYPERTENSION (n 22)	GESTATIONAL HYPERTENSION (n 38)
PGE	3144 (4191)	2170 (4435)	1946 (3211)
PGF	9 (81)	9 (0)	9 (51)
6KETOPGF$_{1\alpha}$	513 (256)	520 (439)	607 (321)
TxB_2	18 (250)	16 (188)	60 (172)

TABLE 4.XI: MATERNAL 4 HOUR URINARY EXCRETION (ng) OF PROSTANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCIES

MEDIAN (Q_3-Q_1)

	NORMOTENSIVE (n 35)	CHRONIC HYPERTENSION (n 22)	GESTATIONAL HYPERTENSION (n 38)
PGE	856 (1890)	459 (1212)	468 (844)
PGF	4 (23)	3 (5)	4 (11)
6KETOPGF$_{1\alpha}$	86 (177)	108 (196)	171 (171)
TxB$_2$	6 (59)	7 (43)	10 (53)

As the prostanoid values were not normally distributed, the effect of hypertension on 4 hour urinary prostanoid excretion was assessed after logarithmic transformation of the data. The mean values in each diagnostic group after logarithmic transformation are shown in Appendix 9 Table II. Although the median excretion rate of PGE was lower in the hypertensive patients these differences were not statistically significant (p 0.29). (Figure 4.30). The total urinary excretion rates of the other prostanoids were also similar between the diagnostic groups. (Figure 4.31 - 4.33).

FIG. 4.30: URINARY PGE EXCRETION RATE IN NORMAL AND HYPERTENSIVE PREGNANCIES

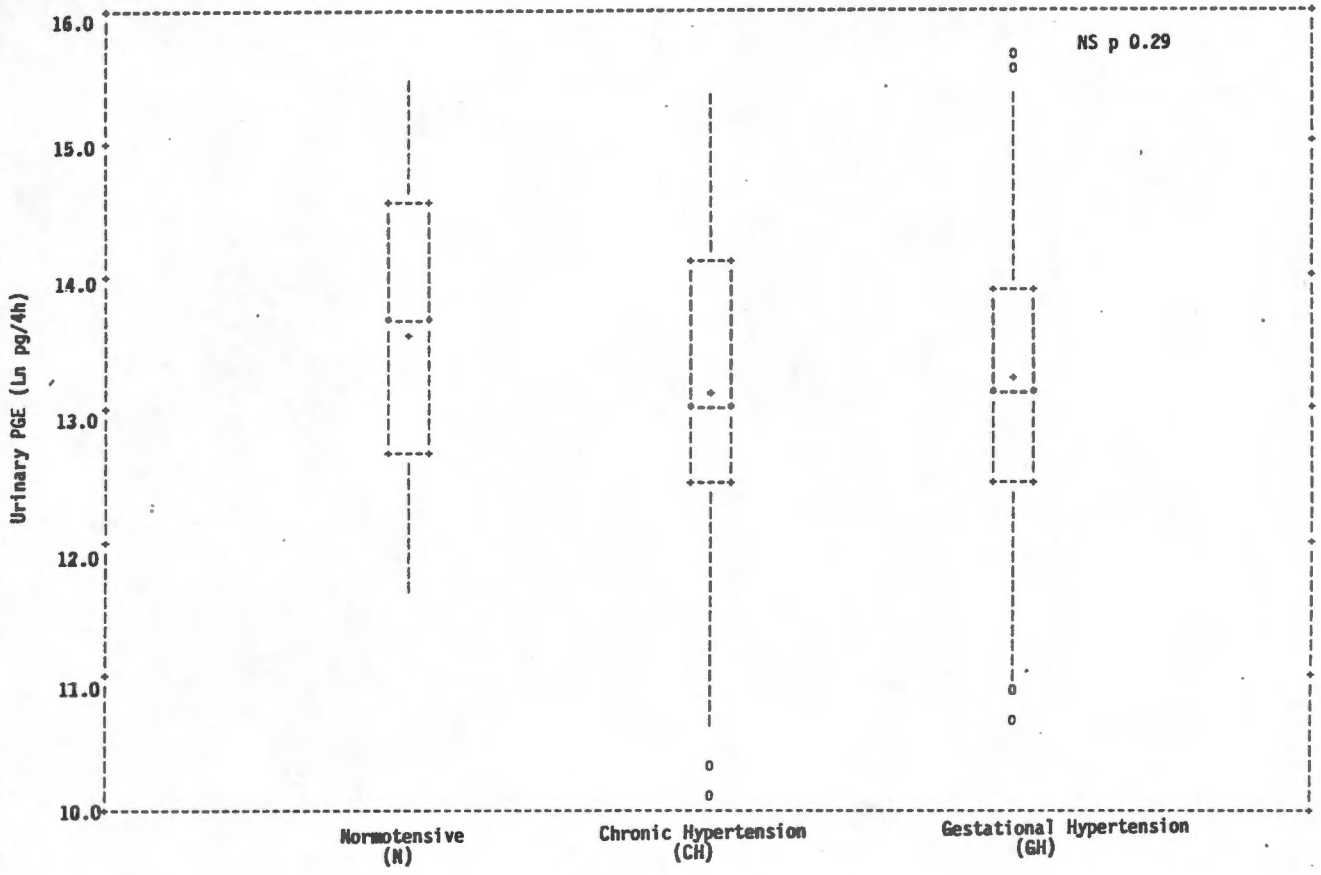


FIG. 4.31: URINARY PGFM EXCRETION RATE IN NORMAL AND HYPERTENSIVE PREGNANCIES

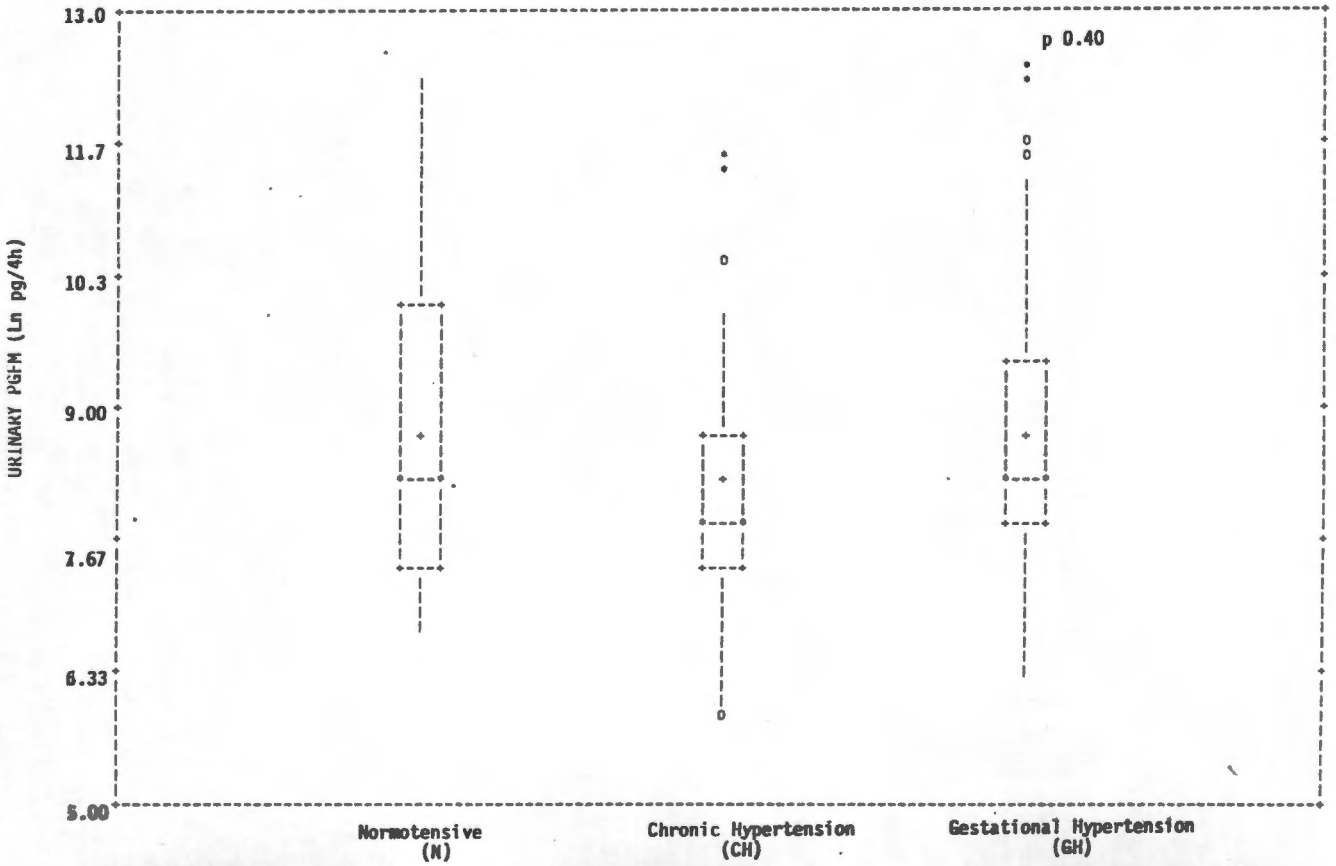


FIG. 4.32: URINARY 6 KETO PGF1α EXCRETION RATE IN NORMAL AND HYPERTENSIVE PREGNANCIES

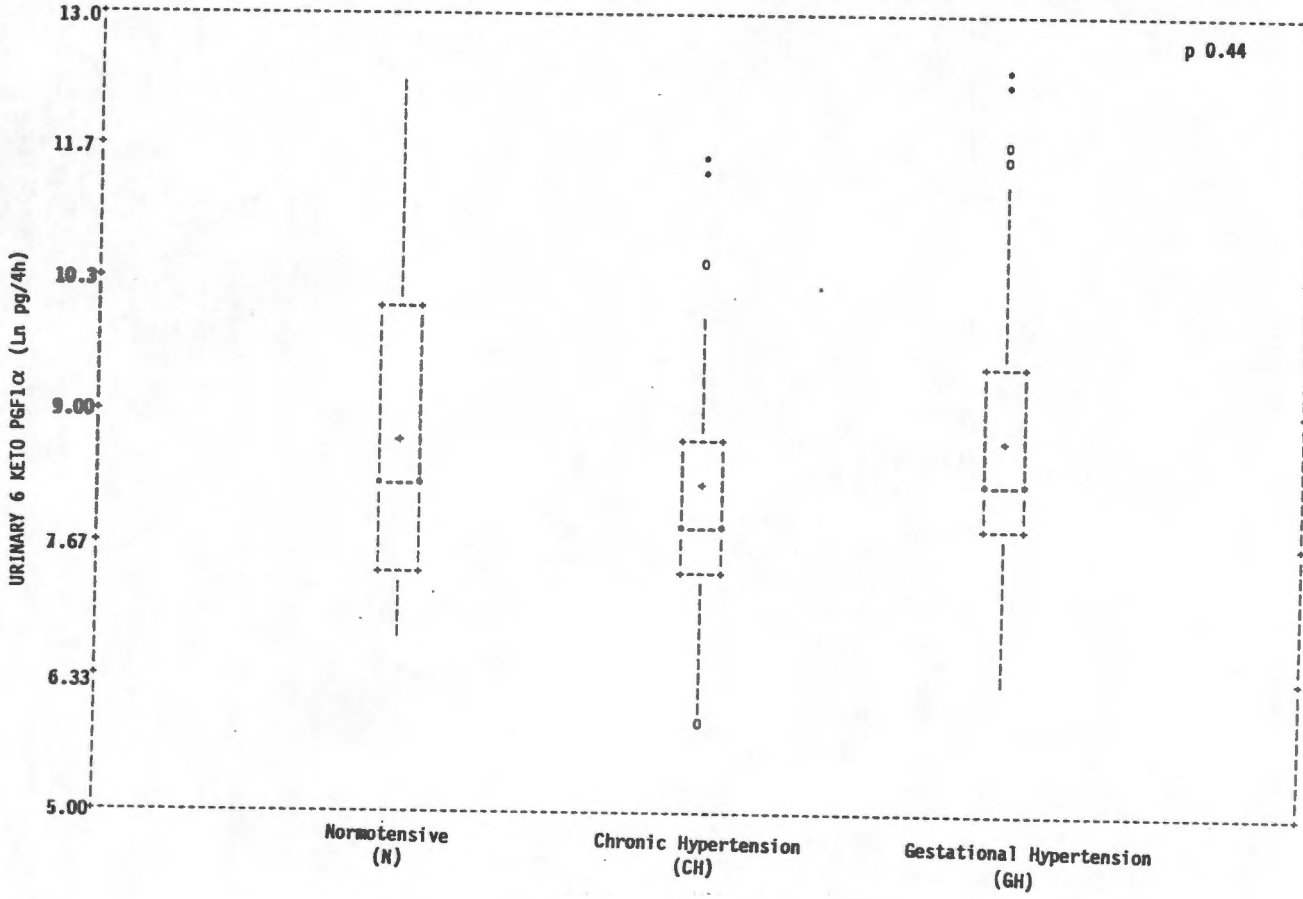
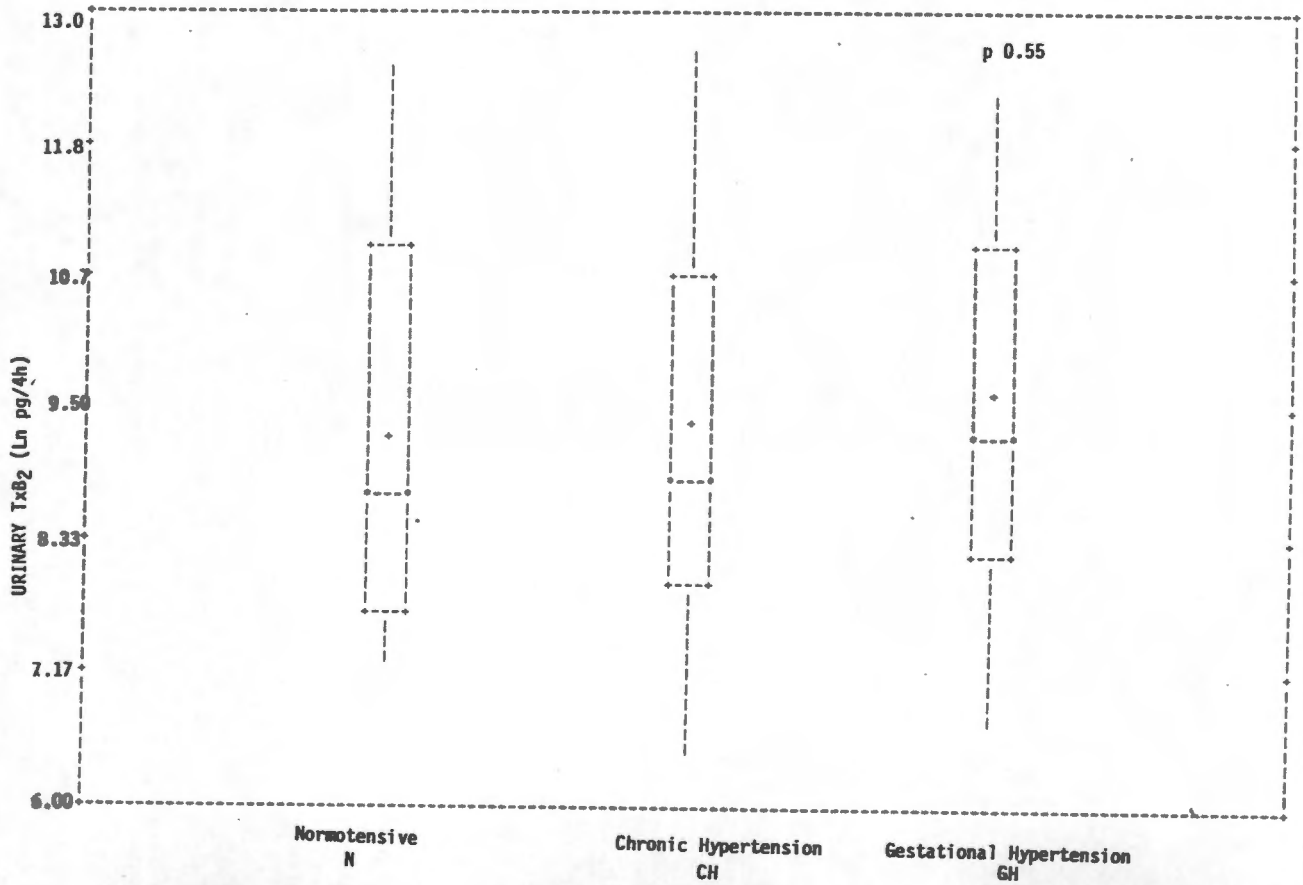


FIG. 4.33: URINARY TxB_2 EXCRETION RATE IN NORMAL AND HYPERTENSIVE PREGNANCIES



4.4.5.6 Effect of blood pressure

Linear regression analysis was performed to assess the relationships of maternal prostanoids to the blood pressure at the time of sampling. Plasma TxB_2 levels had a tendency to a negative correlation with DBP (r -Ø.18 p Ø.Ø3Ø) but plasma concentrations were otherwise unrelated to blood pressure.

In the urine PGE excretion rates had a weak inverse relationship with DBP (r = -Ø.28 p Ø.ØØ5) and SBP (r = -Ø.23 p Ø.Ø19).

4.4.5.7 Prostanoid Ratios in Hypertensive Diseases of Pregnancy

As the vasodilator and vasoconstrictor interaction of the prostanoids may be more important than the absolute levels, the ratios of one to another in plasma or urine were compared between the groups by analysis of variance and covariance. An overall significance level of p = Ø.Ø5 was taken, giving a significance level p = Ø.Ø16 for any comparisons of two of the three groups.

The results, unexpectedly, showed a tendency to an excess of vasodilator prostaglandins over vasoconstrictors in the plasma of patients with hypertension. The 6 keto $PGF_{1\alpha}/TxB_2$ ratio was higher

in both hypertensive groups than normotensive (p Ø.Ø35), with the highest ratios in the chronic hypertensive group. Plasma E_2/TxB_2 ratios were also higher in chronic hypertensives (p Ø.Ø11) and gestational hypertensives (p Ø.Ø17) compared to the controls.

The plasma PGE/PGFM ratio tended to be higher in the gestational hypertensives than normotensives (p Ø.Ø27) with chronic hypertensives having an intermediate value. Plasma 6 keto $PGF_{1\alpha}$/PGFM ratios did not differ between the diagnostic groups. The mean ratios of the plasma prostanoids are shown in Table 4.XII.

TABLE 4.XII: RATIOS OF MATERNAL PLASMA PROSTANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCIES

(Mean adjusted for gestational age)

	n	6 keto $PGF_{1\alpha}$: TxB_2	n	PGE : TxB_2	n	PGE : PGFM	n	6 keto $PGF_{1\alpha}$: PGFM
NORMOTENSIVE	43	0.66	35	1.8	35	6.1	35	1.63
GESTATIONAL HYPERTENSION	31	1.02	21	4.9	21	9.0	21	1.43
CHRONIC HYPERTENSION	68	0.97	38	4.1	38	11.0	34	1.55
OVERALL p		0.034*		0.015*		0.079		0.517

This relative excess of vasodilator over vasoconstrictor prostanoids in patients with hypertension was also found on analysis of the relationships of the plasma prostanoid ratios in all the patients to the blood pressure. The ratio of PGE to TxB_2 was positively correlated to the diastolic and systolic blood pressures (r Ø.24 p Ø.Ø17 and r Ø.26 p Ø.Ø11 respectively). Weak positive correlations of the PGE/PGFM and 6 keto $PGF_{1\alpha}$:TxB_2 to DBP were also demonstrated (r = Ø.25 p Ø.Ø14 and r = Ø.2Ø p Ø.Ø16 respectively).

In the gestational hypertensives the 6 keto $PGF_{1\alpha}$: PGFM ratio was also related to DBP (r Ø.44Ø p Ø.ØØ5).

The ratios of the prostanoids in the urine did not differ between the diagnostic groups (Table 4.XIII). PGE/TxB_2 ratios had a tendency to be lower in gestational hypertensives compared to the normals (mean 36.6 compared to 73.7) but the differences did not achieve statistical significance (p Ø.Ø9). The PGE/TxB_2 ratio also showed a tendency to a weak inverse correlation to DBP (r = -Ø.19 p Ø.Ø61).

TABLE 4.XIII : RATIOS OF MATERNAL URINE PROSTANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCIES

(Mean adjusted for gestational age)

	n	6 keto $PGF_{1\alpha}$: TxB_2	n	PGE : TxB_2	n	PGE : PGFM	n	6 keto $PGF_{1\alpha}$: PGFM
NORMOTENSIVE	35	8.76	35	73.7	35	121.5	35	14.9
CHRONIC HYPERTENSION	22	8.41	22	44.7	22	121.5	22	24.5
GESTATIONAL HYPERTENSION	34	8.25	38	36.6*	38	90.0	34	18.7
Overall p		0.45		0.14		0.83		0.63

* p 0.09 vs Normotensives

4.4.5.8 Relation of Prostanoids to Duration and Severity of Gestational Hypertension

To further evaluate if the changes in prostanoids are secondary to the blood pressure changes rather than the primary initiating factor, the relationship of the prostanoids to the duration of hypertension was studied in the gestational hypertensives. The ratios of plasma PGE:PGFM and plasma 6 keto $PGF_{1\alpha}$:PGFM had a significant positive correlation to the duration of hypertension ($r = 0.38$ p 0.023 and $r = 0.36$ p 0.033 respectively).

This could be interpreted as an increase in vasodilators or suppression of vasoconstrictors in response to the hypertension. It is possible that in the hypertensives the vasoconstrictor PGF is suppressed thus, causing a rise in the ratio with increasing duration of hypertension as plasma PGFM shows a trend to an inverse correlation to the duration of blood pressure elevation (r Ø.32 p Ø.Ø57). The scatter plots of these relationships (Figures 4.34 - 4.36) show however, that these correlations are strongly affected by the few observations in patients with a long duration of hypertension and hence these conclusions may not be valid.

FIG. 4.34: RELATION BETWEEN DURATION OF GESTATIONAL HYPERTENSION AND THE RATIO OF PLASMA PGE TO PGFM

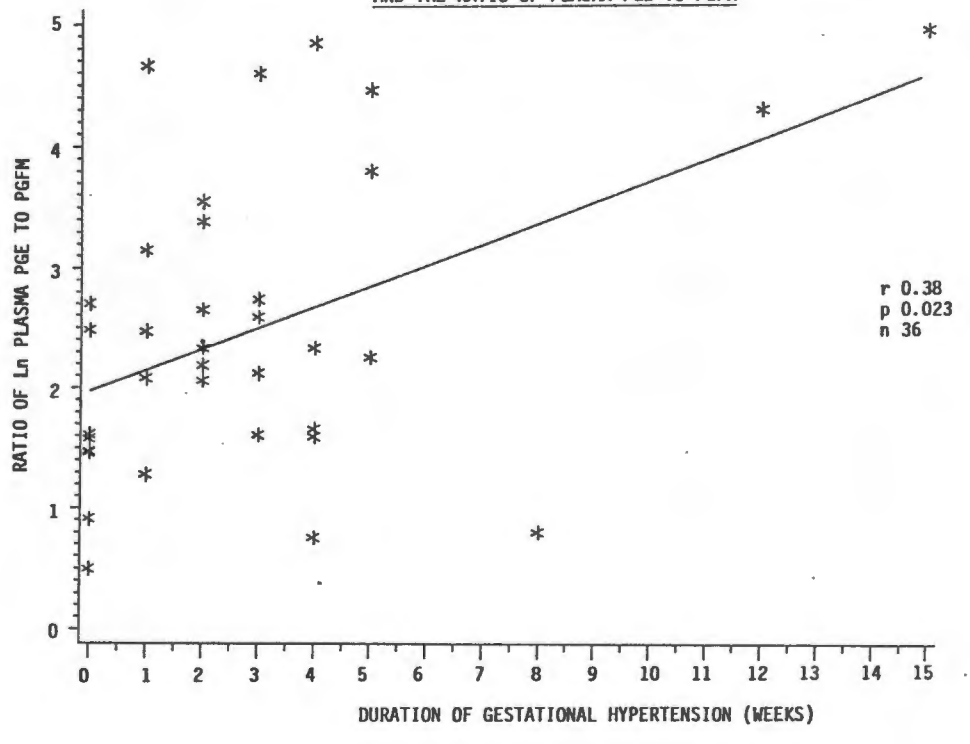


FIG. 4.35: RELATION BETWEEN DURATION OF GESTATIONAL HYPERTENSION AND THE RATIO OF PLASMA 6 KETO PGF1 α TO PGFM

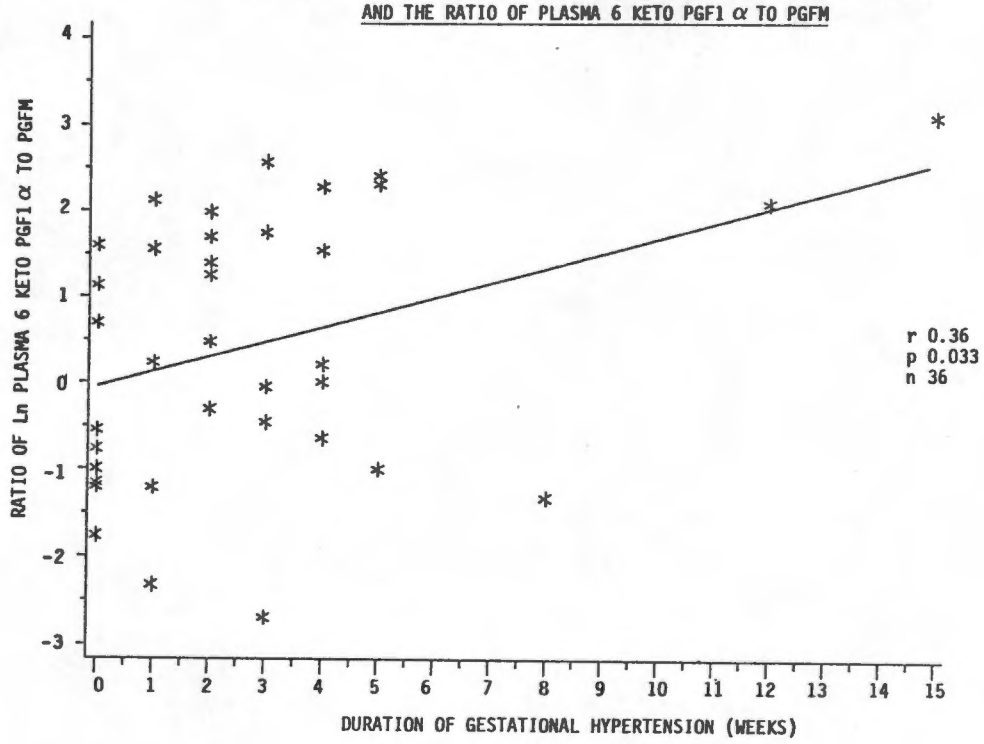
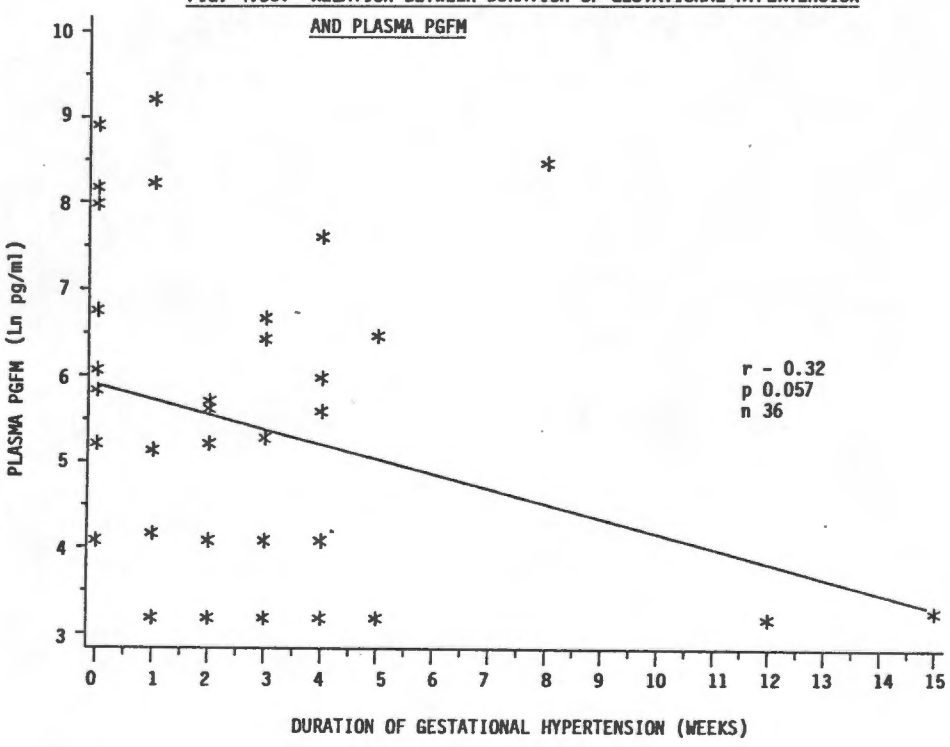


FIG. 4.36: RELATION BETWEEN DURATION OF GESTATIONAL HYPERTENSION AND PLASMA PGFM



The urine prostanoid values showed no relationships to the duration of gestational hypertension.

The presence of proteinuria implies a more severe form of gestational hypertension, so comparative analysis of maternal prostanoid levels between pre-eclamptic and non-proteinuric gestational hypertensives was performed. Prostanoid values however did not seem to be related to the severity of the disease as the median values of all the plasma and urinary prostanoids were similar between the two groups (after gestational age was taken into account where appropriate).

4.4.5.9 Summary of the Relationships of Maternal Prostanoids to the Hypertensive Disease

Thus, overall in the plasma there is evidence of a disturbance of the vasodilator/vasoconstrictor prostanoid balance, in both chronic and gestational hypertensives. This is illustrated by lower levels of thromboxane in hypertensive patients and a weak negative correlation of thromboxane to DBP. This could be interpreted as a secondary response to hypertension to overcome vasoconstriction. In addition, an increase in the ratio of the vasodilator to vasoconstrictor prostaglandins (6-keto $PGF_{1\alpha}$: TxB_2, PGE : TxB_2 and PGE : PGFM) in hypertensive patients suggests that in

these patients the vasodilator elements may be stimulated or vasoconstrictors suppressed. This finding is supported by the observation that the ratio of 6 keto $PGF_{1\alpha}$: TxB_2 and PGE : TxB_2 in the plasma are positively correlated to the DBP.

In the urine the situation is rather different, which is not unexpected as it has already been demonstrated (4.4.5.3) that plasma and urine prostanoids are not related to each other and therefore may have different controlling mechanisms. In the urine there is a tendency to a deficiency of the vasodilator PGE in hypertensive patients which is demonstrated by the negative relationship of PGE and the PGE : TxB_2 ratio to DBP, together with lower values of the PGE : TxB_2 ratio in gestational hypertensives although these relationships were not statistically significant.

4.4.5.1Ø Maternal Prostanoids in Relation to Fetal Growth

The relationships of maternal prostanoids to fetal growth were studied initially by comparing the values in pregnancies with normal weight babies (appropriate for gestational age, AGA) to those with intrauterine growth retardation (IUGR birthweight < 1Øth percentile for gestational age). Birthweight data were available

for 167 patients of whom 27 (16.1%) had babies weighing < 10th percentile for gestational age.

Maternal Plasma Prostanoids and Intrauterine Growth Retardation (IUGR)

In order to differentiate between the effects of hypertension and IUGR on maternal prostanoids analysis of covariance was performed with hypertension and IUGR as covariables. Thus each diagnostic group was subdivided according to the presence or absence of IUGR and the means of the logarithmically transformed values compared between the resulting six groups. There were no differences in the maternal plasma prostanoid levels between these groups (with p values ranging from 0.27 to 0.58).

Association of Maternal Plasma Prostanoids with low birthweight infants (< 25th percentile)

As there were only 3 IUGR babies amongst the chronic hypertensives, the validity of this analysis is questionable. Therefore in order to study the effect of fetal growth on maternal prostanoids, the covariance analysis was repeated after the data was subdivided according to the presence of hypertension and fetal birthweight above or below the 25th percentile for gestational age.

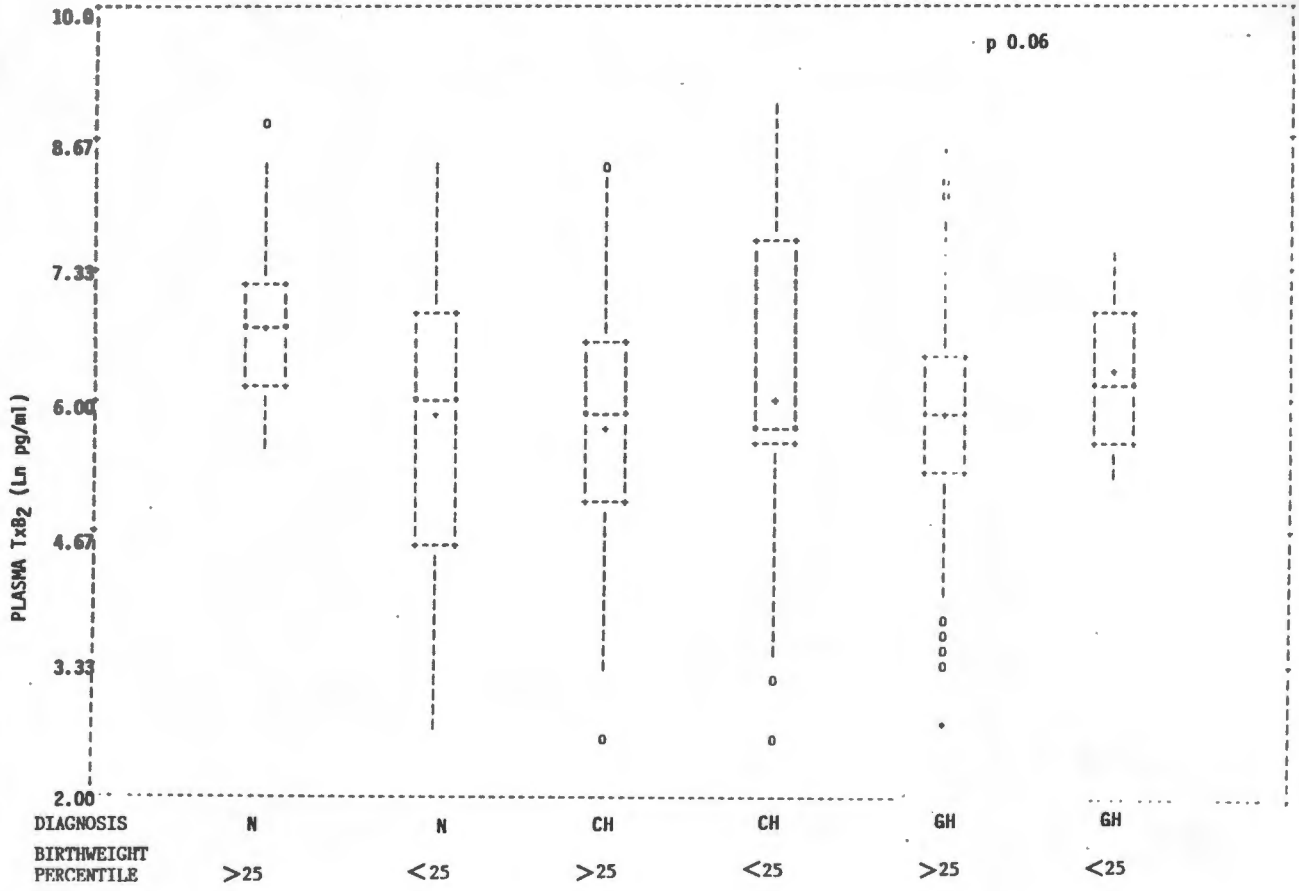
Prostanoid values were therefore compared between the following six groups:

1. Normotensive Birthweight > 25th percentile
2. Normotensive Birthweight < 25th percentile
3. Chronic Hypertension Birthweight > 25th percentile
4. Chronic Hypertension Birthweight < 25th percentile
5. Gestational Hypertension Birthweight > 25th percentile
6. Gestational Hypertension Birthweight < 25th percentile

This method allowed some assessment of trends in maternal prostanoids in relation to fetal weight and evaluation therefore of their clinical value in predicting inadequate fetal growth.

Normotensive patients with babies weighing above the 25th percentile (Group 1) had higher plasma TxB_2 values than all the other groups but these differences did not achieve statistical significance (p Ø.Ø6) (Fig. 4.37). There were no differences in the levels of the other plasma prostanoids in relation to birthweight above or below the 25th percentile (Appendix 9 Fig. 1 - 3).

FIG. 4.37: PLASMA TxB_2 LEVELS IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE



The 6 keto $PGF_{1\alpha}$: PGFM ratio was higher in normotensive and gestational hypertensives with babies weighing below the 25th percentile, but the differences were not statistically significant (p Ø.32 Fig. 4.38).

A study of the plasma prostanoid ratios showed that although normotensive patients with babies weighing below the 25th percentile had a higher mean plasma $PGF1_{\alpha x}$: TxB_2 ratio than those with well grown babies these relationships were also not significant (p Ø.Ø77) (Figure 4.39).

The mean PGE : PGFM ratio in the plasma was also marginally higher in pregnancies with babies weighing below the 25th percentile compared to appropriately grown babies (p Ø.Ø9) (Figure 4.4Ø), particularly in patients with both gestational hypertension and small babies.

The growth of the baby was not related to plasma PGE : TxB_2 ratio (p Ø.48) (Figure 4.41).

In summary, although there were no significant differences in maternal plasma prostanoids in relation to fetal weight for gestational age, there were tendencies to an excess of the vasodilators in pregnancies with smaller infants. This tendency was most apparent in the normotensive patients. The method of statistical analysis used did not allow separate analysis of the normotensive patients, and I was advised by the statistician that repetition of the significance tests on these patients alone would be invalid.

FIG. 4.38: RATIO OF PLASMA 6 KETO PGF1α TO PGFM IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE

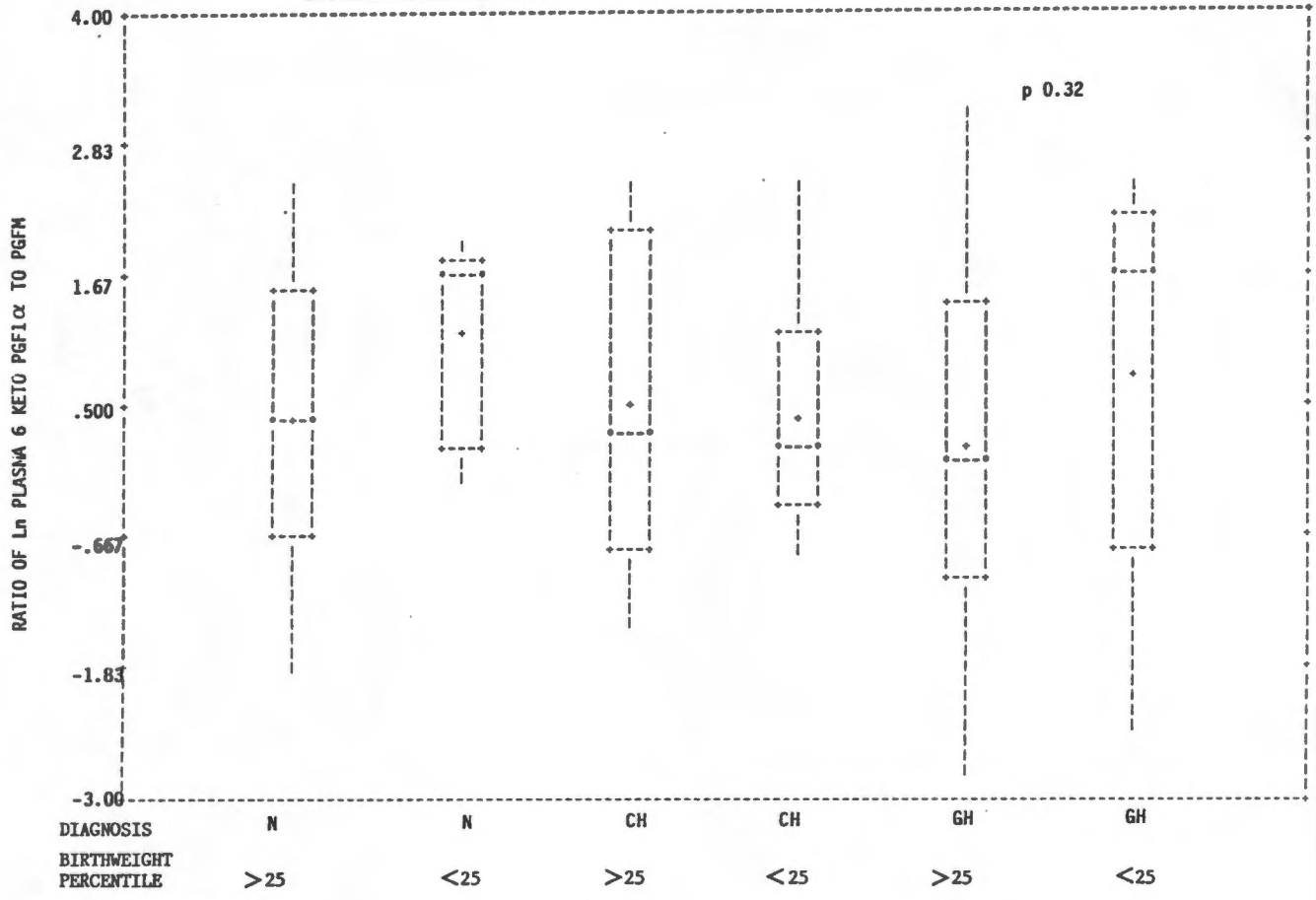


FIG. 4.39: RATIO OF PLASMA 6 KETO PGF1α TO TxB_2 IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE

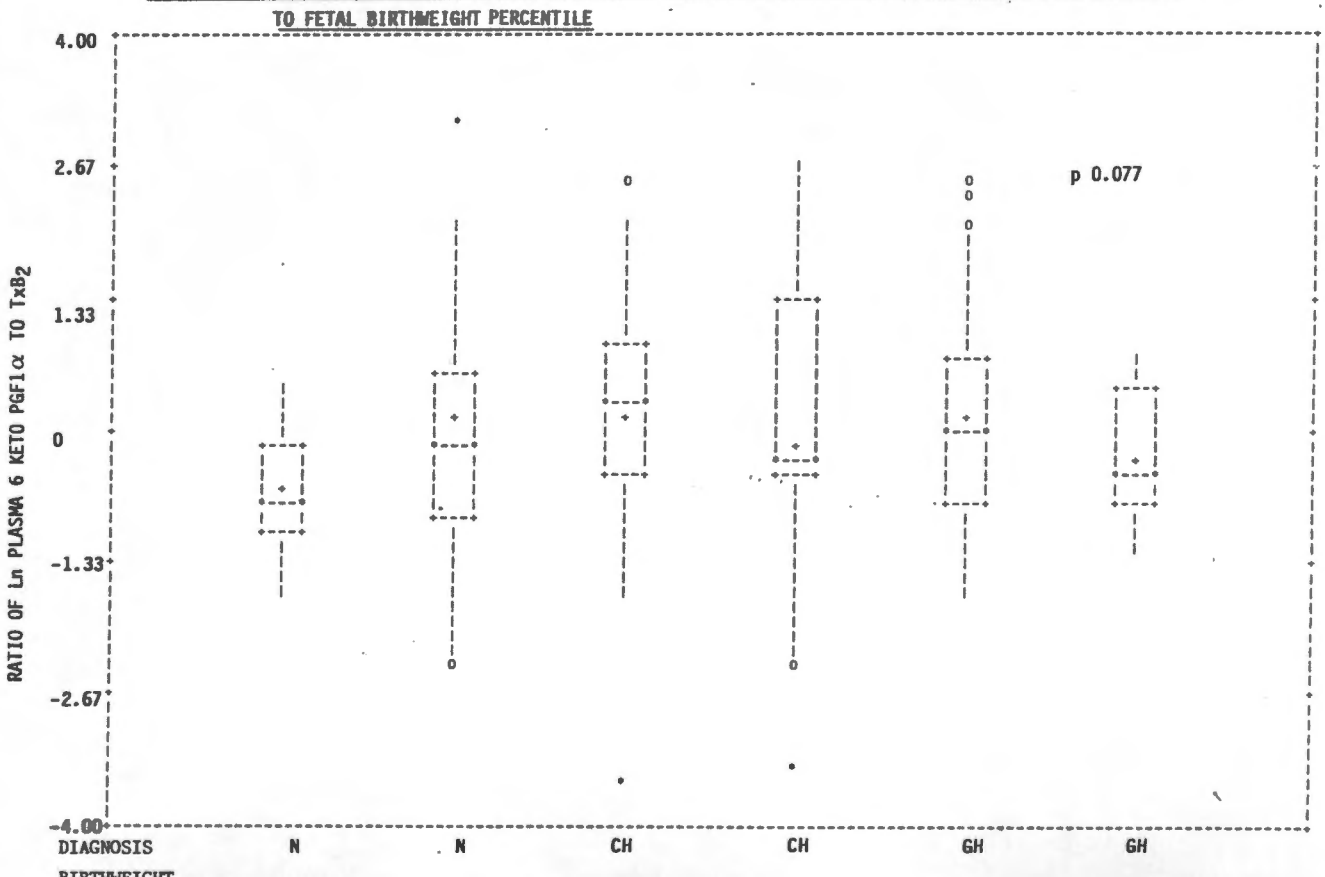


FIG. 4.40: RATIO OF PLASMA PGE TO PGFM IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE

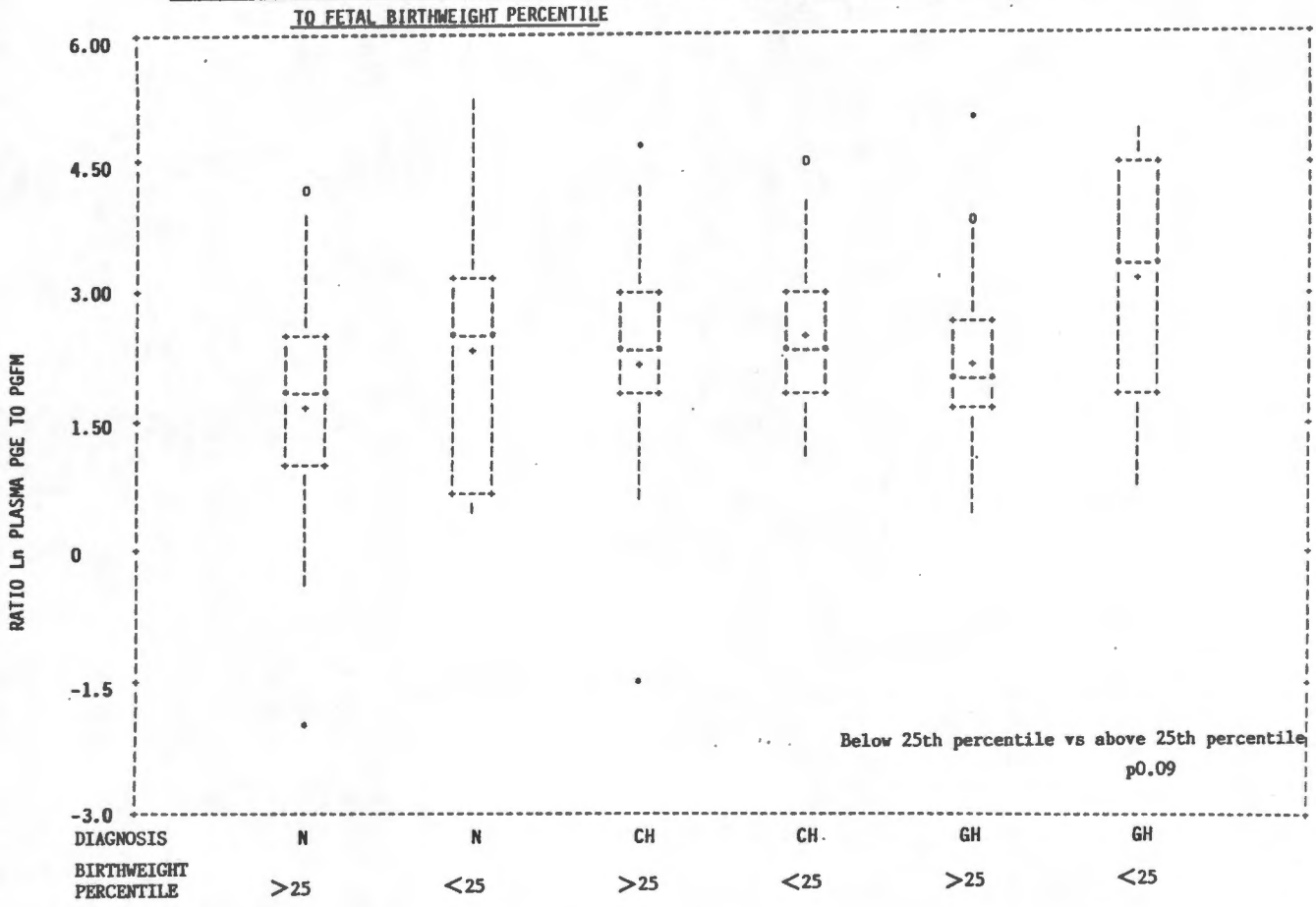
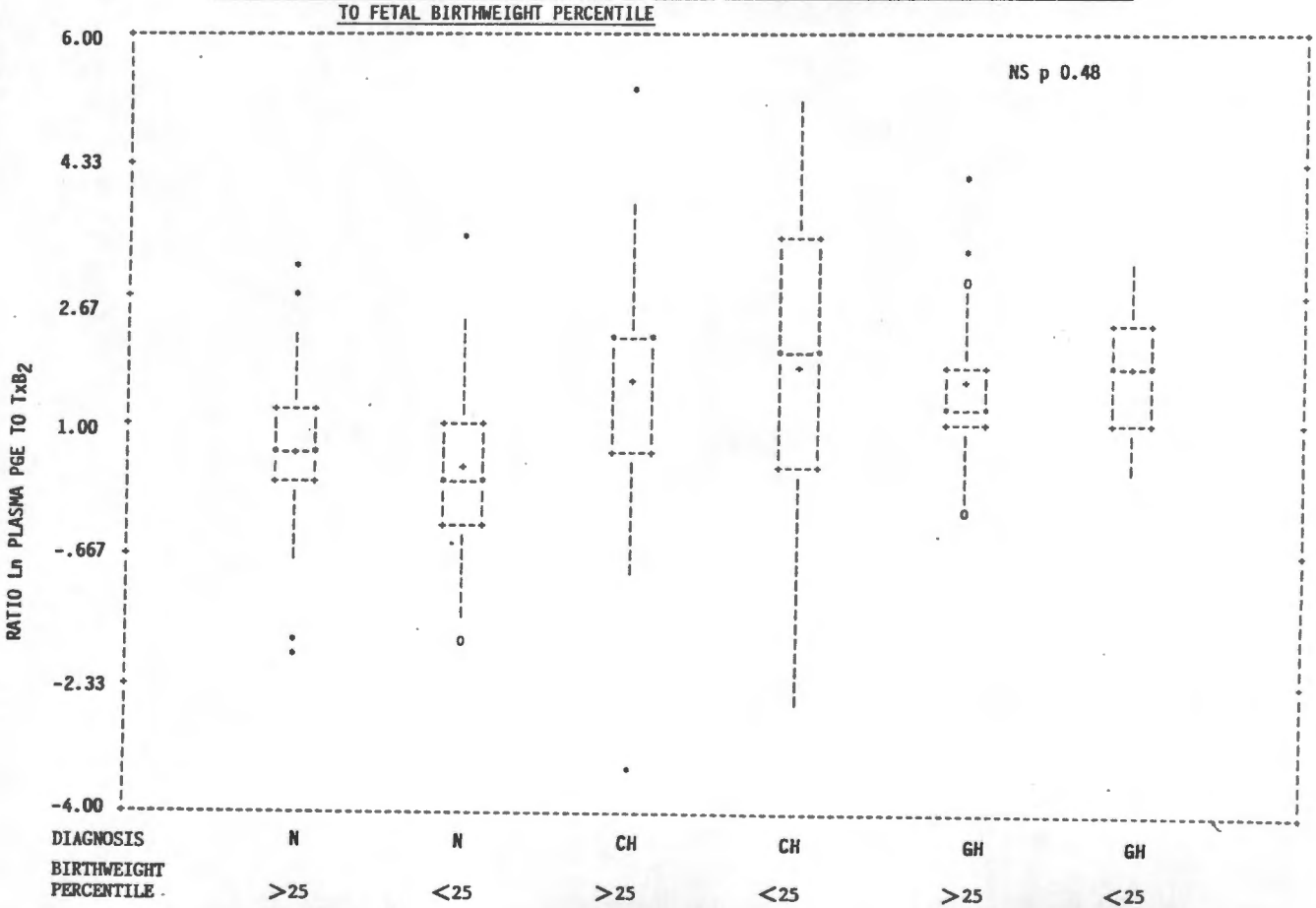


FIG. 4.41: RATIO OF PLASMA PGE TO TxB_2 IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE



Maternal Urinary Prostanoids and Intrauterine Growth Retardation

Statistical analysis was performed by the Median test using Fisher's exact probability, to assess if urinary prostanoid values above or below the median value were more likely to be associated with IUGR babies.

A comparison of maternal urinary excretion of PGE, PGFM and 6 keto $PGF_{1\alpha}$ between pregnancies with appropriately grown babies compared to IUGR (without considering the effect of hypertension) showed no significant differences (Median test p values were 1.0, 0.39 and 0.39 respectively). There was however a tendency for women with IUGR babies to have a higher excretion rate of TxB_2 than those with appropriately grown babies (76.9% of values were above the overall median in women with IUGR babies compared to 46.7% in women with babies of appropriate weight for gestation, p 0.069).

The association of intrauterine growth retardation and hypertension with maternal urinary prostanoids were then evaluated by analysis of covariance but no differences were found in maternal urinary TxB_2 excretion between appropriately grown and IUGR babies in each diagnostic group (p 0.51). There were no differences in maternal urinary excretion of PGE or 6 keto $PGF_{1\alpha}$ in relation to IUGR in

in each diagnostic group (p 0.49 and p 0.69 respectively) but the urinary excretion rate of PGFM was higher in women with IUGR compared to those with appropriately grown babies (p 0.026). This significant relationship was mainly due to the high urinary PGFM values in the three patients with chronic hypertension and IUGR (Fig. 4.42).

Association of Maternal Urinary Prostanoids and Fetal Birthweight < 25th percentile

The analysis of covariance was repeated as described above (page 204) to compare urinary prostanoid excretion in each diagnostic group after subdivision according to birthweight > or < 25th percentile. The higher urinary PGFM levels in association with IUGR were no longer apparent when this less stringent measure of fetal growth was used (p 0.61).

Urinary TxB_2 excretion however tended to be higher in pregnancies with babies weighing below the 25th percentile for gestational age compared to those with babies weighing above the 25th percentile. Closer analysis showed higher urinary TxB_2 excretion particularly in the normotensive patients with smaller babies (Fig. 4.43) but statistical

analysis of all the patients showed the growth of the baby did not significantly affect the TxB_2 excretion (p 0.072).

This excess of vasoconstrictors over vasodilators in the urine of patients with small babies was also illustrated by a lower PGE_2 : TxB_2 ratio in women with babies weighing below the 25th percentile (p 0.025 Fig. 4.44). The mean urinary 6 keto $PGF_{1\alpha}$: TxB_2 ratio was also lower in mothers of smaller infants and as noted previously the change was most apparent in the normotensive patients (Fig. 4.45) with an overall statistical significance of p = 0.082.

FIG. 4.42: URINARY PGFM EXCRETION IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE

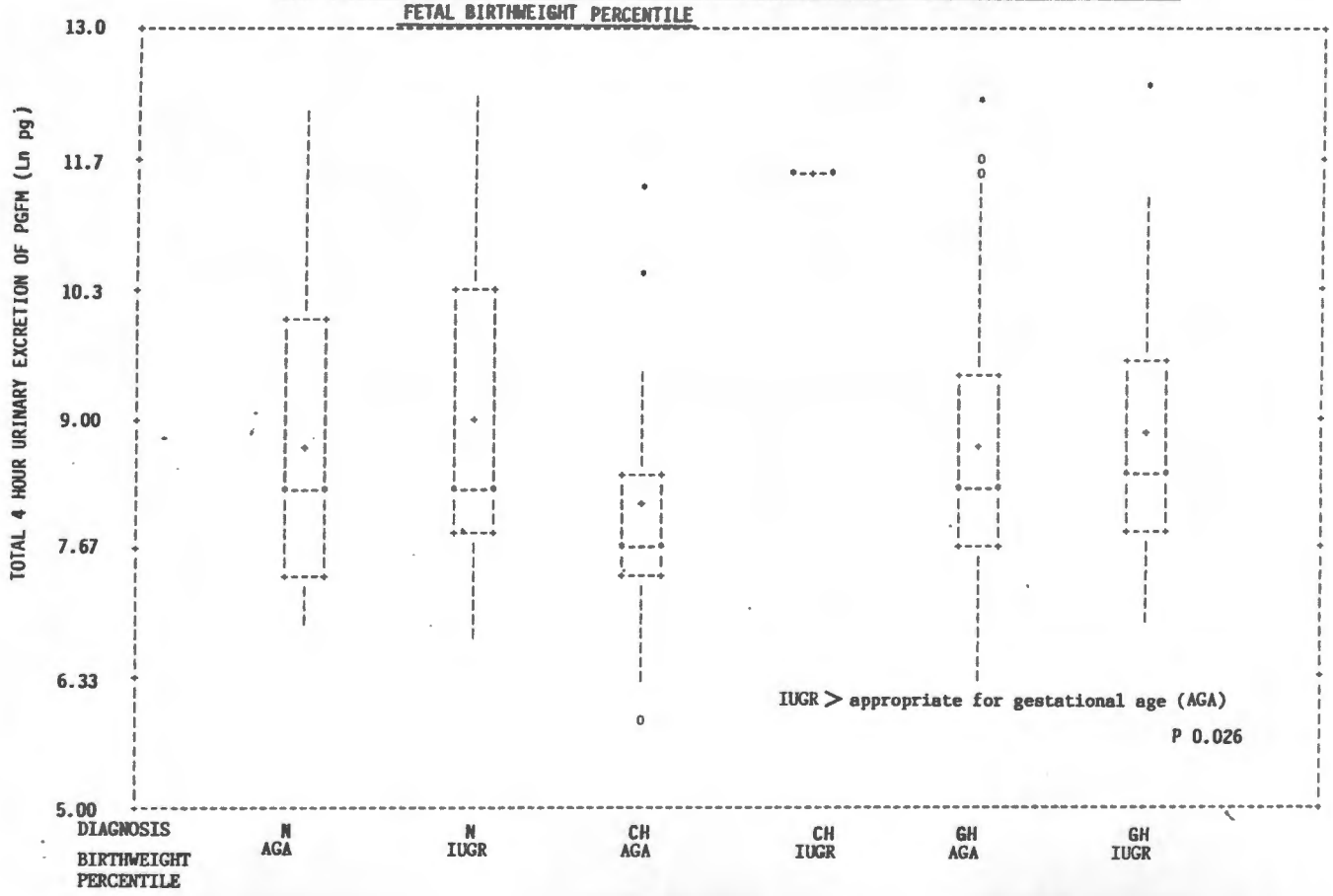


FIG. 4.43: URINARY TxB_2 EXCRETION IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE

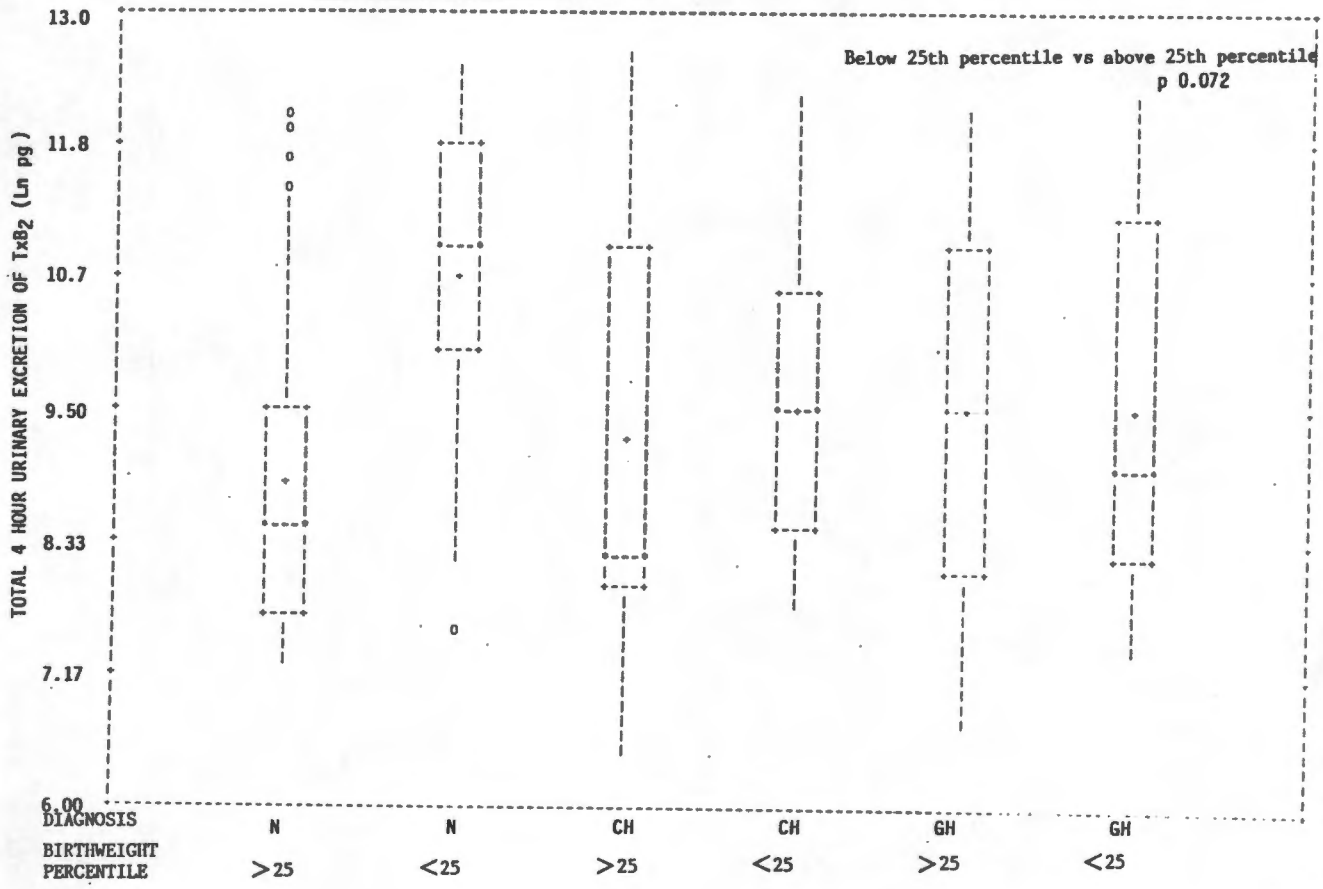


FIG. 4.44: RATIO OF URINARY PGE TO TxB_2 IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE

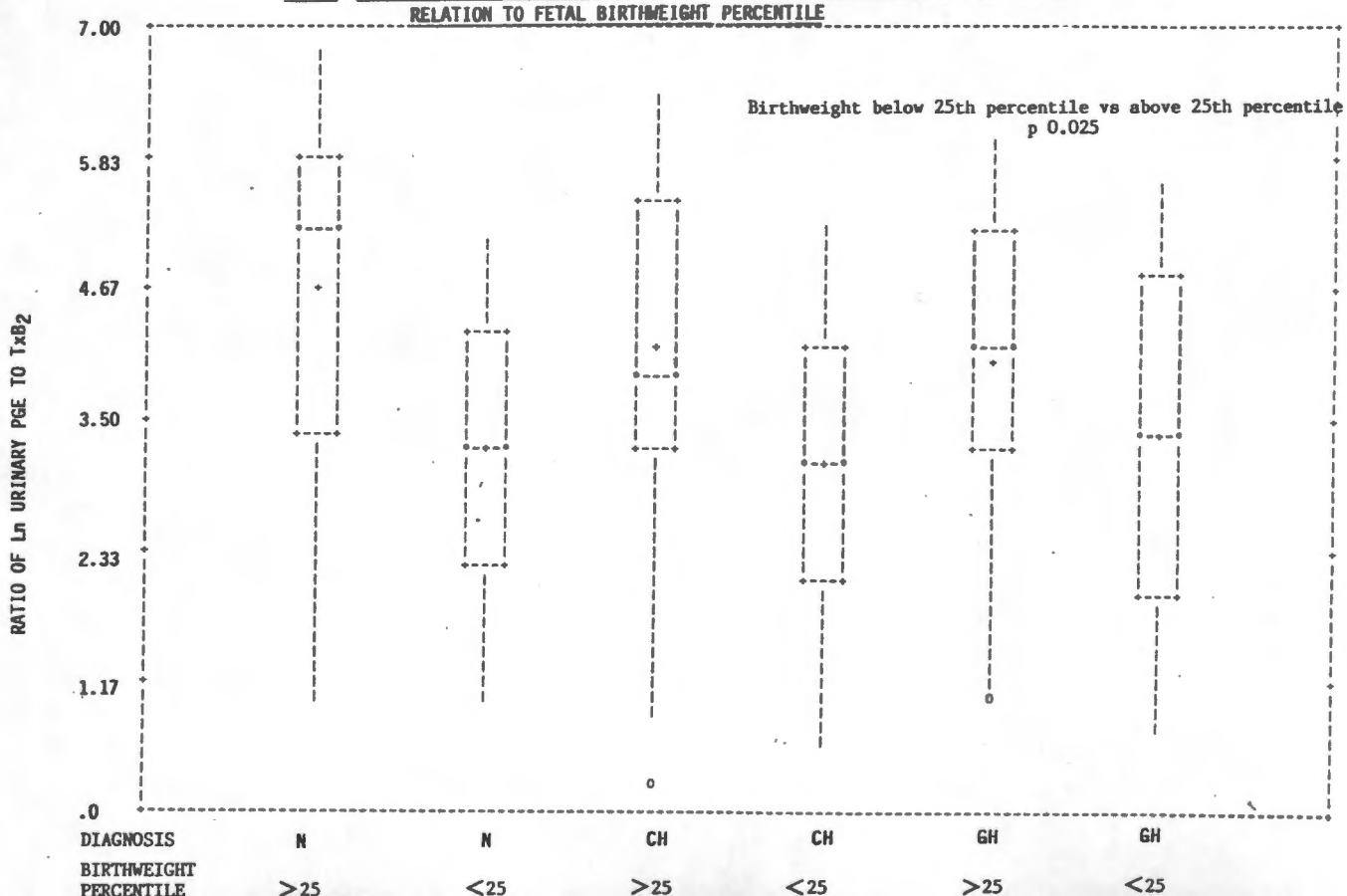
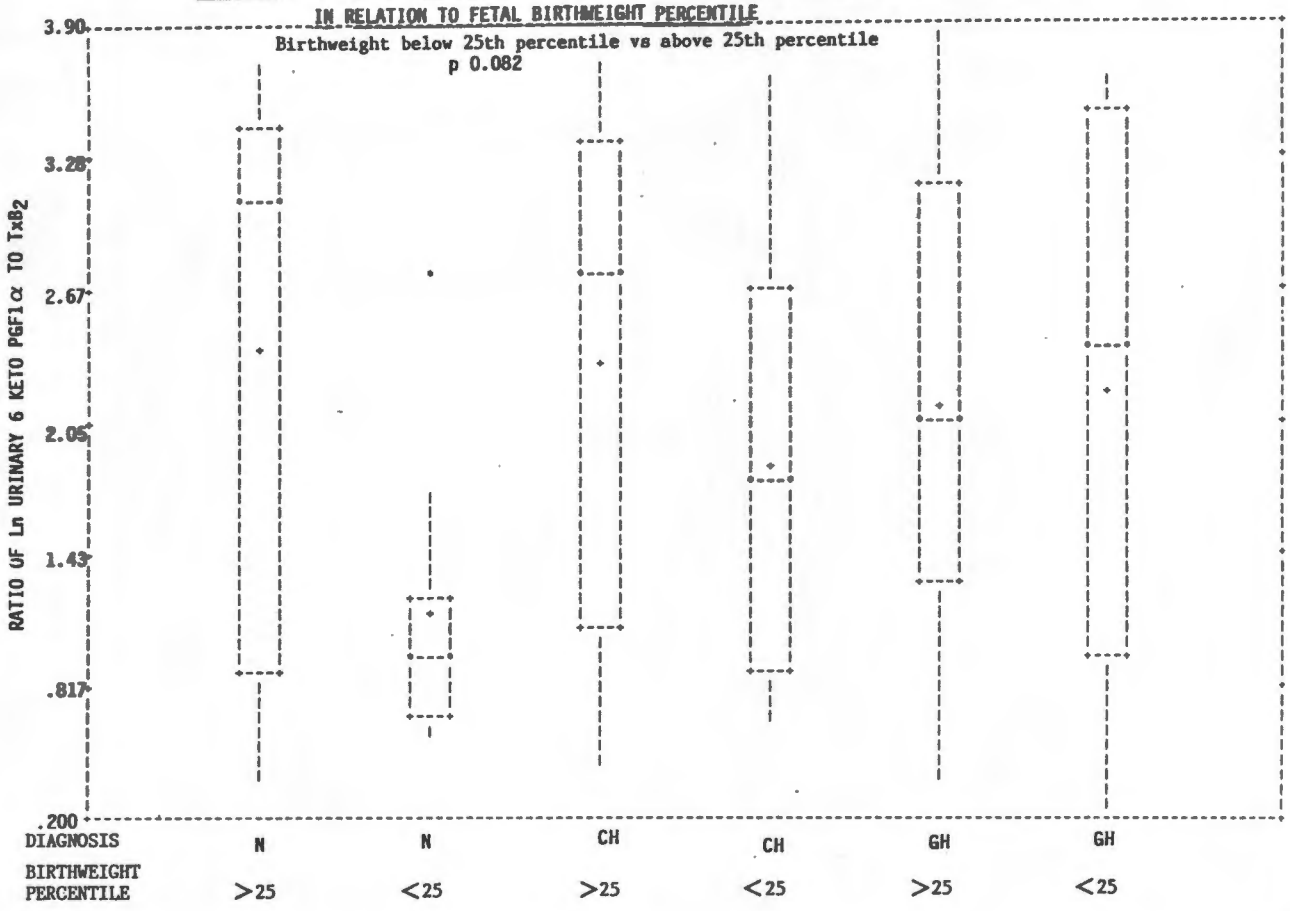


FIG. 4.45: RATIO OF URINARY 6 KETO PGF1α TO TxB_2 IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE



The urinary excretion of the PGE and 6 keto $PGF_{1\alpha}$ were unrelated to the fetal birthweight percentile (Appendix 9 Fig. 4 and 5). Although the median PGE excretion rate was lower in pregnancies with a combination of hypertension and birth weight below the 25th percentile, overall the combined effect of these abnormalities on the PGE excretion was not statistically significant (p Ø.16).

The urinary 6 keto $PGF_{1\alpha}$: PGFM and PGE : PGFM ratios were also not related to fetal growth (p Ø.88 and p Ø.13 respectively. (Appendix 9 Fig. 6 and 7).

In summary patients with infants weighing < 25th percentile for gestational age had an elevated excretion rate of PGFM and a decrease in the PGE : TxB_2 ratio (which presumably relates to the tendency for urinary TxB_2 to be elevated).

4.4.5.11 Summary of the Relationships of Maternal Prostanoids to Fetal Growth

It is of interest, as has previously been observed in relation to the hypertensive disorders of pregnancy that the plasma and urinary prostanoid levels show opposite changes. There was thus a tendency to an decrease in vasoconstrictor prostanoids in the plasma but an increase in vasoconstrictor prostanoids in the urine of women with poor fetal growth. In the plasma there were trends to a decrease in vasoconstrictor prostanoids in women with babies weighing below the 25th percentile. This was evidenced by a tendency to lower TxB_2 levels with higher ratios of PGE : TxB_2, 6 keto $PGF_{1\alpha}$: TxB_2 and PGE : PGFM. Although none of these differences were statistically significant the trends are all consistent with a relative excess of vasodilator over vasoconstrictor prostanoids in women with smaller babies.

In the urine the tendency to an excess of vasoconstrictor prostanoids in pregnancies with poor fetal growth was demonstrated by higher PGFM excretion in women with IUGR infants and a tendency for higher TxB_2 excretion in these women. In addition the ratio of vasodilator to vasoconstrictor prostanoids in the urine was lower in women with babies weighing below the 25th percentile for gestational age, as evidenced by the significantly lower PGE : TxB_2 ratio.

4.4.5.12 Maternal Prostanoids in Relation to Placental Hormones

The relationship of maternal prostanoids to serum oestradiol, progesterone and human placental lactogen (hPL) were studied by least squares linear regression and the results expressed by the Spearman rank correlation coefficients.

Oestradiol

Plasma prostanoids showed no overall relationship to serum oestradiol with correlation coefficients ranging from Ø.Ø44 to Ø.2Ø1. Closer analysis of the diagnostic groups separately however revealed a positive relationship in chronic hypertensive patients between plasma PGFM and serum oestradiol ($r = Ø.57$ p Ø.ØØ7).

The urinary excretion rates of the prostanoids TxB_2 and 6 keto $PGF_{1\alpha}$ were negatively related to oestradiol levels (r -Ø.27 p Ø.Ø1 and r -Ø.21 p Ø.Ø5). Urinary excretion of PGFM and PGE however were unrelated to serum oestradiol (r Ø.Ø4 and r -Ø.Ø8 respectively). Analysis of the results in the different diagnostic groups showed that the relationship of TxB_2 to oestradiol was strongest in women with chronic hypertension (r = Ø.47 p Ø.Ø3).

Progesterone

There was no correlation between plasma prostanoids and serum progesterone either in the overall analysis or in the individual diagnostic groups (r values -Ø.Ø4 to Ø.Ø8).

Urinary TxB_2, 6 keto $PGF_{1\alpha}$ and PGFM however were all negatively correlated to serum progesterone (r -Ø.42 p Ø.ØØØ1, r -Ø.31 p Ø.ØØ3 and r -Ø.24 p Ø.Ø2 respectively). Urinary PGE and serum progesterone were not correlated (r = Ø.ØØ7). These relationships of the prostanoids and serum progesterone were consistent in each diagnostic group.

hPL

Urinary TxB_2 and 6 keto $PGF_{1\alpha}$ excretion were negatively correlated with serum hPL (overall r -0.295 p 0.004 and r -0.29 p 0.005 respectively) and the relationships were the same in each diagnostic group. There were otherwise no associations between the maternal prostanoids and serum hPL (r values ranging from -0.14 to 0.12).

In summary there was a positive correlation of plasma PGFM with oestradiol in chronic hypertensive patients and a negative correlation of urine TxB_2 and 6 keto $PGF_{1\alpha}$ excretions with oestradiol, hPL and progesterone in all the patient groups.

4.5 DISCUSSION

Peripheral vascular resistance is the major determinant of blood pressure in pregnancy (See 2.2.1). Whether or not prostanoids act as modulators of the blood pressure under normal physiological circumstances is controversial. Evidence is accumulating however to support the theory of a generalised reduction in vasodilator prostanoids in pathophysiological states such as hypertension in pregnancy, but particularly affecting those vascular beds which have a high blood flow (See 2.5). Despite the controversies

regarding methodology and the absolute levels of prostanoids in plasma and urine, previous studies have shown differences in circulating and urine prostanoids metabolites in pregnant compared to to the non-pregnant individuals (2.4.3) and between pre-eclamptic and normal pregnancy (2.5). Several workers have reported differences in maternal prostanoid levels between normal pregnancy and pre-eclampsia (see below), but few have compared chronic and gestational hypertension.

4.5.1 Patient Characteristics

Pre-eclampsia (defined as gestational hypertension and proteinuria) is primarily a disease of primigravidae (MacGillivray 1983). In this study however only 48% of patients with gestational hypertension were nulliparous suggesting that some of these patients had latent essential hypertension. One third of the proteinuric patients moreover were multiparous, indicating that pre-eclampsia is not confined to first pregnancies, although in such patients it may be initiated by an underlying hypertensive tendency. This however illustrates the difficulties in defining clinical groups with the different hypertensive diseases in pregnancy.

Patients in the present study who were destined to develop gestational hypertension had a higher midpregnancy blood pressure, confirming previous reports (Gallery et al 1977, MacGillivray 1961). It is however apparent that although there were statistically significant differences in midpregnancy blood pressure between those who remained normotensive (mean 112/65mmHg) compared to those who later developed gestational hypertension (mean 123/75mmHg), the predictive value in individual cases was low.

4.5.2 Renal Function Tests

In pre-eclampsia a failure of the normal increase in glomerular filtration rate (GFR) has been described (Chesley and Duffus 1971a), together with hyperuricaemia which correlates directly with the decrement in plasma volume (Beaufils et al 1981). Hyperuricaemia may precede the change in GFR (Davison 1983) and has been described as a specific feature of pre-eclampsia (Redman et al 1976(b)), but many patients with pre-eclampsia have no detectable impairment of renal function. Serum uric acid levels however correlate with the severity of the histological renal lesion (Pollack and Nettles 1960) and vascular abnormalities in the placental bed (McFadyen et al 1986). Serum uric acid is therefore a sensitive index of renal

impairment and maternal vascular pathology. In this study the mean uric acid levels were elevated in patients with gestational hypertension but the mean urea and creatinine levels were unchanged. These findings are evidence of microangiopathy and abnormal renal function in some of these patients.

As expected, renal function was not detectably impaired in the chronic hypertensive patients (Knuppel et al 1985). As the kidney however has high functional reserves, plasma constituents often remain normal until renal damage is considerable and hence normal serum values do not exclude the possibility of some decrement in renal function.

4.5.3 Platelet count

Thrombocytopaenia may be an early feature of pre-eclampsia in some patients (Redman et al 1978). Large studies indicate that 15 - 18% of patients with pre-eclampsia have a mild decrease in platelet count (Reviewed by Gibson et al 1982) and this is thought to be evidence of an abnormal activation of the coagulation system. However most patients with mild gestational hypertension, and even some with severe pre-eclampsia, do not have evidence of coagulation abnormalities in comparison to normal pregnancy (Chesley 1978) and thrombocytopaenia is not a feature of chronic hypertension (Howie et al 1971) (See 2.2.3 for a summary of the coagulation changes in pre-eclampsia).

The automated platelet count may detect circulating damaged platelets (Khan et al 1978) and platelet counts and behaviour may vary from day to day (Wallenburg 1987). Hence a single platelet count may not be a sensitive indicator of platelet activity and probably fails to detect more subtle changes in platelet function. Some patients with pre-eclampsia have evidence of abnormal platelet activation and consumption when compared to normal pregnancy, which is reflected by elevated β thromboglobulin levels, decreased platelet life span and platelet fragmentation (Douglas et al 1982, Borok et al 1984, Wallenburg and Rotmans 1982(a). However this is usually compensated by increased megakaryocyte activity so that severe thrombocytopaenia is relatively rare (Thiagarajah et al 1984).

In the present series the mean platelet count was reduced in the patients with gestational hypertension but this difference was not statistically significant and as expected chronic hypertensive patients had a normal platelet count.

The failure to demonstrate thrombocytopaenia in these patients with gestational hypertension probably reflects the wide variation of pathological changes in this condition, but does not necessarily imply normal platelet function.

4.5.4 Placental Hormones

Placental hormones all showed the expected increase with gestational age (Coats et al 1977) but a notable feature of the results is the wide range at each stage of gestation. The low predictive value of measures of fetoplacental inadequacy is therefore not surprising. Human placental lactogen (hPL) was found in this study to have an association with inadequate fetal growth in agreement with other workers (Grudzinskas 1986) and appeared to be a more reliable indicator of IUGR than the other placental hormones. The mean hPL values were slightly (but not significantly) lower in the patients with gestational hypertension, probably due to the higher incidence of low birthweight for gestational age. This is in agreement with Sagen et al (1984) and Spellacy et al (1974) who consider that hPL is a useful indicator of fetal condition in gestational hypertension.

One interesting finding was the trend to lower placental hormone levels in chronic hypertensive patients. hPL levels

were lower in chronic hypertensives than normotensives with a mean value after adjustment for gestational age of 5.1µg/ml compared to 6.3µg/ml. This trend however cannot be explained on the basis of growth retardation as the incidence of growth retardation in the two groups was comparable. In the chronic hypertensive patients serum oestradiol levels were also significantly lower. There was thus evidence of poor placental function in terms of hormone production which was not related to fetal growth. These results may indicate that hPL is a less reliable indicator of IUGR in patients with chronic hypertension.

There were no differences in oestradiol levels between normotensive patients and those with gestational hypertension in keeping with the finding of other workers (Rosing and Carlstrom 1984). The study failed to confirm the higher hPL levels found by Obiekwe et al (1984) in pre-eclampsia. Abnormalities of oestradiol and progesterone conjugation may occur in pre-eclampsia (Rosing and Carlstrom 1984, Diaz-Zagoya and Arias 1981) which were not assessed in the present study but no differences in the total progesterone, or total oestradiol-progesterone ratio were detected between the diagnostic groups. Thus the present study does not exclude the possibility of a disturbance in placental hormone catabolism in hypertensive patients which may influence prostanoid production.

Maternal Prostanoids

4.5.5.1 Validity and Significance of Results

In the assessment of the significance of the maternal prostanoid levels and their relationship to hypertension in pregnancy, it is first necessary to discuss the validity of the results.

The initial impression of the prostanoid values in this study is that they are very high. For example, the median plasma 6 keto $PG_{1\alpha}$ of normotensive patients in this study was 415 pg/ml in comparison to the reported range of mean values from similar studies of 100 pg/ml to 266 pg/ml (Ylikorkala et al 1984, Ylikorkala et al1981(a), Yamaguchi and Mori 1984, Ogino et al 1986(b), Lewis et al 1980, Bolton et al 1981, Gallery et al 1984(a)). Median plasma TxB_2 levels were 422 pg/ml compared to the reported results ranging from 56 to 323 pg/ml (Yamguchi and Mori 1984, Ylikorkala and Viinikka 1980, Ylikorkala et al 1984, Ogino et al 1986(b), Mitchell et al 1978(c), Joupilla et al 1985). Similarly plasma PGFM and PGE were also much higher than previously reported values for normotensive pregnant patients. Median plasma PGFM was 242 pg/ml, the reported range being from unrecordably low to 16 pg/ml (Valenzuela et al 1983, Gallery et al 1984, Mitchell et al 1978(a), Yamguchi and Mori 1984, Youssafnejadien et al 1978) and

median plasma PGE_2 1488 pg/ml (reported range 4.8 pg/ml to 443 pg/ml (Mitchell et al 1978(a), Whalen et al 1978, Valenzuela et al 1983).

There have been fewer reports of the levels of prostanoid metabolites in urine in pregnancy, and as they are expressed in different ways, direct comparison is difficult. The only comparable report of urinary 6 keto $PGF_{1\alpha}$ excretion is by Joupilla et al (1985) which did not give precise mean values but the result was in the region of 14 ng/4 hours compared to 86 ng/4 hours in normal patients in the present study. The reported range of PGE excretion in normal pregnancy is 265 to 1581 ng/24 hour (Pedersen et al 1983, Bernheim et al 1986, Kovatz et al 1981, Moutquin and Leblanc 1982) as compared with a median urinary excretion of 540 ng/4 hours or 3242 ng/24 hours in the present study. There are no reports of urinary TxB_2 and PGFM excretion in comparable units.

Thus, the values obtained for all the prostanoids in both blood and urine in the present study are well above previously reported values. This raises several questions. Firstly, what precisely do these measurements reflect and secondly are the results invalidated by their high values?

Accurate measurements of prostacyclin metabolites in blood and urine by GCMS in man indicate that in the non-pregnant state production rates and circulating levels are in the order of < 1pg/ml (Blair et al 1982). These levels are well below the sensitivity of even the most sensitive RIA. Although in pregnancy at term endogenous prostacyclin biosynthesis is increased five fold (Brash et al 1983), circulating measurements assessed by GCMS are still < 8pg/ml (Barrow et al 1983), which is also incompatible with reported values of 6 keto $PGF_{1\alpha}$ by RIA. Thus RIA probably measures a number of prostacyclin metabolites (Belch et al 1983) and as such may give a better guide to biological activity in vivo. This probably also applies to the other prostanoids. Nevertheless, the results obtained in this study are higher than those reported by similar techniques. This may be a reflection on the quality of the commercial assay used.

Could the high measurements be artefacts caused by tissue trauma? This would explain both the high blood values and strong correlation of the prostanoids in plasma, but would not be consistent with the close correlations of the prostanoids in urine as they were obtained non-invasively.

Another possible explanation is that the presence of unidentified interfering substances or some aspect of the treatment of samples caused the results to be falsely elevated. This possibility is difficult to exclude but as plasma and urine samples from each patient were extracted and assayed together, one would anticipate that any such interference would affect both the samples equally. If this were the case urine and plasma values from the same patient should be closely correlated, but they were not related. The sampling and assay techniques were also very similar to those described by other workers (Morris et al 1981) who did not experience this problem. The PGE assay in plasma is particularly suspect as PGE is rapidly inactivated during passage through the lungs (Ferreira 1967) and the high values found in the present study remain unexplained.

Can any valid conclusion be drawn from these results? Exceptional care was taken to avoid traumatic generation of prostanoids and samples were discarded if any difficulty was experienced in venepuncture. The 6 keto $PGF_{1\alpha}$ assay moreover was validated by the prostacyclin infusion study and indicate that this is a true measure of circulating prostacyclin. It is unlikely therefore that the high values were due to the trauma of venepuncture or assay technique.

A major point in favour of accepting the results as a valid measure of the changes in prostanoids in the different patient groups, is the consistency of the changes seen in relation to pregnancy pathology. These changes do not appear random and are therefore worthy of critical analysis.

Thus, in summary, whilst there may be doubts about the validity of the numerical values obtained in the different prostanoid assays, I contend that, with the possible exception of plasma PGE, the results do reflect the biological levels and that comparisons between normotensive and hypertensive patients are valid. Whether measurements of prostanoid levels in biological fluids are of value in assessing biological activity at a local level is a different matter and will be considered in more detail later.

4.5.5.2 Prostanoids in Pregnancy

Prostanoids have long been known to have physiological roles in pregnancy (See 2.4) and although the evidence is contradictory, most of the available evidence supports the concept of a generalised disturbance of prostanoid metabolism and in particular a deficiency of prostacyclin in pre-eclampsia (See 2.5). Although the measurement of prostanoids in biological fluids is controversial, if such

an effect is truly generalised, changes in the levels of stable metabolites are probably a true reflection of changes in prostanoid synthesis and metabolism.

This study has shown a positive correlation among each of the prostanoids in plasma and in urine, but no correlation between blood and urine levels. In plasma this could be interpreted as a generalised stimulation of arachidonic acid metabolism by venepuncture but it could also be indicative of true differences in prostanoid production between individuals. A similar correlation between serum 6 keto $PGF_{1\alpha}$ and TxB_2 was noted by Viinika et al (1981) in non-pregnant individuals and these authors considered that this is a reflection of the physiological balance between these two prostanoids.

Although infusion studies have shown that circulating prostanoids may contribute to the urinary metabolites (Patrono et al 1982, Ciabattoni et al 1979) the lack of association in the present study between plasma and urinary concentrations of the prostanoids is more consistent with the theory that under physiological circumstances the urinary prostanoids reflect intrarenal rather than circulating prostanoids (Fitzgerald et al 1983, Patrono and

Dunn 1987). Dinor and tetranor metabolites are the major urinary products of the prostanoids and may be a better method of quantifying extra-renal prostanoid production (Patrono and Ciabattoni 1985) than the primary metabolites. Thus, as primary prostanoid metabolites were measured in this study and as they did not relate to the plasma levels, it is likely that the urinary prostanoids probably reflected mainly production locally in the kidney.

4.5.5.3 Effect of Gestational Age on Maternal Prostanoid Levels

There has been no consistency in the previously reported studies of the effect of gestational age on either plasma or urinary prostanoid metabolites. Maternal blood 6 keto $PGF_{1\alpha}$ has been reported to increase (Barrow et al 1983), remain stable (Yamguchi and Mori 1984, Mitchell 1981(b)) or decrease (Spitz et al 1983) with gestational age in normal pregnancy. The study of Spitz et al (1983) was the only longitudinal one and hence it may be more reliable. Remuzzi et al (1981) reported a decrease in a plasma stimulator of prostacyclin at term which would be consistent with a fall in prostacyclin in late pregnancy. In the present study there was no relationship of gestational age to plasma 6 keto $PGF_{1\alpha}$ levels but only one blood sample was obtained from each patient and there were relatively few from

patients in late pregnancy. TxB_2 levels in the plasma were similarly unaffected by gestational age in keeping with two reported studies (Koullapis et al 1982, Yamguchi and Mori 1984).

Studies of the circulating PGF metabolite PGFM in pregnancy show that the levels are unaltered with gestation (Dubin et al 1981, Mitchell 1981(a), Koullapis et al 1982, Yamguchi and Mori 1984, Ogino and Jimbo 1986(a)). Although there have been differences of opinion with regard to the validity of plasma PGE_2 measurements, recent assays of the stable metabolite also showed no effect of gestational age (Brennecke et al 1985) which is in accordance with the findings of the present study.

Urinary PGE and PGFM have previously been reported to increase with gestational age, particularly in the third trimester (Bernheim et al 1986). In the present study however urinary PGE and PGFM excretion were unrelated to gestational age. These differences may be accounted for by the fact that the study of Bernheim et al (1986) was longitudinal and also that in the present work the suppression of PGE by the hypertensive diseases would tend to mask the effect of gestational age.

Urinary excretion of 6 keto $PGF_{1\alpha}$ and dinor prostacyclin metabolites have also been shown to increase in pregnancy (Ylikorkala et al 1986(a), Fitzgerald et al 1987) and with gestational age (Ylikorkala et al 1986(a)). Urinary thromboxane levels were unrelated to gestational age in the single reported study (Ylikorkala et al 1986(a)). In the present study 6 keto $PGF_{1\alpha}$ and TxB_2 excretion had a weak negative correlation to gestational age. Again this may be accounted for by the fact that the present study was not longitudinal by cross-sectional. In fact, in the groups taken individually the excretion of neither metabolite was related to gestational age and the correlation coefficients in the whole group were low (TxB_2 $r = -0.20$ and 6 keto $PGF_{1\alpha}$ $r = -0.28$). The findings are therefore in keeping with the conclusions of Ylikorkala et al (1986(a)) that gestational age has little effect on thromboxane excretion but do not confirm their findings of an increase in 6 keto $PGF_{1\alpha}$ excretion with increasing gestational age.

4.5.5.4 Maternal Plasma Prostanoids in the Hypertensive Diseases of Pregnancy

In pre-eclampsia several workers have produced evidence of alterations in intrauterine prostanoid production although the findings are not consistent (See 2.5). However circulating levels in pre-eclampsia have in general been

found to be comparable with normal pregnancy and in the present study peripheral plasma concentrations of PGE, PGFM and 6 keto $PGF_{1\alpha}$ did not differ between normotensive and hypertensive patients. Although the plasma levels in the study are high, the comparative data are consistent with a number of previously reported studies (Valenzuela et al 1983, Ylikorkala et al 1981(a), Ogino et al 1986(c), Brown et al 1987).

There has been much debate with regard to the contribution of placental prostanoids to the peripheral circulation. Although depressed intrauterine prostacyclin production in pre-eclampsia has been convincingly demonstrated (See Table 2.VIII), consistent changes have not been found in maternal blood.

In severe gestational hypertension however some workers have demonstrated reduced circulating 6 keto $PGF_{1\alpha}$ (Lewis et al 1981, Moodley et al 1984(b), Yamguchi and Mori 1985). There is thus some evidence that major alterations in intrauterine prostanoid production are reflected in circulating levels. Further evidence for this is found in the observation that circulating prostanoid levels are higher in the pregnant than non pregnant state and that

elevated plasma PGFM levels reflecting intrauterine changes, are found in labour (Mitchell 1981(a)). Martensson and Wallenburg (1984) have shown an increase in uterine venous and umbilical venous TxB_2 concentrations in pre-eclampsia which also suggests that the uteroplacental circulation may contribute prostanoids to the maternal circulation.

In the present study although both groups of hypertensive patients had lower levels of 6 keto $PGF_{1\alpha}$ than normotensive patients, the differences were not significant. The reported studies showing reduced maternal plasma prostacyclin metabolites in pre-eclampsia have all involved patients with severe disease (Lewis et al 1981, Moodley et al 1984(b), Yamguchi and Mori 1985) whereas other studies have shown no differences (Ylikorkala et al 1981(a)). It was considered possible that the failure to demonstrate differences may have been due to the heterogenous nature of the gestational hypertensive group with a mixture of mild and severe cases. However, after re-analysis of the data dividing this group into proteinuric and non proteinuric cases, the pre-eclamptic patients were still found to have comparable values of 6 keto $PGF_{1\alpha}$ to the normotensives.

Although depressed placental PGE activity has been reported in pre-eclampsia (Demers and Gabbe 1976, Robinson et al 1979), the results are not consistent (Hillier and Smith 1981) and plasma PGE levels have generally been found to be comparable to normal (Yamguchi and Mori 1985, Valenzuela et al 1983). Although Moodley et al (1984(b)) did report depressed central venous plasma PGE levels in eclamptic patients, the patients were on treatment with magnesium sulphate and this may have affected the results. None of the patients in the present study were receiving any medication except iron supplementation. Thus, although the measurement of plasma PGE must be viewed with circumspection the findings of the present study would indicate that plasma PGE levels do not relate to the hypertensive diseases in pregnancy.

Circulating PGFM levels also in keeping with previous studies, (Yamguchi and Mori 1985, Moodley et al 1984(b)) were comparable between normotensive and hypertensive patients.

The plasma TxB_2 was however significantly lower in both groups of hypertensives and was negatively correlated to diastolic blood pressure.

The finding of a lower plasma TxB_2 in hypertensive pregnant women was unexpected and is contrary to previous studies. Although Mitchell et al 1978(c) and Ylikorkala et al 1984 demonstrated lower mean plasma TxB_2 levels in pre-eclampsia the differences were not significant. In the studies of Koullapis et al (1982) and Yamguchi and Mori (1985) mean TxB_2 levels were higher in pre-eclampsia, although only in the former study were the differences significant. It is possible that the findings of the present study occurred by chance, but although there was a considerable overlap in values between diagnostic groups, there are obvious differences between normotensives and both hypertensive groups (Fig. 4.26). The validity of these observations is strengthened by the negative correlation of plasma TxB_2 to DBP. The data appear to contradict the many reports on prostacyclin deficiency or a relative thromboxane excess in pre-eclampsia.

There is some evidence of increased catabolism of circulating prostacyclin and thromboxane in pre-eclampsia (Martensson and Wallenburg 1984). It is therefore possible that this may mask changes in the circulating levels.

Although the suspected intrauterine prostanoid imbalance in pre-eclampsia is reflected in uterine venous blood (Martensson and Wallenburg 1984), this study shows that it is not demonstrable in peripheral blood.

So how can the demonstrated low thromboxane levels in hypertensive patients be explained? The production of thromboxane in vitro by placentae from pre-eclamptic patients has been found to be higher than placentae from normotensive patients (Makila et al 1984(b), Walsh 1985(a)). The findings suggest that intrauterine prostanoids may not be a major factor in the control of maternal blood pressure or that systemic prostanoids are stimulated in response to the prostanoid abnormalities generated by a primary placental pathology. Interaction of the prostaglandins and other circulating vasoactive hormones such as Angiotensin II (AII) are undoubtedly important in pregnancy both in control of blood pressure and the regulation of uterine and renal blood flow (See 2.2.1.4). A possible interpretation of the results is that TxB_2 is suppressed in an attempt to overcome the effects of some other circulating vasoconstrictor.

The placenta may be a major site of AII formation (Glance et al 1984) and also produces thromboxane (Mitchell et al 1978(c)). Uterine venous blood obtained from women with severe pre-eclampsia demonstrates higher levels of AII than normal pregnant patients (Broughton Pipkin et al 1981). In peripheral blood however AII levels are decreased in hypertensive compared to normal pregnancy (Beilin et al 1983). These findings suggest that in pre-eclampsia vasoconstrictors produced by the placenta may enter the maternal circulation but that peripherally changes occur to attenuate their effects. One could speculate that in the peripheral vessels the pressor effects of these vasoconstrictors are modulated by alterations in the prostanoids. The results of the present study are consistent with such a mechanism by the evidence of lower thromboxane levels and a relative excess of vasodilator prostanoids in the plasma of hypertensive patients.

4.5.3.5. Maternal Plasma Prostanoids and Fetal Growth

Although umbilical arteries have been shown to have a reduced ability to generate prostacyclin in pre-eclampsia (Remuzzi et al 1980(b), Downing et al 1980, Makila et al 1984(a)), it has been suggested that this may not be a unique feature of pre-eclampsia as it also occurs in other states of chronic placental insufficiency (including chronic hypertension and IUGR)

(Stuart et al 1981). It has been considered that intrauterine prostanoid production is primarily of importance in local regulation of the placental and fetal blood flows (Elder et al 1985). However circulating plasma prostanoids in the current study were unrelated to fetal growth.

Intrauterine blood flow may be decreased in pregnancies with inadequate fetal growth or maternal hypertension (Nylund et al 1983, Kaar et al 1980). The possible role of prostanoids in controlling uteroplacental blood flow and the association of decreased umbilical and placental prostacyclin with IUGR have been discussed in Part 2.7. Although there were no significant differences in plasma prostanoids in relation to birthweight it is of interest that there were consistent tendencies to a vasoconstrictor deficiency in patients with small babies. Normotensive patients with appropriately grown babies tended to have higher plasma TxB_2 (p 0.06) levels than all the patients with pregnancy complications and these patients also tended to have a lower 6 keto $PGF_{1\alpha}$: TxB_2 ratio. The plasma prostanoid ratios also showed tendencies to an excess of vasodilator prostanoids in pregnancies with small compared to appropriately grown infants, but these changes were marginal.

Plasma prostanoids are therefore of no clinical value in the prediction of pre-eclampsia or poor fetal growth but this does not preclude a major role for the prostanoids at local level in blood pressure control or in the control of umbilical blood flow. It is possible that small local alterations are occurring which are not detectable in the maternal circulation. However the decrement of umbilical prostacyclin production in pre-eclampsia is greater than in maternal vessels (Remuzzi et al 1980(b)), implying that the prostacyclin deficiency is a more important factor in the umbilical than maternal circulations. It is likely that while prostanoid imbalance may be generalised it is not uniform and that some vascular beds are more affected than others.

Recent work showing a fall in the 6 keto dinor urinary metabolites of prostacyclin prior to the development of+ the clinical signs of pre-eclampsia (Fitzgerald 1987) is further evidence that whilst prostacyclin deficiency could play an important aetiological role in pre-eclampsia, it may not be the direct cause of the hypertension.

Although the absolute levels of the plasma prostanoids with the exception of TxB_2, did not show any differences in relation to hypertension or growth retardation, there were some differences in the ratios of vasodilator to vasoconstrictor prostanoids. There was some evidence in plasma of an excess of the vasodilator element in patients with hypertension as the plasma $PGF_{1\alpha}$: TxB_2 and PGE_2 : TxB_2 ratios were higher in hypertensive patients. A relative excess in the maternal plasma of the vasodilator over vasoconstrictor prostanoids in relation to the level of blood pressure was also found as the ratios of PGE : TxB_2, PGE : PGFM and 6 keto $PGF_{1\alpha}$: TxB_2 were all positively correlated to the DBP. There is also the suggestion that the balance shifts further to the vasodilator side with increasing duration of gestational hypertension as the plasma PGE : PGFM and 6 keto $PGF_{1\alpha}$: PGFM ratios were positively correlated to the duration of hypertension.

4.5.5.6 Maternal Urinary Prostanoid Excretion in Hypertensive Diseases of Pregnancy

Although urinary PGE excretion was lower in hypertensive pregnancies and particularly pre-eclampsia, as the range of values was so wide statistical analysis did not confirm the suppressed PGE excretion seen in other studies (Rathaus et al 1982,

Pedersen et al 1984, Moutquin and Leblanc 1982). Although Pedersen et al (1984) showed no relationship of PGE to blood pressure, the present study has demonstrated a significant negative correlation of urinary PGE excretion rate to diastolic blood pressure, which is evidence of a vasodilator deficiency in hypertensive patients. This is also reflected by the lower ratio of urinary PGE : TxB_2 in gestational hypertensives although these differences were not statistically significant.

Urinary PGFM excretion was not related to hypertension, confirming previous studies (Pedersen et al 1984, Rathaus et al 1982). Some studies of urinary PGE have shown that both pre-eclamptic and chronic hypertensives demonstrate a deficiency (Moutquin and Leblanc 1982) whereas other have found the decrement to be present only in pre-eclampsia (Rathaus et al 1982, Kovatz et al 1981). The latter workers however did find an impairment of the PGE : PGFM ratio in chronic hypertensives. The present study has shown that mean urinary PGE values in chronic hypertensives fall between the values for gestational hypertensive and normal patients.

Urinary prostacyclin metabolite deficiency in pre-eclampsia, but not chronic hypertension has been documented (Ylikorkala et al 1986(a), Fitzgerald et al

1987). Reduced urinary 6 keto $PGF_{1\alpha}$ excretion was not apparent in the current study in either gestational or chronic hypertensive women. Both these previous studies measured dinor metabolites of prostacyclin which as the major prostacyclin metabolites (Fitzgerald et al 1987) may be a more appropriate means of detecting a generalised prostacyclin deficiency.

Urinary thromboxane B_2 excretion in the present study was higher in gestational hypertensives, in keeping with the findings of Ylikorkala et al 1986(a), but these differences were not statistically significant. There was nevertheless a significant weak negative relationship of the PGE/TxB_2 ratio to the blood pressure and a non-significant trend for the PGE:TxB_2 ratio to be lower in pre-eclampsia (p 0.09).

Thus urinary prostanoid excretion tends to reflect deficiencies of the vasodilator prostanoids which may be an index of reduced renal prostanoid production.

4.5.5.7 Maternal Urinary Prostanoids and Fetal Growth

In the urine there were trends to an excess of the vasoconstrictors as evidenced by higher PGFM levels in patients with IUGR infants (p 0.026). Normotensive patients with infants weighing below the 25th percentile for gestational age had higher urinary TxB_2 than those infants weighing more than the 25th

percentile (Fig. 4.42). The urinary PGE_2 : TxB_2 ratio was also lower in patients with infants weighing below the 25th percentile (Fig. 4.44).

4.5.5.8 Maternal Prostanoids and Maternal Serum Placental Hormones

Work in animals and in vitro experiments has suggested interrelationships between oestradiol and the prostanoids and in particular that oestradiol may stimulate intrauterine production of vasodilator prostanoids (See 2.8). In the present study no relation was found between plasma placental hormones and plasma prostanoids. There were negative correlations of urinary 6 keto $PGF_{1\alpha}$ and TxB_2 to serum oestradiol and although the relationship was statistically significant, the correlation coefficients were very low and would suggest that these relationships are not of major physiological importance. Although low oestradiol concentrations in this study appear to be a specific feature of chronic hypertension, there is no evidence that this relates to circulating prostanoids. The failure to demonstrate a correlation between serum oestradiol and the

prostanoids does not however exclude the possibility that their interactions may be important locally in the uterus.

4.5.5.9 Summary

In summary it has been shown that plasma prostanoids do not provide a good reflection of intrauterine pathology, and are of no clinical value in prediction of inadequate fetal growth. The observation of an excess of circulating vasodilator prostanoids in relation to hypertension a trend opposite to the expected, does not preclude their activity at a local level in regulation of blood pressure and regional blood flows. However it would suggest that vasoactive prostanoids produced by the uteroplacental unit are not the primary cause of systemic hypertension. It is possible that the alterations in the balance of the prostanoids to a vasodilator excess demonstrated in peripheral blood are compensatory to another factor causing the hypertension. The prostanoid activity locally in different vascular beds appear to be independant of each other so that a deficiency of the vasodilator prostanoids in the kidney and hence the urine may coexist with a vasodilator prostanoid excess in the peripheral circulation.

Plasma prostanoids did not reflect the expected changes in uteroplacental or fetal prostanoid production. However, a deficiency of vasodilator or excess of vasoconstrictor prostanoids has demonstrated in the urine of patients with small infants. The combination of inadequate fetal growth and hypertension did not appear to be additive and in fact the highest urinary thromboxane excretion and lowest urinary PGE : TxB_2 ratio was in normotensive patients with babies weighing below the 25th percentile. It is interesting that growth retardation should have a more marked effect on urinary prostanoids than gestational hypertension. Renal blood flow and function are unaffected in intrauterine growth retardation in the absence of associated disease, so one would anticipate that the renal contribution to urinary prostanoids would be unaltered.

The expected tendency to a vasodilator deficiency in hypertension and inadequate fetal growth has been demonstrated in the urine by this study. Urinary prostanoids provide a better predictor of inadequate fetal growth than of hypertension but the combination of hypertension and growth retardation has not been shown to be associated with a more marked disturbance of the vasoactive prostanoid imbalance.

It is of interest that in the urine the vasodilator deficiency tended to be more marked in pre-eclampsia than chronic hypertension, suggesting differences in pathophysiology between pre-eclampsia and chronic hypertension. In the plasma, however, the evidence for thromboxane suppression was equally apparent in both groups. The effect on thromboxane in plasma seemed more strongly related to the level of blood pressure than the underlying pathology. This is consistent with the theory, that the lower plasma TxB_2 levels in hypertensive patients were a secondary response to some other vasoconstrictors rather than the primary abnormality.

The findings of this study suggest that the interrelationship of the vasodilator to vasoconstrictor prostanoids may be of more importance than the absolute levels. The present study provides strong evidence that elevation of the maternal blood pressure initiates attempts to overcome this by alterations in the plasma of the balance of vasodilator to vasoconstrictor prostanoids. There may therefore be differences in the vasoactive prostanoids acting locally in the different vascular beds. A possible explanation is that the vasodilator prostanoid deficiency in the uteroplacental circulation in pre-eclampsia does not initiate the hypertension. If some other factor then initiates the hypertension there may be subsequent alterations in the

production of the vasoactive prostanoids locally in the maternal peripheral circulation to oppose this.

PART 5

STUDY B - PROSTACYCLIN AND THROMBOXANE IN AMNIOTIC FLUID AND UMBILICAL ARTERIAL BLOOD IN NORMAL AND HYPERTENSIVE PREGNANCY

5.1 INTRODUCTION

In vitro studies suggest that prostacyclin may be important in placentation (Rakoczi et al 1983) and there is evidence of a deficiency of prostacyclin in conditions associated with defective placentation such as pre-eclampsia and intrauterine growth retardation (reviewed in Parts 2.5 and 2.7).

In Part 4 a study of maternal prostanoids in pregnancy has been described. In hypertensive patients maternal plasma TxB_2 levels were significantly depressed and the ratios of vasodilator to vasoconstrictor prostanoids were elevated. Circulating prostanoid levels, however, may not reflect the activity locally in the tissues.

Urinary prostanoid excretion in contrast showed trends to a deficiency of vasodilator prostanoids in women with hypertension and a significant decrease in the vasodilator to vasocontrictor prostanoid ratio in women with poor fetal growth. These findings probably

represent a localised alteration in prostanoid synthesis and metabolism in the kidney but there may also possibly be a contribution from other tissues to urinary prostanoid levels.

The differences in the findings in blood and urine suggest that local production of the various prostanoids may differ between different vascular beds and that the vasodilator prostanoid deficiency which has been demonstrated in pre-eclampsia may not be uniform throughout the body.

There is good evidence that the uterus, decidua, placenta, fetal membranes and vessels produce prostacyclin and thromboxane in vitro but the in vivo activities of these substances are less well documented (See Part 2.4). It is possible that intrauterine rather than maternal prostanoid levels may provide a more reliable reflection of the imbalance in prostanoid production in pregnancy complicated by hypertension, particularly if the primary abnormality is in the fetoplacental unit. A study was therefore undertaken of the prostacyclin and thromboxane metabolites in amniotic fluid and umbilical arterial blood from normal and hypertensive pregnant women.

It has been shown that amniotomy and labour increase amniotic fluid prostacyclin and thromboxane metabolite

levels (Mitchell et al 1978(h), Mitchell et al 1979, Makarainen and Ylikorkala 1984) and therefore to avoid these possible effects amniotic fluid was obtained by amniocentesis from patients without evidence of uterine activity.

It has also been shown that PGE and PGF metabolites in umbilical plasma are increased after vaginal delivery as compared to elective caesarean section (Bibby et al 1979), and that the amnion and umbilical artery in vitro produce more 6 keto $PGF_{1\alpha}$ (the prostacyclin metabolite), in patients delivered vaginally compared with those delivered by elective caesarean section (Mitchell et al 1978(e), Makila et al 1984(a)). To avoid any possible effect of labour on umbilical prostanoid levels, this study was therefore confined to patients delivered by elective caesarean section.

5.2 Patients and Methods

Thirty patients due to undergo elective caesarean section were studied, of whom 14 were normotensive and 16 hypertensive. The indications for operation in the normotensive patients were cephalopelvic disproportion, breech presentation or previous caesarean section. In the hypertensive patients caesarean section was performed for deteriorating gestational hypertension, previous caesarean section, suspected IUGR or a combination of these.

Patients with uterine contractions, a recent (< 24 hours) vaginal examination or a history of ingestion of a drug known to affect prostaglandin metabolism were excluded from study, together with patients with any general medical condition such as diabetes which might be associated with a disturbance of the prostaglandins.

Before induction of anaesthesia the blood pressure was measured in the right arm with standard technique with the patient lying with a 30° right lateral tilt. Korotkoff phase 4 sounds were used for measurement of diastolic blood pressure (DBP).

Amniotic fluid was obtained by amniocentesis immediately prior to skin incision. Any samples contaminated with blood or meconium were discarded. Immediately after delivery the placenta was placed on ice and umbilical arterial blood withdrawn by direct arterial puncture. If difficulty was experienced in obtaining arterial blood the sample was discarded.

All samples were placed into chilled polypropylene tubes containing 240µl theophylline EDTA solution (540mg% theophylline and 10g% EDTA) at pH 7 and 120µl acetylsalicylic acid solution (12g% in methanol) and separated within 1 hour by centrifugation at 3000g for 15 minutes at 4°C. Extraction, storage and radioimmunoassay

for 6 keto $PGF_{1\alpha}$ and TxB_2, the stable hydrolysis metabolites of prostacyclin and thromboxane, were performed as described previously (Part 4.3.3 and Appendix 6).

Statistical analysis

The prostanoid levels were not normally distributed and the data were therefore analysed statistically by the non-parametric Mann Whitney test to evaluate the differences between normotensive and hypertensive patients. The effect of gestational age on prostanoid levels was assessed by least squares linear regression and the results are expressed as the Spearman rank correlation coefficient, r. Possible differences in gestational age between the normotensive and hypertensive patients were tested by the non parametric Kruskal Wallis test and the results expressed as the Kruskal Wallis test statistic (K-W). A statistical level of $p = 0.05$ was regarded as "significant".

The 6 keto $PGF_{1\alpha}$ and TxB_2 immunoreactivity are expressed as the median value, with the interquartile range from the 75th to the 25th percentile ($Q_3 - Q_1$) as the measure of variability.

5.3 RESULTS

Amniotic fluid specimens were obtained from 30 patients of whom 14 were normotensive and 16 hypertensive, but

prostanoid assays were only performed on arterial samples from 11 normotensive and 8 hypertensive patients due to difficulties in obtaining umbilical arterial blood. Thirteen of the hypertensive patients had gestational hypertension (7 of whom were proteinuric) and 3 had chronic hypertension. All the hypertensive patients were grouped together for statistical analysis as the numbers were small.

5.3.1 Effect of Gestational Age

The levels of TxB_2 in amniotic fluid were positively correlated to the gestational age of the fetus at the time of sampling (r 0.45 p 0.01). 6 keto $PGF_{1\alpha}$ levels in amniotic fluid also tended to increase with increasing gestational age (r 0.35) although the relationship did not quite achieve statistical significance (p 0.054). Umbilical arterial blood TxB_2 and 6 keto $PGF_{1\alpha}$ however were unrelated to gestational age (r 0.019 p 0.45 and r -0.18 p 0.44 respectively).

5.3.2 Amniotic Fluid

6 keto $PGF_{1\alpha}$ levels in amniotic fluid were significantly lower in hypertensive patients (443 (277) pg/ml) than normotensives (621 (409) pg/ml), with a probability of 0.034 (Mann Whitney u 4.50) (Fig. 5.1). Levels of TxB_2 in amniotic fluid did not differ significantly between the groups (p 0.056 u 3.64), with median values of 207.5 (237) pg/ml in the hypertensive group and 237 (386) pg/ml

in normotensive patients (Fig. 5.2). The ratios of 6 keto $PGF_{1\alpha}$: TxB_2 in amniotic fluid however did not differ between the diagnostic groups (p 0.36) in spite of the lower 6 keto $PGF_{1\alpha}$ levels.

It is possible that the lower 6 keto $PGF_{1\alpha}$ values may have been due to earlier delivery in the hypertensive patients as the mean gestational age at delivery was 35.0 (SD 2.5) weeks in hypertensive patients and 38.2 (SD 2.4) weeks in normotensive patients (p 0.0009; K-W 11,01; df1). The small number of patients in the study unfortunately did not permit evaluation of the effect of gestational age by covariance analysis.

FIG. 5.1: AMNIOTIC FLUID 6 KETO PGF1 α LEVELS IN NORMAL AND HYPERTENSIVE PREGNANCIES

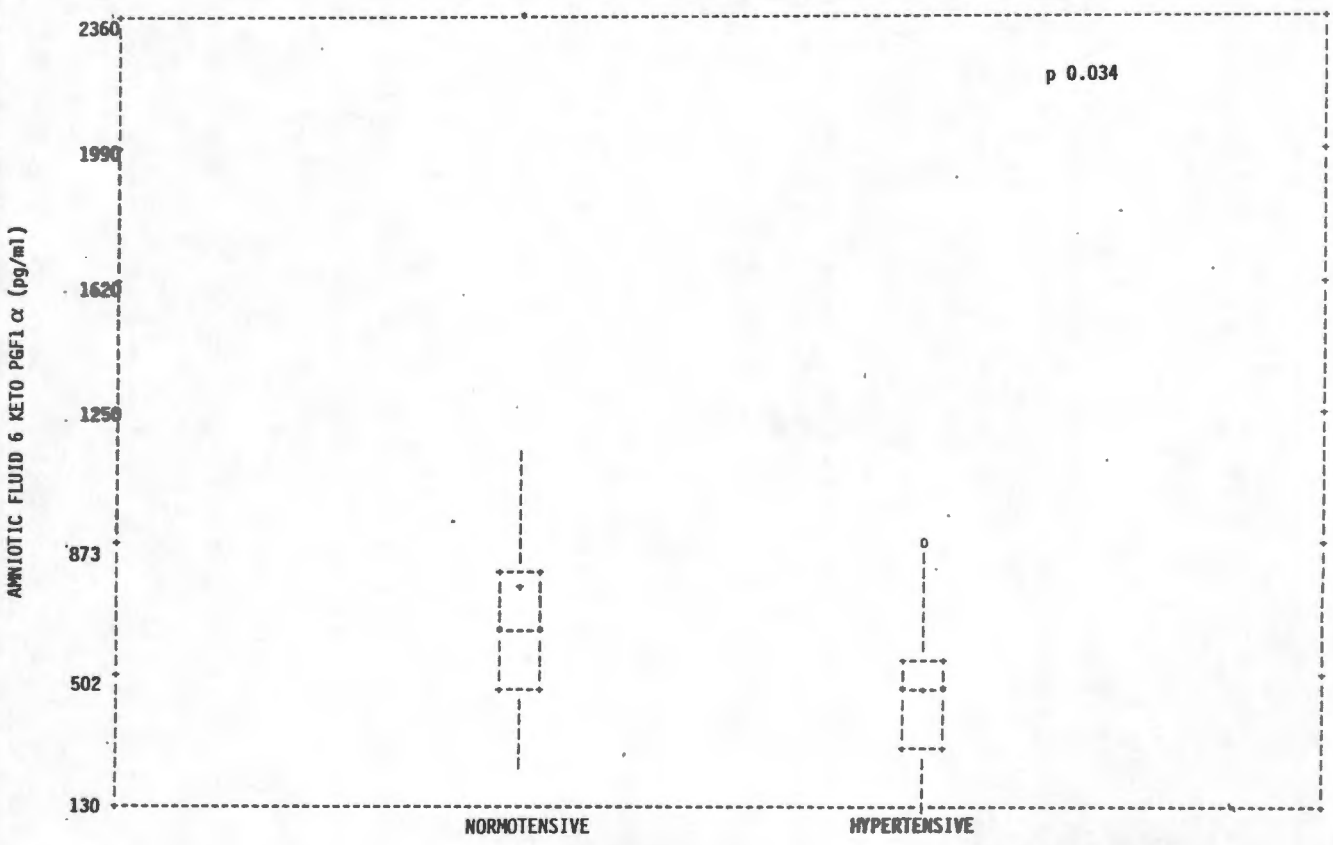
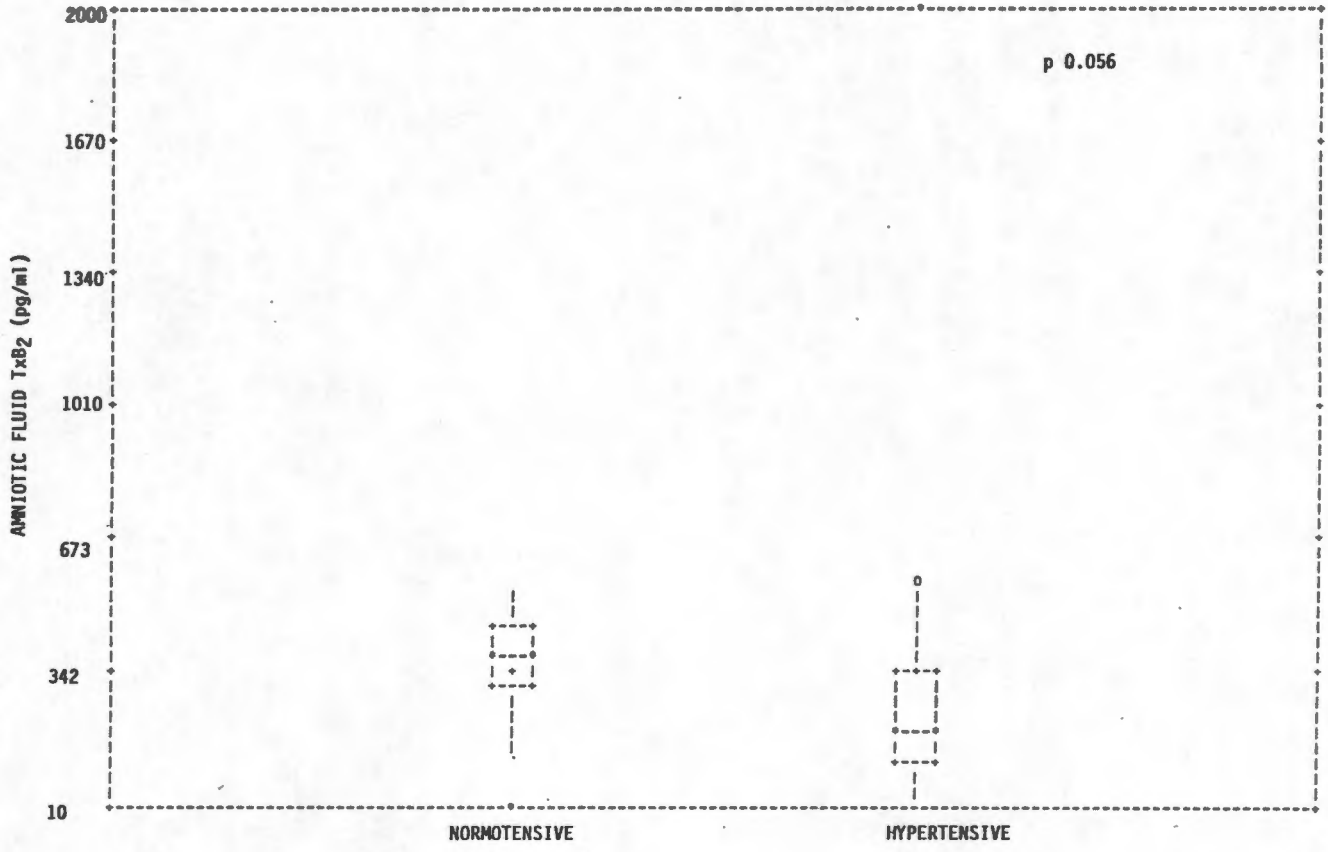


FIG. 5.2: AMNIOTIC FLUID TxB_2 IN NORMAL AND HYPERTENSIVE PREGNANCIES



The effect of fetal growth on 6 keto $PGF_{1\alpha}$ and TxB_2 levels was studied by comparison of the values in amniotic fluid in patients delivered of infants weighing more than and less than the 25th percentile for gestational age (Lubchenco 1963). There were no differences in the levels of either of the prostanoids or the ratio of one to the other in amniotic fluid (Table 5.I). The small number of patients unfortunately did not permit the use of stricter criteria of inadequate fetal growth.

TABLE 5.I : THE EFFECT OF FETAL GROWTH ON AMNIOTIC FLUID 6 KETO $PGF_{1\alpha}$ AND TxB_2 LEVELS (MEDIAN $Q_3 - Q_1$)

FETAL BIRTH-WEIGHT PERCENTILE	6 KETO $PGF_{1\alpha}$ pg/ml	p	TxB_2 pg/ml	p
> 25TH PERCENTILE n = 23	497 (332)	Ø.89	314(275)	Ø.59
< 25TH PERCENTILE n = 7	457 (3Ø7)		279(193)	

5.3.3 Umbilical Arterial Blood

TxB_2 levels in umbilical arterial plasma were very high with median values of 6286 (9433) pg/ml in normotensive patients and 7Ø2 (1591) pg/ml in hypertensive patients (Fig. 5.3). The values were significantly lower in hypertensive patients (p Ø.Ø17 u 5.74). This marked difference could not be accounted for by differences in gestational age between the two groups as umbilical cord prostanoid levels were not related to gestational age. Umbilical plasma levels of 6 keto $PGF_{1\alpha}$ were similar in the normotensive and hypertensive groups (p Ø.8Ø u Ø.Ø6), the median 6 keto $PGF_{1\alpha}$ for normotensives being 821 (2214) pg/ml and for hypertensives 716 (87Ø) pg/ml (Fig. 5.4). The ratios reflected the significantly higher TxB_2 levels with unaltered 6 keto $PGF_{1\alpha}$ levels as the median ratio of 6 keto $PGF_{1\alpha}$ to TxB_2 was Ø.3Ø (Ø.65) in the normotensive compared to 1.Ø9 (1.43) in the hypertensive patients (p Ø.Ø39 u = 4.26).

FIG. 5.3: UMBILICAL ARTERIAL PLASMA 6 KETO PGF1α IN NORMAL AND HYPERTENSIVE PREGNANCIES

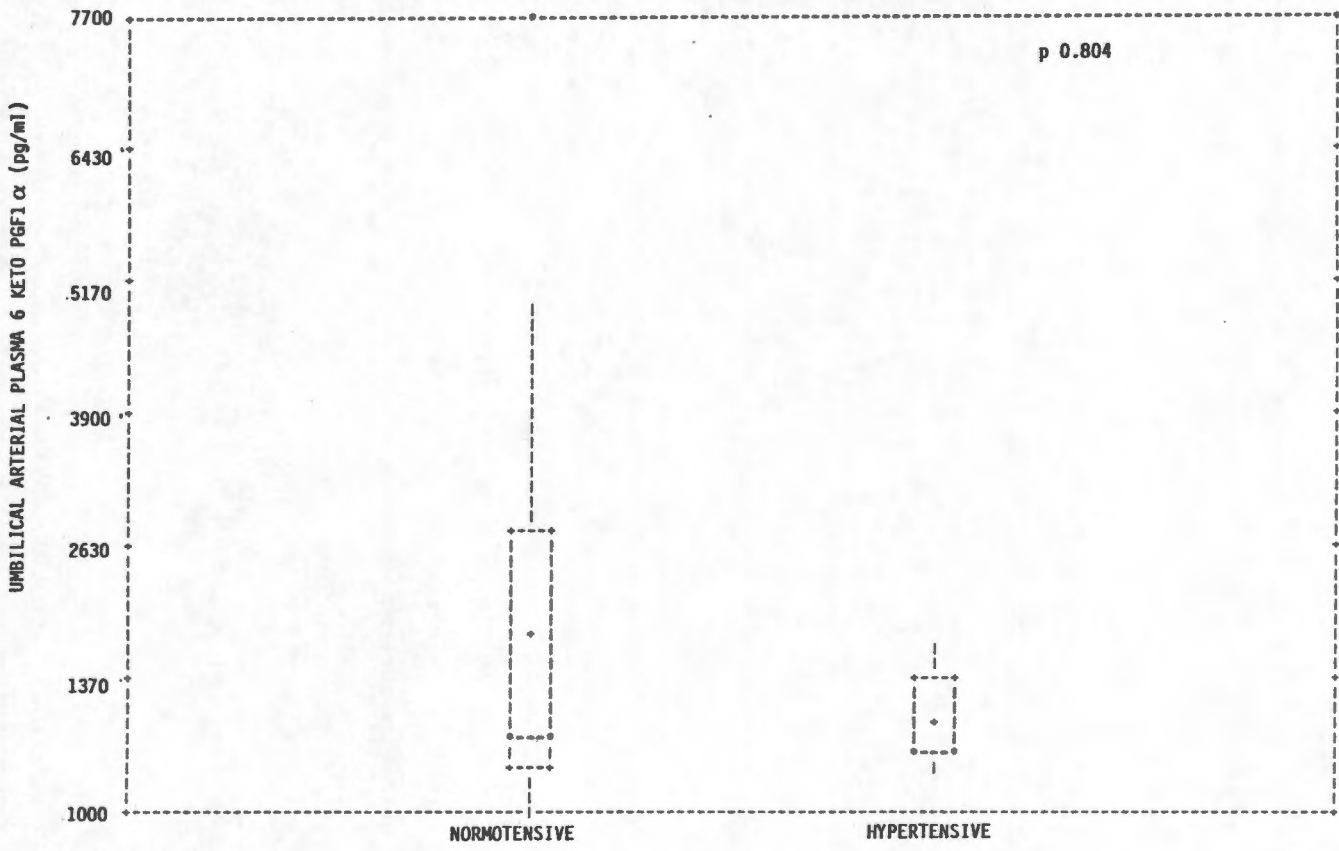
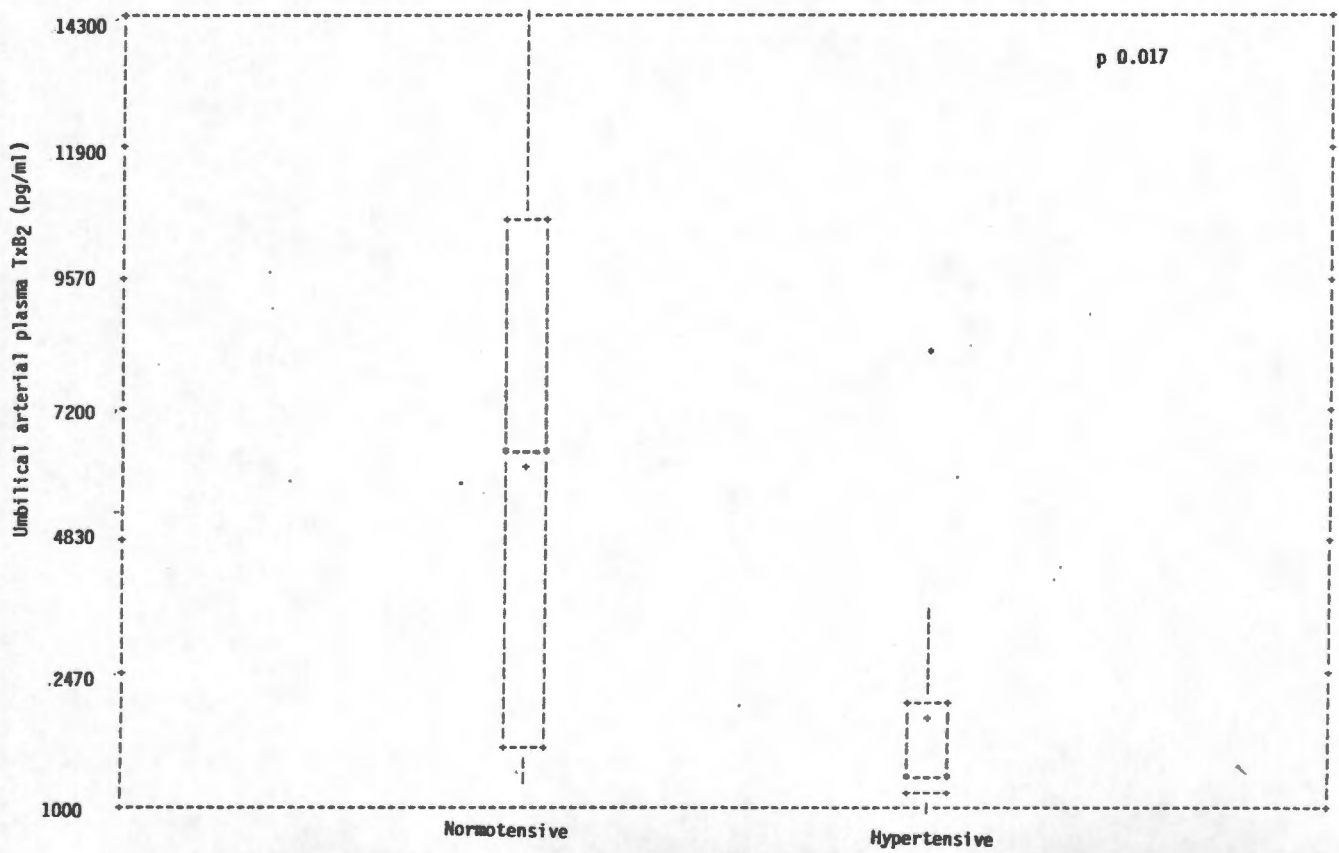


FIG. 5.4 : UMBILICAL ARTERIAL PLASMA TxB_2 IN NORMAL AND HYPERTENSIVE PREGNANCIES



Umbilical arterial blood levels of 6 keto $PGF_{1\alpha}$ and TxB_2 were not related to fetal growth (Table 5.II).

TABLE 5.II : THE EFFECT OF FETAL GROWTH ON UMBILICAL ARTERIAL PLASMA 6 KETO $PGF_{1\alpha}$ AND TxB_2 LEVELS (MEDIAN Q_3-Q_1)

FETAL BIRTH-WEIGHT PERCENTILE	6 KETO $PGF_{1\alpha}$ pg/ml	p	TxB_2 pg/ml	p
> 25TH PERCENTILE n = 14	821 (878)	Ø.43	2429(7173)	Ø.78
< 25TH PERCENTILE n = 5	621 (738)		2ØØØ(7Ø68)	

5.4 DISCUSSION

Amniotic Fluid Prostanoids

Source of amniotic fluid prostanoids

Prostanoids in amniotic fluid may be derived from the fetal membranes (Keirse and Turnbull 1976, Mitchell et al 1978(d)), with an additional contribution from the fetal kidney via the fetal urine (Casey et al 1983, Norman et al 1981). Umbilical arteries synthesise prostanoids in vitro (Tuvemo et al 1976, Takagi and Den 1985) and are another potential source of amniotic fluid prostanoids. It has been suggested that prostanoids synthesised in the decidua may appear in amniotic fluid (Willman et al 1976). Recently, however, it has been shown (Glance et

al 1986) that there is very little transfer of prostanoids across the placenta, which suggests that maternal prostanoids make little contribution to the levels in amniotic fluid. The amniotic fluid prostanoids therefore probably reflect prostanoid production by the membranes, fetal side of the placenta, umbilical vessels and fetal kidneys.

Amniotic fluid 6 keto $PGF_{1\alpha}$

The present study confirms that prostacyclin in amniotic fluid measured as its metabolite 6 keto $PGF_{1\alpha}$, is reduced in pregnancies complicated by hypertension (Ylikorkala et al 1981(c), Makarainen and Ylikorkala 1984). Moodley et al (1984(a)) however in a very similar study were unable to demonstrate any differences. It is possible that the differences in the present study were partly related to the lower gestational age in the hypertensive patients. Amniotic fluid prostacyclin activity has been shown to be related to gestational age by some workers (Wilcox et al 1983, Ylikorkala et al 1981(c)) but not by others (Mitchell et al 1979). In this study although the relationship to gestational age was not statistically significant it may have had some effect. An attempt was made to assess this by dividing the patients into two groups according to gestational age. The effect of hypertension was still apparent but the numbers in each group were too small for valid statistical analysis.

This study, though with relatively small numbers, confirms the finding that prostacyclin deficiency in amniotic fluid is specific to the hypertensive diseases (Ylikorkala et al 1981(c)) and is not related to fetal growth.

Amniotic fluid TxB_2

In common with other workers (Ylikorkala et al 1981(c), Moodley et al 1984(a), Brown et al 1987) no difference was found in amniotic fluid TxB^2 levels between normal and hypertensive patients. Although Ylikorkala et al (1981(c)) described a lower 6 keto $PGF_{1\alpha}$ to TxB^2 ratio in amniotic fluid in pre-eclampsia, this was not apparent in the present study nor those of Moodley et al (1984(a)) and Brown et al (1987). The Finnish workers (Ylikorkala et al 1981(c)) in a later report however (Makarainen and Ylikorkala 1984) were also unable to detect any differences in the 6 keto $PGF_{1\alpha}$: TxB_2 ratio in pre-eclamptic patients before labour, but the difference became apparent as prostanoid production increased in labour. If thromboxane production in the placenta is increased in gestational and chronic hypertension, as suggested by some workers (Makila et al 1984(b), Walsh 1985(a)) these differences do not appear to be reflected in amniotic fluid.

Umbilical arterial blood prostanoids

Decreased prostacyclin production by umbilical and placental vessels is well documented in vitro (Remuzzi et al 1980(b), Downing et al 1980, Carreras et al 1981, Bussolino et al 1980). Although depressed prostacyclin production by placental tissue in pre-eclampsia and intrauterine growth retardation have been described (Walsh 1985(a), Walsh et al 1985(b), Jogee et al 1983), other workers have found placental prostacyclin production to be normal in hypertensive pregnancy (Makila et al 1984(b)).

Umbilical arterial blood samples were shown to have 6 keto $PGF_{1\alpha}$ and TxB_2 levels which were extremely high and almost certainly do not reflect the circulating fetal levels. Very high levels of 6 keto $PGF_{1\alpha}$ have been reported previously (Takagi and Den 1985, Ylikorkala et al 1981(b), Ogino et al 1986(b)). As the umbilical vessels have even greater potential than maternal vessels to respond to trauma by prostanoid production (Keirse 1979) it is likely that umbilical blood samples were subject to considerable artefact and hence may not reflect the true situation in the fetus in utero. Trauma is particularly likely to cause elevation of TxB_2 levels (Morris et al 1981).

A recent study using the specific GCMS technique (Jacqz et al 1985) has demonstrated that umbilical cord blood 6 keto $PGF_{1\alpha}$ levels increase significantly between delivery of the baby and expulsion of the placenta. After delivery of the placenta the levels of 6 keto $PGF_{1\alpha}$ increased further, rising from 345 pg/ml at 1 minute after placental delivery to 3678 pg/ml at 10 minutes. In the present study the median 6 keto $PGF_{1\alpha}$ of 821 pg/ml in umbilical cord blood samples from normotensive patients a few minutes after placental delivery would be consistent with these findings. The concentrations of PGE, PGF and PGFM in umbilical cord plasma have also been found to increase continuously from that time of delivery of the baby but with no significant changes in relation to clamping of the cord or delivery of the placenta (Mitchell et al 1978(g)). This marked increase immediately after delivery of the fetus therefore probably applies to all prostanoids including TxB_2 and may be of physiological importance in closure of the umbilical cord.

Umbilical arterial plasma 6 keto $PGF_{1\alpha}$

Under normal physiological constitution the umbilical arteries produce mainly prostacyclin (Mitchell et al 1980) which is believed to be a major regulator of fetoplacental blood flow (Tuvemo 1980, Makila et al 1983(a)). In pre-eclampsia the umbilical arteries in

vitro produce less prostacyclin than normal but thromboxane production is unaffected (Remuzzi et al 1980(b), Downing et al 1980, Takagi and Den 1985, Makila et al 1983(a)). Although Takagi and Den (1985) found reduced levels of the prostacyclin metabolite 6 keto $PGF_{1\alpha}$ in umbilical plasma from pre-eclamptic compared to normal women, their reported mean levels for normal patients (1630 pg/ml) seem very high. In the present study contrary to expectations the umbilical arterial blood 6 keto $PGF_{1\alpha}$ levels were similar in normotensive and hypertensive women (median values 821 and 716 pg/ml respectively).

Umbilical arterial blood TxB_2

The high levels of TxB_2 in the umbilical arterial blood from the normotensive pregnancies in the present study suggest artefactual synthesis or release of thromboxane by the sampling method. However, this does not explain the significantly higher TxB_2 levels in normotensive compared to hypertensive women. Factors which control prostanoid synthesis may be activation of the cyclo-oxygenase or PGH synthetase enzymes (Downing et al 1981, Keirse et al 1985) or the availability of precursor essential fatty acids (Ongari et al 1984, Ogburn et al 1984). It is possible that the results reflected an overall impairment of prostanoid synthesis in hypertensive patients, with the umbilical cord of hypertensive patients having a reduced capacity to

respond to trauma.

The lower umbilical arterial plasma TxB_2 levels in hypertensive patients and the subsequent alteration in the balance of 6 keto $PGF_{1\alpha}$: TxB_2 ratio towards the vasodilator side, could alternatively suggest suppression of the vasoconstrictor element in response to inadequate umbilical blood flow. The findings of decreased TxB_2 with unchanged 6 keto $PGF_{1\alpha}$ levels in umbilical arterial blood from hypertensive patients contrast with the reduced capacity of umbilical arteries from pre-eclamptic patients to produce prostacyclin in vitro. The capacity of the umbilical arteries to produce prostanoids in vitro may not reflect the situation in the fetus in utero and furthermore the prostanoids in umbilical arterial blood samples may derive from the fetus itself as well as the umbilical arteries. It is possible that the fetus responds to excessive vasoconstrictors from the placenta and umbilical cord tissues by suppression of TxB_2 in the arterial blood flowing through the umbilical cord.

Umbilical arterial blood samples have been reported to show elevation of vasodilator prostanoids in response to acute fetal distress in labour (Ogino et al 1986(b)).

These findings are therefore consistent with the theory that adaptation may occur in response to unfavourable conditions such as hypoxia or reduced blood flow, by alterations in the balance of the vasodilator to vasoconstrictor prostanoids.

The observations agree with Doppler ultrasound studies which have demonstrated that in hypertensive pregnancy despite increased resistance in the placenta the fetus shows some tolerance to these effects. Studies of umbilical arteries in pregnancies complicated by hypertension show abnormal waveforms with a low diastolic velocity particularly in those pregnancies associated with poor fetal growth (Trudinger et al 1985). This implies increased placental resistance in pregnancies with IUGR. Fetal aorta waveforms however show no abnormalities unless the pregnancy is complicated by both fetal growth retardation and hypertension (Joupilla and Kirkinen 1986). These findings suggest that up to a point the fetus is capable of maintaining its circulation in spite of increased placental resistance. Other studies have demonstrated a process of selective vasoconstriction and vasodilatation in the growth retarded fetus as flow velocity waveforms studies using pulsed Doppler show increased peripheral resistance in

the fetal aorta with decreased resistance in the cerebral vessels (Wladimiroff et al 1986). Thus the fetus may be capable of redirection of its blood flow in the presence of hypoxia. It is possible that in relatively mild hypertension the fetus is able to compensate for an increased placental resistance and reduced umbilical artery blood flow by vasodilatation. If however the hypertension becomes more severe and hypoxia ensues, then redistribution of the circulation may occur with peripheral vasoconstriction and consequent growth retardation but with preferential cerebral vasodilatation to maintain brain growth. The findings of the present study suggest that a reduction of the fetal thromboxane production may be a mechanism to maintain fetal blood flow in the face of a prostacyclin deficiency in the placenta and umbilical artery.

Umbilical artery prostanoid levels were not related to fetal growth but the numbers were not sufficient to assess differences using the accepted measure of the 10th birthweight percentile. It would be of interest to study a larger group of hypertensive patients to test the above hypothesis of the fetal response to hypertension.

5.5 SUMMARY

Amniotic fluid 6 keto $PGF_{1\alpha}$ levels were lower in pregnancy complicated by hypertension than in normal pregnancy but TxB_2 levels were comparable in the two groups. The origin of prostanoids in amniotic fluid is unknown but the source is likely to be intrauterine. These findings therefore confirm the evidence from in vitro studies of a deficiency of prostacyclin production in the uterus in hypertensive pregnancy. This disturbance of prostanoid synthesis and metabolism may be the primary abnormality leading to deficient placentation or may be the result of placental abnormalities.

Umbilical arterial blood TxB_2 levels were significantly lower in hypertensive patients but the 6 keto $PGF_{1\alpha}$ levels were similar in hypertensive and normotensive women. These differences were also reflected in the higher 6 keto $PGF_{1\alpha}$: TxB_2 ratio in the hypertensive. These findings may indicate a response of the fetus to the deficiency of the vasodilator prostacyclin by suppression of the vasoconstrictor thromboxane. By this mechanism the fetus may minimise the effects of the vasodilator deficiency.

PART 6

STUDY C - THE EFFECT OF PLASMA VOLUME EXPANSION ON PLASMA PROSTACYCLIN AND THROMBOXANE METABOLITES IN HYPERTENSIVE PREGNANCY PATIENTS

6.1 INTRODUCTION

Normal pregnancy is associated with a physiological expansion of the plasma volume (Pirani et al 1973, Hytten and Paintin 1963). In pre-eclampsia the blood volume is relatively decreased compared to normal pregnant values at a comparable gestational age (Chesley 1972). It has been suggested (Assali and Vaughan 1978) that the low plasma volume is secondary to vasoconstriction whereas other workers in contrast feel that the hypovolaemia itself is at least partly a cause of poor organ perfusion in pre-eclampsia (Cloeren and Lippert 1972). Plasma volume expanders have been used by a number of workers to increase plasma volume in pre-eclamptic patients and a fall in blood pressure, improved renal function and a fall in systemic resistance have been reported (Gallery et al 1981, Cloeren and Lippert 1972, Groenendijk et al 1984). In addition an increase in fetal blood flow velocity measured by pulsed doppler has been reported by Siekmann et al (1986) and some believe that sufficient

reversal of the pathophysiological changes occurs for this treatment to be used for long term therapy in pre-eclampsia (Rasmussen et al 1984).

The treatment of pregnancy hypertension with plasma volume expanders is further described in Part 2.9.

Rapid expansion of the plasma volume causes vasodilatation and animal studies have suggested that distension and stretch may stimulate local prostacyclin release in isolated arteries (Pace-Asciak et al 1978). It is possible that plasma volume expansion by mechanical distension of the blood vessels may initiate prostacyclin release or alternatively suppress thromboxane production causing vasodilatation and a fall in blood pressure.

The aim of this study was to investigate the relationship of plasma volume to circulating blood levels of prostacyclin and thromboxane metabolites, to study the acute effect of plasma volume expansion on these metabolites in pregnant hypertensives and to further evaluate their role in the vasoconstriction commonly seen in these patients.

6.2 PATIENTS AND METHODS

Twenty-one hypertensive pregnant patients from the hypertension antenatal ward at Groote Schuur Hospital

were studied. All patients who satisfied the selection criteria during the study period were included (Table 6.I). Every third patient selected for study was allocated to the control group.

Table 6.I: Selection criteria for plasma volume expansion study

Inclusion criteria:

1. Diastolic blood pressure 90 - 110 mmHg (average of 4 readings over a 24 hour period on the 2nd day after admission to hospital.
2. Gestational age greater than 20 weeks.
3. Free informed written consent.

Exclusion criteria:

1. Antihypertensive medication.
2. Antiprostaglandin therapy in the previous 2 weeks.
3. Other medical or obstetric disorders.
4. Recent (< 24 hours) vaginal examination.
5. Uterine activity.

The timetable of investigation is shown in Table 6.II.

Table 6.II: Timetable of Investigation

Day 1: Admission to Hospital.

Day 2: 6 hourly blood pressure measurement. Overnight fast.

Day 3: (a) Baseline blood pressure and pulse.
Blood sampling for 6 keto $PGF_{1\alpha}$ and TxB_2.
Plasma volume estimation.

(b) Treatment Patients:
Plasma volume expansion with 500 ml stabilised human serum (SHS) infused over 90 min.

Control Patients:
Slow running saline infusion of approximately 20 ml over 90 min.
Measurement of blood pressure and pulse every 15 min.

(c) 2 hours after the start of plasma volume expansion blood sampling for plasma 6 keto $PGF_{1\alpha}$, TxB_2 and plasma volume measurement was performed.
Measurement of blood pressure and pulse.

All patients had a 16 G intravenous cannula inserted into the antecubital fossa for blood sampling. Samples for measurement of the prostanoids were taken, treated and assayed as described previously (Section 4.3.3).

The stable metabolites of prostacyclin and thromboxane, 6 keto $PGF_{1\alpha}$ and TxB_2 were measured in plasma before and after volume expansion. Blood pressure measurements were obtained with a Bresco computerised manometer to exclude observer bias. Plasma volume was measured by the Evans Blue dilution technique (Chesley and Duffus 1971(b)). An initial sample was taken as a serum blank after which a known volume of Evans Blue dye (approximately 3 ml) was injected and plasma volume calculated from the dye concentration in the 10 min. blood sample.

The patients in the two groups underwent the same observations.

Plasma volume expansion was performed over 90 min. with stabilised human serum (SHS) (Western Province Blood Transfusion Service) the main constituents of which are shown in Table 6.III.

Table 6.III: Composition of Stabilised Human Serum

Total Protein	50 g +/- 5 g/l
Albumin	31 g +/- 2 g/l
Immunoglobulins	11 g/l
Osmolarity	260 +/- 20 mOsm/kg

No preservatives

Statistical Analysis

The differences in blood pressure and plasma volume before and after treatment in individual patients were analysed by paired Students t-test and the differences between control and treatment groups by unpaired Students t-test. Prostanoid results were analysed by the Sign test for differences before and after treatment and the Mann Whitney test for differences between the control and treatment groups.

The relationships of initial plasma volume to plasma prostanoid levels and the changes in these measurements after plasma volume expansion were assessed by Spearman rank correlation. The plasma volume measurements were adjusted for maternal height prior to this analysis.

6.3 RESULTS

The initial blood pressure and plasma volume measurements in the two groups were not significantly different.

6.3.1 Plasma Volume

After plasma volume expansion however there was a rise in plasma volume in the volume expansion group ($p < 0.001$) but not in the control group (Table 6.IV).

Table 6.IV : Plasma volume measurements before and 2 hours after volume expansion

Mean (SD)

	Before	After	Paired t-test
Volume expansion group	3.94(0.59)	5.79(1.40)	$p < 0.001$
Control group	3.83(0.70)	4.37 (0.90)	$p > 0.1$
Unpaired t-test	$p > 0.5$		

6.3.2 Blood Pressure

After plasma volume expansion there was a significant drop in diastolic ($p < 0.001$) but not systolic blood pressure ($p > 0.5$) in the treatment group. There were no significant changes in blood pressure in the control group (Table 6.V).

Table 6.V: Systolic and diastolic blood pressures (mmHg) before and at 2 hours after volume expansion (Mean SD)

	Before	After	Paired t-test
Volume expansion group			
Systolic BP	148 (16)	142 (19)	p > Ø.1
Control group			
Systolic BP	133 (2Ø)	131 (16)	p > Ø.5
Unpaired t-test	p < Ø.1		
Volume expansion group			
Diastolic BP	1Ø3 (9)	86 (9)	p < Ø.ØØ1
Control group			
Diastolic BP	1ØØ (14)	96 (14)	p > Ø.1
Unpaired t-test	p > Ø.5		

6.3.3 Plasma Prostanoid Levels

The plasma levels of 6 keto $PGF_{1\alpha}$ and TxB_2 were not related to the first plasma volume measurement (r -Ø.Ø3 p Ø.92 and r -Ø.15 p Ø.54 respectively).

The levels of 6 keto $PGF_{1\alpha}$ were significantly increased in the treatment group after treatment (p Ø.Ø33). In the control group 6 keto $PGF_{1\alpha}$ levels increased slightly but the differences were not statistically significant (p Ø.446) (Table 6.VI).

Table 6.VI: Maternal plasma 6 keto $PGF_{1\alpha}$ levels (pg/ml) before and 2 hours after plasma volume expansion

Median (interquartile range Q_3–Q_1)

	n	Before	After	Effect of Treatment (Sign test)
Volume expansion group				
6 keto $PGF_{1\alpha}$	11	600(228)	671(486)	p 0.33*
Control group				
6 keto $PGF_{1\alpha}$	7	614(465)	629(643)	p 0.466

Control vs treatment

Mann Whitney test p 0.751

The levels of thromboxane B_2 were significantly decreased in the treatment group (p 0.0329) after plasma volume expansion. In the control group in contrast, TxB_2 levels increased but the increase was not statistically significant (p 0.075) (Table 6.VII). The change in the plasma volume expansion group was significantly different to the control group (p 0.01).

Table 6.VII: Maternal plasma TxB_2 levels (pg/ml) before and 2 hours after plasma volume expansion

Median (Q_3-Q_1)

	n	Before	After	Effect of Treatment (Sign test)
Volume expansion group				
TxB_2	13	264(187)	214(104)	p 0.032*
Control group				
TxB_2	7	228(22)	243(178)	p 0.075

Control vs Treatment

Mann Whitney Test p 0.553

The difference in plasma volume between the first and second measurements was significantly correlated to the change in plasma TxB_2 (r -0.55 p 0.014). This indicates that an increase in plasma volume is associated with a significant fall in plasma TxB_2 levels. The alteration in plasma volume was not however correlated to the change in plasma 6 keto $PGF_{1\alpha}$ levels (r -0.28 p 0.25).

The ratio of 6 keto $PGF_{1\alpha}$ to TxB_2 tended to increase in the treatment group and decrease in the control group but the differences were not statistically significant (p 0.10 and p 0.27 respectively (Table 6.VIII).

Table 6.VIII: Ratio of maternal plasma 6 keto $PGF_{1\alpha}$: TxB_2 before and 2 hours after plasma volume expansion

Median (Q_3-Q_1)

	n	Before	After	Effect of Treatment (Sign test)
Volume expansion group Ratio 6 keto $PGF_{1\alpha}$: TxB_2	11	2.35(2.2)	3.22(1.9)	p Ø.1Ø
Control group Ratio 6 keto $PGF_{1\alpha}$: TxB_2	7	2.69(Ø.9)	2.67(Ø.8)	p Ø.27
Control vs treatment Mann Whitney test		p Ø.62	p Ø.17	

6.4 DISCUSSION

This study confirms previous findings (Gallery et al 1981, Gallery et al 1984(a)), that plasma volume expansion is accompanied by a significant decrease in diastolic blood pressure. This suggests that not only is there a passive increase in vascular capacity but that there is an active arterial or arteriolar vasodilatation in response to plasma volume expansion.

The consistency of the fall in blood pressure implies that this is not an anaphylactoid reaction as suggested in normotensive patients (Ring and Messmer 1977, Bland et al 1983), but is a normal haemodynamic response to vasodilatation in pregnant women.

The observation of an increase in circulating 6 keto $PGF_{1\alpha}$ and a decrease in TxB_2 levels is evidence that plasma volume expansion stimulates prostacyclin or suppresses thromboxane release. The only previous comparable investigation of prostacyclin response to plasma volume expansion in hypertensive pregnancy (Gallery et al 1984(a) and (b)), reported results in contrast to the present study. These workers found mean 6 keto $PGF_{1\alpha}$ levels were decreased by plasma volume expansion (Gallery et al 1984(b)). In the latter studies and this one, the increments of change were small, but the dilutional effect of plasma volume expansion increases the significance of the increase in 6 keto $PGF_{1\alpha}$ which has been demonstrated in the present study.

The initial plasma volume measurement was not related to the plasma levels of 6 keto $PGF_{1\alpha}$ and TxB_2. However the second plasma volume measurement had a significant negative correlation to the plasma TxB_2 level. This is

evidence that alterations in maternal TxB_2 production are important in control of vasodilatation and as a result, important in the regulation of blood pressure in pregnancy. The change in plasma volume over the period of the study was not related to the alteration in plasma 6 keto $PGF_{1\alpha}$. Although the study of Gallery et al (1984(b)) reported a fall in 6 keto $PGF_{1\alpha}$ levels in relation to plasma volume expansion, they were also unable to relate the changes to the increase in plasma volume.

Another explanation for the increase in prostacyclin metabolites and the vasodilator effect of volume expansion in the current study is that SHS contains prostacyclin or 6 keto $PGF_{1\alpha}$ and that the effects would be similar to prostacyclin infusion which produces vasodilatation and hypotension (Fidler et al 1980). It was planned to measure the 6 keto $PGF_{1\alpha}$ and TxB_2 content of SHS, but the samples were inadvertently omitted from the assay. Nevertheless it can be calculated from the data of the prostacyclin infusion study presented earlier in this thesis (See Part 4.3.3.3) that at the infusion rate used in the present study a physiological response would only be expected

if the expansion fluid contained at least 10 ng/ml of prostacyclin, which would appear very unlikely.

The release of prostacyclin locally in arteries and arterioles by distension has been described in vitro in animal studies, the effect being more marked in hypertensive rats (Botha et al 1980, Pace-Asciak et al 1978). A deficiency of prostacyclin has been demonstrated in fetal samples from pre-eclamptic pregnancies (See Part 2.5). Maternal plasma prostacyclin metabolite levels do not usually reflect these changes (See Part 4.4.5.4) (Ylikorkala et al 1981(a)). Only a small increase in plasma 6 keto $PGF_{1\alpha}$ levels has been demonstrated, which would not seem sufficient to cause the haemodynamic changes. It is probable that the effect of plasma volume expansion in lowering blood pressure is by local stimulation of prostacyclin in the arteriolar walls and the true effect is much greater than can be detected in peripheral plasma.

The decrease in circulating thromboxane following plasma volume expansion has not previously been described. The suppression of TxB_2 levels may be due to a direct suppressor effect on platelet thromboxane

production or it may represent a generalised tendency to the vasodilator side of the prostacyclin/thromboxane balance in response to a fluid infusion.

Another possible explanation is that the synthesis or metabolism of prostacyclin and thromboxane is affected by volume expansion with subsequent alterations in their biological effects.

6.5 SUMMARY

This study provides evidence that the vasodilatation and fall in blood pressure induced by plasma volume expansion in hypertensive pregnant patients are mediated by alterations in vasoactive prostanoids. Although the initial plasma volume was not related to the plasma prostanoid levels, the change in plasma volume was significantly negatively correlated to plasma TxB_2 levels. This suggests that changes in plasma TxB_2 play a role in the regulation of intravascular volume.

Peripheral resistance in the different vascular beds is important in control of blood pressure in pregnancy. Vasoconstrictors with increased vascular resistance in peripheral organs particularly the uterus and kidney are recognised features of gestational hypertension.

The increase in plasma 6 keto $PGF_{1\alpha}$ and decrease in plasma TxB_2 levels in patients treated with volume expansion adds further evidence that the balance of circulating prostacyclin and thromboxane are important regulators of vascular resistance and blood pressure in hypertensive pregnancies.

PART 7

STUDY D - LOW DOSE ASPIRIN AND DIPYRIDAMOLE IN THE PREVENTION OF PRE-ECLAMPSIA - THE EFFECT ON PLASMA PROSTACYCLIN AND THROMBOXANE METABOLITES

7.1 INTRODUCTION

The best approach to pregnancy hypertension would be its prevention. Various combinations of low dose aspirin and dipyridamole have been reported to be effective in preventing pre-eclampsia (Crandon and Isherwood 1979, Beaufils et al 1985, Wallenburg et al 1986). The mechanism by which this is achieved is poorly understood but it has been suggested that these agents restore the disturbed prostacyclin/thromboxane balance which may be one of the main aetiological factors in the development of pre-eclampsia. The primary aim of this study was to evaluate the effect of low dose aspirin and a low dose aspirin/dipyridamole combination on circulating prostacyclin and thromboxane metabolites, and at the same to conduct a randomised controlled study of aspirin and dipyridamole in pregnant patients judged to be at increased risk of pre-eclampsia.

7.2 BACKGROUND AND RELEVANCE OF STUDY

Most of the maternal and fetal risks of hypertension in pregnancy are mediated through the development of proteinuric pre-eclampsia (Redman 1980, Dunlop 1966). Several controlled trials have addressed the question of whether antihypertensive therapy prevents progression of mild to moderate non-proteinuric hypertension to pre-eclampsia. Redman et al (1980) found that treatment of chronic hypertensive patients with methyldopa did not affect the incidence of pre-eclampsia. Two other studies, however, (Walker et al 1982, Rubin et al 1983) have claimed a reduction in the incidence of progressive hypertension and the development of proteinuria in patients treated with beta blockers. The evidence that antihypertensive agents prevent the development of pre-eclampsia is controversial and in addition there have been reports that these agents, particularly beta blockers, may be associated with fetal side effects (Lieberman et al 1978, Woods and Morrell 1982, Fidler et al 1983). The evidence that antihypertensive treatment results in a significant overall improvement in fetal outcome is scanty (reviewed by Walker 1987), so that any agent which will prevent pre-eclampsia would be a major advance in management.

7.2.1 The Effects of Low Dose Aspirin

Aspirin has become established as an antithrombotic agent in the prevention of thrombo-embolic disease and was chosen initially by virtue of its action on platelets. Platelet aggregation and adhesion of platelets to the vessel wall are believed to play an important role in the development and progression of thrombotic lesions (Hess et al 1985). In pre-eclampsia platelet hyperactivity, fibrin deposition and uteroplacental arterial thrombosis, are important features (See Part 2.2.3 and Lindheimer and Katz 1981) possibly due to an imbalance of prostacyclin and thromboxane (Part 2.5.4). Theoretically therefore a treatment which inhibits platelet activation may prevent the development of pre-eclampsia or reduce the progression of the disease.

Aspirin inhibits the second phase of platelet aggregation by irreversibly binding the cyclooxygenase enzyme thereby inhibiting TxA_2 formation in the platelets. As the platelet is unable to generate a new enzyme system the effect lasts for the duration of the life span of the platelet (4 - 7 days) (Weiss 1982). The endothelial cell cyclooxygenase system is also suppressed by aspirin, which by decreasing the cyclic endoperoxide production in the endothelium, will reduce

the production of prostacyclin. Platelet cyclooxygenase however is more sensitive to inhibition by aspirin (Hess et al 1985) and the effect on endothelial cyclooxygenase may be of shorter duration than the effect on the platelet enzyme (Masotti et al 1979). The differences in sensitivity of the platelet and endothelial cell cyclooxygenase may be a reflection of the more rapid turnover of the endothelial cells (Jaffe and Weksler 1979, Smith et al 1980) or may be due to differences in drug distribution to the different tissues. There nevertheless appear to be differences in sensitivity to inhibition by aspirin in different tissues (Baenziger et al 1977). Aspirin in low doses may increase endogenous prostacyclin production by redirection of platelet endoperoxides to the endothelium (Weselcouch et al 1985).

This differential suppression of prostacyclin and thromboxane by aspirin has led to its wide use in the management of occlusive arteriosclerosis. However the dose of aspirin which will produce maximum suppression of the proaggregatory vasoconstrictor thromboxane without inhibiting the antiaggregatory, vasodilator prostacyclin is still uncertain. Studies performed by Hanley et al (1985) have suggested that doses of aspirin is excess of 80mg a day substantially block

both thromboxane and prostacyclin synthesis. This is in agreement with Davi et al (1983) who demonstrated that a single dose of 100mg of aspirin substantially inhibited both prostacyclin and thromboxane production in whole blood, whereas a 20mg dose produced a preferential suppression of thromboxane. Masotti et al (1979) however showed that prostacyclin output was unaffected by a dose of aspirin of 2mg/kg (although thromboxane activity was completely inhibited by this dose) and these workers recommended a dose of 3.5mg/kg at 3 day intervals to induce maximum inhibition of platelet aggregation with minimal effect on endothelial prostacyclin production.

Most reported studies showing aspirin to be effective in the prevention of vascular occlusive diseases have used doses far in excess of this (300mg - 1300mg/day) (Editorial Lancet 1985). However it must be recognised that in prevention of thromboembolism other antithrombotic effects may be as important as the role of prostacyclin and thromboxane. ADP produced by platelets has a proaggregatory effect. Aspirin may inhibit prostaglandin dependant ADP release from the platelets and stimulate conversion of ADP released by prostaglandin independant pathways to adenosine (itself an antiaggregatory substance) (Lewis et al 1977). In

thromboembolism high dose aspirin may therefore be effective whilst totally blocking prostacyclin synthesis. In the prevention of pre-eclampsia the effect of aspirin in inhibiting release of thromboxane whilst avoiding prostacyclin inhibition is likely to be more important than prevention of platelet aggregation per se. Thus the use of a low dose of aspirin and the avoidance of high dose may be particularly important.

There is evidence that the effect of aspirin on prostanoid synthesis may be cumulative (Hoogendijk and Tencate 1980) although Heavey et al (1985) in contrast, showed that endothelial cell prostacyclin production is suppressed for only 6 hours even with a large dose of aspirin (600mg). The optimum dose of aspirin in prevention both of vascular disease and of pre-eclampsia has therefore still to be established but it is generally accepted that the doses utilised in most studies are too high to produce the optimum effects on prostanoid synthesis. The choice of dose is also complicated by observations that there may be considerable differences in response to aspirin between different individuals (Hoogendijk and Tencate 1980, O'Brien 1980). A recent editorial (Lancet 1986) advocated a dose of 300mg/day for prevention of myocardial infarction and stroke, but acknowledged that

for optimum effect on the prostacyclin/thromboxane balance the dose may need to be as low as 30 - 40mg on alternate days.

7.2.2 The Effects of Dipyridamole

The addition of dipyridamole to aspirin therapy is based on the observation that in vitro at least the drugs have different but complementary effects on platelet activity. Dipyridamole acts primarily as an inhibitor of platelet phosphodiesterase with consequent elevation of platelet cyclic adenosine monophosphate (cAMP) and inhibition of platelet aggregation (Mills and Smith 1971, Weiss 1982). Dipyridamole also stimulates adenylate cyclase in the platelets increasing conversion of ATP to cAMP and it has been suggested that this effect may be mediated by stimulation of endogenous prostacyclin (Moncada and Vane 1978) or inhibition of adenosine uptake (Gresele et al 1986). The stimulation of endogenous vasodilator and antiaggregatory substances in vivo may explain the increased effectiveness of dipyridamole as an antiaggregatory agent in vivo compared to in vitro (Mehta et al 1982).

Aspirin and dipyridamole work through different mechanisms to inhibit platelet aggregation and there is some evidence that the drugs work synergistically

(Gresele et al 1985). Theoretically the combination of aspirin and dipyridamole should be a more effective antithrombotic agent than aspirin alone in preventing thrombosis, but studies in vivo of aspirin and dipyridamole separately and in combination have utilised widely differing doses and it is difficult to draw conclusions. Hess et al (1985) reported that the combination was more effective than aspirin alone in prevention of occlusive peripheral arterial disease but Bousser et al (1983) were unable to demonstrate this effect in the prevention of cerebral ischaemia. In view of this uncertainty, aspirin alone and in combination with dipyridamole were compared to no treatment in the present study.

7.2.3 Aspirin and Dipyridamole in Prevention of Pre-Eclampsia

The first evidence that aspirin may be effective in preventing pre-eclampsia was the observation of Crandon and Isherwood (1979) that women who took aspirin at least every two weeks in pregnancy had a lower incidence of pre-eclampsia than women with no history of aspirin ingestion.

Beaufils et al (1985) reported a randomised controlled study of 102 patients (mainly multiparous) judged to be at high risk of pre-eclampsia mostly on the grounds of

a poor obstetric history. Those patients who received 150mg aspirin and 300mg dipyridamole daily from 3 months gestation had a significantly lower incidence of pre-eclampsia and growth retardation than patients who received no treatment. There was also evidence of prevention of some of the physiological abnormalities of pre-eclampsia as both platelet count and plasma volume were higher in treated patients.

Wallenburg et al (1986) reported a lower incidence of pre-eclampsia in primigravid patients treated with 60mg aspirin daily in a placebo controlled randomised double blind study. The patients were selected on the basis of a positive angiotensin sensitivity test at 28 weeks gestation (which defines a group of patients at high risk of pre-eclampsia (Gant et al 1973)). This dose of aspirin caused 90% inhibition of platelet malondialdahyde (a stable product of thromboxane synthesis) and although prostacyclin activity was not measured in this study, the authors speculated that the treatment worked by producing a favourable alteration in the prostacyclin-thromboxane ratio.

The study of Beaufils et al (1985) reported a significantly lower incidence of intrauterine growth retardation (IUGR) in treated patients which may

reflect a reversal of the increased platelet consumption and thrombus formation and consequently increased blood flow in the uteroplacental arterial bed.

The rationale for the use of aspirin and dipyridamole treatment in the prevention of pre-eclampsia relates primarily to the disturbance of prostacyclin and thromboxane in these patients (See 2.5). This disturbance may be responsible for intravascular coagulation and platelet activation as well as the increased angiotensin sensitivity. Restoration of the prostacyclin-thromboxane balance may prevent the development of the disease.

The aim of the present study was primarily to investigate the effects of aspirin and dipyridamole on the circulating prostacyclin and thromboxane metabolites 6 keto $PGF_{1\alpha}$ and TxB_2. Although circulating metabolites may not reflect the local activity of these prostanoids, the findings of the plasma volume expansion study (Part 6) provide evidence that major changes may be detectable.

Although Wallenburg et al (1986) detected 90% inhibition of thromboxane activity with a 60mg daily dose of aspirin, Viinikka et al (1981) only detected

suppression of serum TxB_2 with doses in excess of 160mg combined with 75mg dipyridamole and reported no effect of aspirin and dipyridamole even in high doses on serum 6 keto $PGF_{1\alpha}$. However these findings do not exclude a response locally in the tissues, as in vitro studies on the veins of volunteers have shown that a single 300 mg dose of aspirin suppresses prostacyclin release (Pareti et al 1980).

7.2.4 Maternal and Fetal Effects of Aspirin and Dipyridamole

A prerequisite of any of preventative therapy is that the beneficial effects should outweigh the side effects. In the studies of Beaufils et al (1985) and Wallenburg et al (1986) no adverse side effects were reported as a result of aspirin and dipyridamole therapy although the duration of pregnancy was significantly longer in treated patients in the former study. The importance of this is difficult to estimate as control patients, who developed pre-eclampsia may have been electively delivered earlier.

A review by Collins (1981) of the available literature failed to find any evidence that aspirin in normal analgesic doses (300 - 600mg) is teratogenic. Large doses of aspirin (800mg to 6g daily) however have been shown to be associated with a higher incidence of

prolonged pregnancy, antepartum and postpartum haemorrhage than patients with no aspirin exposure (Collins and Turner 1975) although the babies in the same study had no increased haemorrhagic tendency (Turner and Collins 1975). Stuart et al (1982) in contrast reported bleeding problems in neonates whose mothers ingested aspirin in analgesic doses within 5 days of delivery but there was no evidence of clinical bleeding if more than 5 days had elapsed since the last dose. These studies, although not consistent, suggest that aspirin administered in late pregnancy in doses much higher than in the present study may cause haemorrhagic problems in the mother and neonate.

A study of over 41,000 women reported no increase in perinatal mortality in mothers who ingested aspirin in pregnancy (Shapiro et al 1976) although large doses may be associated with increased perinatal mortality (Collins and Turner 1975).

Neonatal pulmonary hypertension due to closure of the ductus arteriosus may be caused by non-steroidal anti-inflammatory agents (Rudolph 1981). These effects however seemed to be related to the dosage and duration of therapy and the author suggested that an interval of several days or weeks after the last dose should allow spontaneous reversal of the effects.

No maternal or fetal problems have been reported with low dose aspirin and dipyridamole. However to avoid any possible maternal haemorrhagic problem at delivery or neonatal complications, therapy was discontinued at 37 weeks gestation in the present study.

Summary

The initial reports of the value of antiplatelet therapy in the form of aspirin alone and with dipyridamole in prevention of pre-eclampsia are encouraging. The beneficial effect is presumed to be as a result of favourable effects on prostanoid production and prevention of platelet aggregation. Low dose aspirin has been shown to suppress thromboxane production but the effect of these agents on prostacyclin synthesis in pregnancy hypertension has still to be established.

7.3 AIMS OF THE STUDY

The primary aim of the study was to assess the effect of low dose aspirin and low dose aspirin/dipyridamole combination on circulating 6 keto $PGF_{1\alpha}$ and TxB_2 in women at risk of pre-eclampsia.

The secondary aims were to assess

1. the effects of these two treatments in prevention of proteinuric pre-eclampsia,
2. the occurrence of any side effects in the mother, and
3. fetal outcome.

The study was planned with the assistance of the Institute of Biostatistics at the South African Medical Research Council. It was estimated that 300 patients would be required to demonstrate a reduction of the incidence pre-eclampsia from the expected 25% in patients selected for study to 10% with a statistical significance of $p < 0.05$. The main aim of the study was however to determine the effect of treatment on prostanoid levels and from data collected in the earlier parts of the study it was calculated that 60 patients would be sufficient to demonstrate a significant change in plasma prostanoids. The assessment of the incidence of pre-eclampsia in the different treatment groups was however considered important, to assess the efficacy of treatment in our patients and as a pilot study to a larger investigation which is currently under way.

7.4 METHODS

7.4.1 Design of Study

Patients were randomly allocated to one of three treatment groups by means of a computer generated randomisation. Written informed consent was obtained from the patients before allocation to a treatment group. The three treatments were:

A. Aspirin 81mg daily

B. Aspirin 81mg daily and dipyridamole 100mg twice daily

C. No treatment

The lowest dose of aspirin available in South Africa was chosen, to avoid possible suppression of prostacyclin by aspirin and the potential side effects of higher doses. A dose of 200mg dipyridamole (Persantin, Boehringer-Ingelheim) was selected to avoid possible side effects which might reduce compliance.

7.4.2 Patient Selection

Patients were selected from the antenatal clinic and wards at Groote Schuur Hospital on the basis of an elevated midtrimester blood pressure which has been shown to be associated with an increased risk of the later development of pre-eclampsia (Page and Christianson 1976, Gallery et al 1977)). Although

angiotensin sensitivity is a more accurate predictor of the later development of pre-eclampsia (Elder 1986), the midtrimester blood pressure is a simple non-invasive means of selecting patients who may be at risk of pre-eclampsia. It also has a potentially wider clinical application. The selection and exclusion criteria are set out in Tables 7.I and 7.II.

An upper limit of diastolic blood pressure of 105mmHg was chosen to avoid the requirement for antihypertensive therapy which might influence prostanoid measurements and maternal and fetal outcome.

Table 7.I : Patient Selection Criteria

1. 12 - 28 weeks gestation
2. Diastolic blood pressure 80 - 105mmHg
3. No antihypertensive therapy at the start of the trial
4. Normal singleton pregnancy
5. Informed consent

Table 7.II : Exclusion Criteria

1. Other medical problems
2. Obstetric problem requiring urgent delivery
3. Proteinuria
4. Contraindication to aspirin or dipyridamole therapy eg. known sensitivity, past history of haemorrhagic disorders, peptic ulcer, haemorrhagic disease of the newborn
5. Recent aspirin therapy (in previous 2 weeks).

Although antiplatelet treatment commenced in the third trimester has been reported to be effective in prevention of pre-eclampsia (Wallenburg et al 1986), it was considered preferable to commence treatment in the second trimester as 36% of patients in our population who have proteinuric pre-eclampsia develop it before 28 weeks gestation (Anthony unpublished observations).

The study was approved by the Ethics and Research Committee of the University of Cape Town.

7.4.3 Investigations and Observations

All observations and investigations were performed on the antenatal ward immediately before commencing the study and were repeated 1 and 4 weeks later. Patients were followed up at the Hypertension Antenatal Clinic

and admitted to the antenatal ward if elevated blood pressure, proteinuria or any other complication developed. No patient was on antihypertensive therapy during the four weeks of the study, although some patients subsequently required antihypertensive agents (in the form of increasing doses of methyl dopa up to 1g b.d. and prazosin up to 10mg b.d. until diastolic blood pressures were maintained at < 110 mmHg).

The following observations were made at each visit:

1. Blood pressure by standard technique (See Appendix 3).
2. Urinalysis by salicylsulphonic acid "cold" test. If this showed proteinuria, a 24 hour urine collection was obtained and if significant proteinuria (> 300mg/24 hours) was present the patient was withdrawn from the study.
3. Gestational age.
4. Assessment of fetal wellbeing.
5. Assessment of compliance with therapy. Each patient on treatment was supplied with sufficient tablets for exactly 28 days. At each visit any remaining tablets were counted. Patients were considered to be compliant with therapy if at least 75% of the prescribed medication had been taken.
6. Maternal side effects.

Immediately before the start of treatment and 7 and 28 days later blood samples were obtained from a large bore cannula inserted into an antecubital fossa vein without constriction. The following investigations were performed -

1. Plasma 6 keto $PGF_{1\alpha}$ (the stable hydrolysis product of prostacyclin)
2. Plasma TxB_2 (the stable metabolite of thromboxane A_2)
3. Hb, platelet count
4. Urea, creatinine, urate

The sampling methods and techniques of analysis used were the same as in Study A and have been described in Parts 4.3.2. and 4.3.3.

7.4.4 Management of Pregnancy

After the initial blood sampling the patients were started on the treatment or given no medication according to the randomisation. Patients were advised not to take any therapy other than that prescribed and specifically not to use aspirin or any proprietary compound analgesics.

At completion of the four week study period, the patients continued with routine antenatal care (Appendix 3). Antiplatelet therapy was continued until

37 weeks gestation unless there were complications necessitating prior withdrawal from treatment. (Table 7.III)

Table 7.III : Withdrawal Criteria

1. Significant side effects of therapy.
2. Confirmed proteinuria on 24 hour urine specimen (> 300mg).
3. Attainment of 37 weeks gestation.
4. Medical or obstetric complications contraindicating antiplatelet therapy or likely to warrant urgent delivery.
5. Patient request.
6. Poor compliance.

A rise in blood pressure requiring antihypertensive therapy was not considered an indication to withdraw patients from the trial unless proteinuria developed.

At the end of the pregnancy the mode of delivery, presence or absence of proteinuric pre-eclampsia and any evidence of maternal bleeding were noted. Details of the gestational age, birthweight, condition and progress of the infant were also recorded. All the data was then entered onto collection sheets (Appendix 8) and subsequently computerised for statistical analysis.

7.4.5 Statistical Analysis

Continuous variables were analysed by the Kruskal-Wallis test (the statistical test result being expressed as K-W) and discrete variables by the Chi square (X^2) test.

The effects of treatment after 1 week and 4 weeks were measured by the difference from the initial value. The Sign test was used to evaluate whether the effect of treatment within a particular group was significant. The effects of treatment in the three groups were then compared using the Kruskal-Wallis test. A statistical significance of $p = 0.05$ was used.

7.5 RESULTS

7.5.1 Patient Characteristics

Forty four patients were entered into the study, 15 treated with aspirin only (Group A), 15 treated with aspirin and dipyridamole (Group B) and 14 received no treatment (Group C). Due to escalating costs of the RIA kits for prostanoid assay, sufficient funds were not available to recruit the planned total of 60 patients.

Eleven (25%) of the patients were nulliparous. The mean age was 29.6 (SD 6.4) years. The age and parity distributions were similar in each treatment group. There was a history of chronic hypertension in 18 (40.9%) patients, gestational hypertension in a previous pregnancy in 21 patients (47.7%) and a family history of hypertension in 21 patients (52.3%). X^2 analysis showed no difference in the incidence of these variables (which could affect the incidence of pre-eclampsia) between the treatment groups.

The mean gestational age at the start of the trial was 19.3 weeks in Group A, 20.3 weeks in Group B and 23.2 weeks in Group C, which were not statistically different (p 0.154; K-W 3.14; df2).

7.5.2 Side-effects of Treatment and Withdrawals from Study

In Group A no patient complained of any side effects of treatment and with two exceptions treatment was continued until 37 weeks gestation. Two patients were withdrawn from aspirin treatment, one because of the development of proteinuric pre-eclampsia after 12 weeks of treatment. The other patient in Group A who was withdrawn commenced aspirin at 14 weeks gestation but due to uncontrolled hypertension required methyl dopa

at 18 weeks. Four weeks later she developed jaundice which was attributed to methyl dopa and both treatments were discontinued.

In group B only 7 patients however took the treatment as prescribed until 37 weeks gestation. In 3 patients the treatment was discontinued when they developed proteinuric pre-eclampsia. Treatment was stopped in 2 other patients, at their request, because of severe headaches. Another patient also complained of headache which responded to halving the dose of dipyridamole and the remaining two patients in Group B defaulted from medical care after one week of treatment.

In Group C two patients failed to attend for the follow up visits at 1 and 4 weeks.

7.5.3 Prostacyclin and Thromboxane Immunoreactivity

The initial plasma 6 keto $PGF_{1\alpha}$ and TxB_2 values did not differ significantly between the treatment groups (p Ø.97 and p Ø.55 respectively).

Plasma 6 keto $PGF_{1\alpha}$ was unaltered by treatment at either 1 or 4 weeks (Table 7.IV) and there was no difference in treatment effects between the three groups.

TABLE 7.IV : 6 KETO $PGF_{1\alpha}$ LEVELS (pg/ml)

MEDIAN (INTERQUARTILE RANGE, Q_3-Q_1)

	n	GROUP A (ASPIRIN)	n	GROUP B (ASPIRIN + DIPYRIDAMOLE	n	GROUP C (CONTROL)
BEFORE TREATMENT	15	133(130)	15	152(103)	14	164(107)
1 WEEK	14	108(143)	12	178(86)	12	148(204)
4 WEEKS	13	150(132)	10	148(107)	12	139(77)
INITIAL ASSESSMENT VS WEEK 1 (SIGN TEST)		p 0.44		p 0.11		p 0.78
INITIAL ASSESSMENT VS WEEK 4 (SIGN TEST)		p 0.57		p 0.61		p 0.84

There was some evidence of suppression of TxB_2 after 1 week of aspirin and dipyridamole therapy (Group B) with a mean reduction of 48.2 pg/ml (p 0.07), but this difference was no longer apparent after 4 weeks of treatment (p 0.91). Plasma TxB_2 values were higher after 1 week in group C (p 0.05) but were no differences in plasma TxB_2 in group A at either 1 or 4 weeks. The changes in plasma TxB_2 values between the groups differed significantly at 1 week (p 0.002; K-W 12.19; df2) but at 4 weeks the differences in each group from the initial value were comparable (p 0.85).

TABLE 7.V : PLASMA TxB_2 LEVELS (pg/ml)

MEDIAN (Q_3-Q_1)

	n	GROUP A	n	GROUP B	n	GROUP C
BEFORE TREATMENT	15	94(81)	15	135(161)	14	85(67)
1 WEEK	14	136(57)	12	107(90)	12	187(105)
4 WEEKS	13	108(66)	10	119(47)	12	120(47)
INITIAL ASSESSMENT VS 1 WEEK (SIGN TEST)		p 0.10		p 0.07		0.05*
INITIAL VS ASSESSMENT 4 WEEKS (SIGN TEST)		p 0.83		p 0.90		0.41

After 1 week of treatment the fall in plasma TxB_2 levels with unaltered plasma 6 keto $PGF_{1\alpha}$ levels of patients in Group B was reflected in a significant rise in the mean 6 keto $PGF_{1\alpha}$: TxB_2 ratio from 1.06 (SD 0.57) to 1.57 (SD 0.80) (Sign test p 0.0054). In Groups A and C after 1 week the ratios of plasma 6 keto $PGF_{1\alpha}$: TxB_2 were slightly lower than the initial value but these differences did not achieve statistical significance (Sign test p 0.10 and p 0.09 respectively). These differences in the plasma prostanoid ratios after 1 week of the study between the 3 study groups were statistically significant (p 0.02 K-W 7.82; df 2).

After 4 weeks of the study, the ratios of plasma 6 keto $PGF_{1\alpha}$: TxB_2 were similar in each study group to the initial values and the 3 groups did not differ significantly from each other ($p > 0.05$).

7.5.4 Haemoglobin and Platelets

The haemoglobin was slightly lower in the control group at the start of therapy (Table 7.VI) but after 1 and 4 weeks there were no differences between the groups.

TABLE 7.VI : HAEMOGLOBIN (gm/dl)

MEAN (SD)

	n	GROUP A	n	GROUP B	n	GROUP C
BEFORE TREATMENT	15	12.0(0.7)	15	12.2(0.7)	14	11.4(0.9)*
1 WEEK	14	11.5(0.8)	12	11.3(0.9)	12	10.8(1.0)
4 WEEKS	13	11.5(0.8)	10	11.5(0.8)	12	10.7(1.3)

* K-W 5.84 p 0.05

Platelet counts did not differ between groups and were unaffected by treatment ($p > 0.05$) (Table 7.VII).

TABLE 7.VII : PLATELETS (10^9/l)

MEAN (SD)

	n	GROUP A	n	GROUP B	n	GROUP C
BEFORE TREATMENT	15	265(57)	13	272(44)	14	310(65)
1 WEEK	14	234(64)	12	278(50)	12	286(62)
4 WEEKS	13	257(52)	10	295(48)	12	318(82)

7.5.5 Renal Function Tests

The renal function as measured by urea, creatinine and urate was similar in the groups before the start of the trial and was unaffected by treatment ($p > 0.05$).

7.5.6 Blood Pressure

The blood pressure tended to increase over the four weeks of the study in all groups of patients (Table 7.VIII and 7.IX). Statistical analysis of the blood pressures of the patients remaining in the study showed the alterations in blood pressure were similar between the different groups ($p > 0.05$). The results at 4 weeks are possibly biased, however, by the withdrawals due to non-compliance particularly in Group B and by the exclusion of two patients who developed pre-eclampsia during the study period.

TABLE 7.VIII : SYSTOLIC BLOOD PRESSURE (mmHg)

MEAN (SD)

	n	GROUP A	n	GROUP B	n	GROUP C
BEFORE TREATMENT	15	134(21)	15	128(12)	14	130(15)
1 WEEK	15	147(19)	13	144(19)	12	136(20)
4 WEEKS	14	143(19)	10	140(12)	12	139(18)
INITIAL VALUE VS 1 WEEK (SIGN TEST)		p Ø.Ø6		p Ø.Ø7		p Ø.16
INITIAL VALUE VS 4 WEEKS (SIGN TEST)		p Ø.1Ø		p Ø.Ø9		p Ø.21

TABLE 7.IX : DIASTOLIC BLOOD PRESSURE (mmHg)

MEAN (SD)

	n	GROUP A	n	GROUP B	n	GROUP C
BEFORE TREATMENT	15	85(15)	15	86(1Ø)	14	84(11)
1 WEEK	15	95(12)	13	93(17)	12	84(13)
4 WEEKS	14	89(13)	1Ø	92(9)	12	89(1Ø)
INITIAL VALUE VS 1 WEEK (SIGN TEST)		p Ø.Ø2*		p Ø.28		p Ø.16
INITIAL VALUE VS 4 WEEKS (SIGN TEST)		p Ø.23		p Ø.23		p Ø.Ø4*

7.5.7 Incidence of Pre-Eclampsia

Although the primary aim of the study was to assess the effect of treatment with aspirin and dipyridamole on circulating maternal prostacyclin and thromboxane metabolites, maternal and fetal complications of therapy and the incidence of pre-eclampsia were also analysed. Patient compliance and withdrawals due to side effects or failure of treatment are major factors in determining the applicability of the preventive therapy to large populations. One patient was excluded from the analysis because there was insufficient information regarding delivery.

Eight of the 43 patients for whom the pregnancy outcome was known developed pre-eclampsia (defined as a blood pressure exceeding 140/90 mmHg on two occasions with more than 300mg proteinuria/24 hours). Four of these patients were in the control group (C) 3 were taking aspirin and dipyridamole (Group B) and 1 taking aspirin only (Group A) (Table 7.X). The four patients who developed pre-eclampsia whilst on treatment were believed to be compliant with therapy. Thus the incidence of pre-eclampsia was 6.7% in Group A, 21.4% in Group B and 28.6% in Group C. These differences were not statistically significant but the number of patients was small (p 0.30; X^2 2-54 df 2).

TABLE 7.X : DETAILS OF PATIENTS WITH PRE-ECLAMPSIA

TREATMENT	GESTATIONAL AGE AT ENTRY TO TRIAL (WEEKS)	GESTATIONAL AGE AT DEVELOPMENT OF PRE-ECLAMPSIA (WEEKS)	OUTCOME	FETAL WEIGHT (g)	ASSESSMENT OF FETAL GROWTH
C	26	36	IOL	2850	AGA
C	17	37	IOL	2520	10TH PERCENTILE
C	27	38	LSCS	2800	AGA
C	26	28	LSCS	1150	AGA
B	24	34	LSCS	2100	AGA
B	27	28	IUD IOL	800	IUGR
B	28	35	IOL	2120	IUGR
A	20	32	IOL	1900	AGA

LSCS = Lower segment caesarean section
IOL = Induction of labour
AGA = Birthweight appropriate for gestational age
IUGR = Birthweight 10th percentile for gestational age
IUD = Intrauterine death

7.5.8 Pregnancy Outcome

The mean gestation at delivery was slightly earlier in Group B than the other patients (mean 36.0 (SD 3.2)) weeks in Group B compared to 37.4 (1.9) weeks in Group A and 37.3 (2.7) weeks in Group C) but these differences were not statistically significant (p 0.35; K-W 2.1; df 2). Bleeding problems at delivery were experienced by 5 mothers, 1 in Group A, 3 in Group B and 1 in Group C. Statistical analysis showed no difference between groups and the incidence of bleeding was not higher than expected in patients on antiplatelet therapy (p 0.38; X^2 1.94; df 2).

7.5.9 Perinatal Problems

Although no baby had any evidence of haemorrhage, there were significant differences in perinatal outcome between the groups (p Ø.Ø2; X^2 8.Ø6; df 2). Six infants (42.9%) in Group B had perinatal complications compared to 1 each in the other two groups (Table 7.XI).

TABLE 7.XI : PERINATAL COMPLICATIONS

TREATMENT	COMPLICATION	GESTATION AT DELIVERY (WEEKS)	OUTCOME
B	ABRUPTIO PLACENTAE	4Ø	IUD
B	PRE-ECLAMPSIA IUGR	29	IUD
B	IUGR	34	NO MAJOR NEONATAL PROBLEMS
B	IUGR	35	GROUP B STREPTOCOCCAL MENINGITIS. SURVIVED
B	IUGR	36	JAUNDICE PHOTOTHERAPY
B	ABO INCOMPATABILITY	38	JAUNDICE PHOTOTHERAPY
C	SMALL BABY (1ØTH PERCENTILE)	34	IUD
A	ABO INCOMPATABILITY	38	JAUNDICE PHOTOTHERAPY

There were 3 intrauterine deaths, 1 associated with pre-eclampsia and IUGR, 1 with abruptio placentae and 1 with a small baby (10th percentile for gestational age). The first two of these occurred in patients in group B and the third in Group C. There were no perinatal losses in Group A.

Four patients in Group B had IUGR babies (including the intrauterine death with pre-eclampsia mentioned above). No babies in the other two groups weighed below the 10th percentile for gestational age. The mean birth weight percentile was significantly higher in Group A (53.7 (25.8) %) than Group B (32.3 (26.8) %) or Group C (28.0 (22.1) %) (p 0.027 K-W 7.23, df 2).

Two infants developed jaundice due to ABO incompatability and therefore unrelated to treatment.

7.6 DISCUSSION

In recent years more pieces have been added to the complex jigsaw of the pathophysiology of pre-eclampsia and although the picture is still not entirely clear, these observations have formed the theoretical basis for the use of antiplatelet therapy in the prevention of the disease. Pre-eclampsia is associated in some cases with a microangiopathy as evidenced by acute

atherosis, fibrinoid necrosis and thrombosis in the uteroplacental arteries (Robertson and Khong 1987, Brosens and Renaer 1972) and glomerular endotheliosis and mesangial cytoplasmic activity in the kidney (Sheehan and Lynch 1973, Robson 1977), which may be initiated by intravascular coagulation. These observations stimulated attempts to improve the clinical course of the disease by the use of anticoagulants (Howie et al 1975, Fairley et al 1976, Valentine and Baker 1977) but these attempts were not entirely successful. Thrombin induced coagulation abnormalities are however relatively rare in pre-eclampsia whereas changes in platelet activity not mediated by thrombin occur more commonly (Borok et al 1984, Wallenburg 1987). There is evidence (See Part 2.5) of decreased prostacyclin and increased thromboxane production in pre-eclampsia which may be involved in this platelet activation and could also account for the vasoconstriction and the increased sensitivity to infused angiotensin in these patients. Antiplatelet therapy may inhibit platelet consumption by blocking platelet cyclooxygenase and hence TxA_2 release.

In the present study low dose aspirin had no effect on the circulating metabolites of either prostacyclin or thromboxane. The ratio of the two metabolites was also

unaffected by treatment. Wallenburg et al (1986) described a study of aspirin 60mg/day in prevention of pre-eclampsia and found suppression of platelet thromboxane synthesis (evidenced by reduced thrombin induced production of malondialdehyde). These workers however did not measure prostacyclin metabolites and assumed them to be unaltered.

It is possible that the method used in the present study was less sensitive to changes in thromboxane synthesis than that described by Wallenburg et al (1986). The results after 1 week of treatment nevertheless indicate that thromboxane synthesis tends to be suppressed by a combination of aspirin and dipyridamole, leaving prostacyclin unaffected. These changes were also reflected in the favourable alteration of the 6 keto $PGF_{1\alpha}$: TxB_2 ratio after 1 week of aspirin and dipyridamole treatment, with a relative excess of the vasodilator 6 keto $PGF_{1\alpha}$.

It was suggested (Hoogendijk and Tencate 1980) that the effects of antiplatelet therapy may be cumulative, but this was not supported by other workers (Pedersen and Fitzgerald 1984), nor by the results of the present study. In fact after 4 weeks of treatment both 6 keto $PGF_{1\alpha}$ and TxB_2 levels were comparable to the initial

values in all three groups of patients. Although compliance with therapy was thought to be good, it is possible that the failure to demonstrate continued suppression of thromboxane levels in Group B after 4 weeks of treatment reflects a lower compliance in the second observation period. This may be explained by the fact that during the first week of the study the patients were observed in hospital but were then discharged to continue therapy at home.

It is reassuring that prostacyclin metabolite levels were not suppressed by the dosages of drugs used but it is nevertheless possible that changes in prostacyclin production at tissue level would not be detected by this method. Measurement of urinary prostacyclin metabolites may be a more sensitive means of assessing this.

In the study of the effects of plasma volume expansion (Part 6) a drop in blood pressure related to favourable alterations in the plasma levels of 6 keto $PGF_{1\alpha}$ and TxB_2 was demonstrated. In the current study the blood pressure was not affected by treatment. This would suggest that if changes in prostanoids do occur in relation to antiplatelet therapy they are of smaller

magnitude than those seen in the plasma expansion study. As plasma prostanoid levels probably only reflect major changes, the failure to demonstrate any effect of aspirin on circulating prostanoids does not exclude the possibility of beneficial effects at tissue level.

Neither aspirin nor the aspirin and dipyridamole combination had any effect on the platelet count over the duration of the study. Beaufils et al (1985) described significantly higher platelet counts at the end of pregnancy in patients treated with aspirin and dipyridamole, but this effect was only apparent after several months of treatment.

Renal function tests were similarly unaffected by treatment but all patients had normal urea, urate and creatinine prior to commencement of the study. If the therapy did improve renal blood flow, it is unlikely that this would produce a major change in these relatively insensitive measures of renal function.

It is of interest that the incidence of pre-eclampsia in Group B (3/15) was similar to that in the control group (4/14) whereas only 1 patient of the 15 treated with aspirin alone developed pre-eclampsia. Although

the numbers are too small for meaningful statistical analysis the failure rate of aspirin and dipyridamole in prevention of pre-eclampsia in the present study is higher than the study of Beaufils et al (1985) who described no pre-eclampsia in 48 patients treated with a similar regime (compared to 6 patients with pre-eclampsia of 45 in their control group). There are several possible reasons for these differences. Firstly, the patients in the present study commenced treatment at a mean pregnancy duration of 20.3 weeks compared to 12 weeks in the study of Beaufils et al. Secondly, a number of patients were withdrawn from treatment due to side effects and it is uncertain that the others were fully compliant. Thirdly, the definitions of pre-eclampsia in the two studies were different. In the study of Beaufils et al (1985) gestational hypertension occurred in over 40% of patients in both study groups, but pre-eclampsia was only diagnosed if proteinuria > 1.5gm/day was found in association with hypertension. As the authors do not state how many patients had less severe pre-eclampsia, a direct comparison is not possible.

There is no evidence from the present study that the addition of dipyridamole to low dose aspirin increases the effectiveness of treatment in the prevention of

pre-eclampsia. The results suggest that the addition of dipyridamole may reverse the possible favourable effect on clinical outcome, in spite of the evidence that the combination suppresses thromboxane synthesis.

The babies of the mothers in Group B had significantly more perinatal problems than the other two groups, mainly related to IUGR and there was evidence that aspirin alone produced a significant improvement in fetal birthweight. The high incidence of IUGR in the aspirin and dipyridamole group was worrying although it is not certain that it was related to treatment. It was considered possible that these differences may have been related to differences between the groups, but the incidence of risk factors for the development of pre-eclampsia and the gestational age at onset of the trial were similar in the three groups. Furthermore the incidence of pre-eclampsia was similar in Group B and the control group.

Although high dose aspirin was found to be related to IUGR by Turner and Collins 1975, no such association was found by Shapiro et al (1976). The study of Beaufils et al (1985) utilised similar drug doses to the present study and noted larger babies in the treatment group. Although in this latter study these

differences may have been related to later delivery in treated patients, there was a significantly higher incidence of IUGR in the control group. The study of Wallenburg et al (1986) noted that although the incidence of IUGR was higher in the placebo group, the differences in fetal weight between aspirin and placebo groups were not statistically significant. The results of the current study are at variance with the observations of both these workers and need confirmation by larger studies.

7.7 SUMMARY

Antiplatelet therapy may help to prevent pre-eclampsia in a number of ways. It may decrease platelet activation and intravascular coagulation. However only a minority of patients have evidence of overt intravascular coagulation and suppression of platelet aggregation by aspirin and dipyridamole is probably of minor importance in the prevention of pre-eclampsia. Its main mode of action in reducing the incidence of pre-eclampsia if substantiated probably relates more to the suppression of thromboxane release from the platelets and possibly by stimulation of endogenous prostacyclin release. Although an imbalance of prostacyclin and thromboxane is believed to be important in the pathophysiology of pre-eclampsia, it is uncertain whether this is the cause or the result of

the defective placentation which is a characteristic feature of this disease. It is likely however that if the balance could be restored that a reversal or prevention of the physiological abnormalities may occur by causing vasodilatation, improving uterine blood flow and the prevention of thrombosis in the uteroplacental circulation.

There is a suggestion from this study that low dose aspirin may bring about these effects with a resultant improvement in fetal growth. Low dose aspirin therapy did not produce any changes in plasma 6 keto $PGF_{1\alpha}$ or TxB_2 levels but it is possible that the treatment produced beneficial effects locally to improve choriodecidual blood flow. Low dose aspirin and dipyridamole in combination however did produce the expected increase in the ratio of 6 keto $PGF_{1\alpha}$ to TxB_2 but there was a tendency for patients in this group to have a higher incidence of IUGR and the incidence of pre-eclampsia was furthermore not reduced. These findings together with the incidence of side effects with dipyridamole, suggest that the addition of dipyridamole to low dose aspirin does not confer any specific benefit in prevention of pre-eclampsia and may even be detrimental.

In spite of small numbers this study shows that low dose aspirin therapy is acceptable to patients, is not associated with significant side effects and that a larger study of its applicability to the prevention of pre-eclampsia is warranted. Until the aetiology of pre-eclampsia is finally unravelled, manipulation of endogenous prostacyclin and thromboxane seems the most realistic therapeutic approach.

PART 8

CONCLUSIONS

Gestational hypertension is associated with a number of physiological abnormalities.

Systemic hypertension results primarily from an increased peripheral resistance in both the venous and arterial vascular beds. Alterations in peripheral resistance are likely to be mediated either by alterations in the levels of vasoactive substances or changes in the sensitivity to their effects.

Pre-eclampsia is characterised by a failure of the secondary wave of trophoblast invasion of the spiral arterioles in the placental bed which normally occurs in the second trimester. Other pathophysiological abnormalities also precede the hypertension, including an increased sensitivity to the effects of infused angiotensin II and an inadequate expansion of the plasma volume. These changes result in increased resistance in the uteroplacental bed, generalised vasoconstriction, reduced blood flow to the various organs of the body and ultimately in hypertension.

Interactions between the renin-angiotensin system and the vasodilator prostanoids are important in the regulation of systemic blood pressure and also in the control of regional blood flow particularly to the uterus and kidneys. Uteroplacental prostanoids produced in pregnancy may play a role in augmentation of uterine blood flow not only by their vasodilator effects but also by opposing the vasoconstrictor effects of angiotensin II.

A possible theory for the pathogenesis of pre-eclampsia is that as a result of inadequate erosion of the spiral arteries, renin and hence angiotensin II release are stimulated in the placenta and myometrium. Vasoactive substances produced locally within the uterus are likely to play major roles in the control of uteroplacental and fetoplacental blood flows. An excess of vasoconstrictors may then result in a rise in maternal systemic pressure and stimulation of vasodilator prostanoids in order to maintain adequate uterine blood flow and tissue perfusion.

This adjustment to overcome the effects of the placental abnormality could explain the relatively good outcome in most pregnancies complicated by mild or moderate gestational hypertension. If however the

production of vasodilator prostanoids is inadequate vasoconstriction and inadequate regional perfusion will result. In more severe cases of gestational hypertension there may be failure of vasodilator prostanoid production in various tissues resulting in the characteristic increased sensitivity to angiotensin II, reduced renal, uteroplacental and umbilical blood flow.

How can the findings of the present study be linked to this theory? In the first part of the study in maternal plasma there was evidence of a decrease in the circulating thromboxane metabolite TxB_2 together with an increase in the ratios of 6 keto $PGF_{1\alpha}$ to TxB_2 and PGE to TxB_2 in hypertensive patients. The plasma levels of TxB_2 correlated inversely with the level of the diastolic blood pressure and the ratios of vasodilator to vasoconstrictor prostanoids were positively correlated with the diastolic blood pressure. These findings are therefore consistent with the theory that the hypertension stimulates changes in the balance of vasodilator to vasoconstrictor prostanoids in an attempt to counteract the unfavourable physiological effects of a vasoconstrictor excess. There was also evidence that with increasing duration of gestational hypertension the balance of

vasodilator to vasoconstrictor prostanoids in maternal plasma was stimulated further to the vasodilator side.

The suppression of vasoconstrictor prostanoids in hypertension was seen in both gestational and chronic hypertension and would suggest that alterations occur to counteract the effects of hypertension regardless of the underlying cause.

Most of the patients in the study had mild to moderately severe pre-eclampsia and it is possible that had patients with more severe disease been studied that a deficiency of circulating vasodilator prostanoids would have then become apparent.

Plasma volume expansion in hypertensive pregnant women was shown to cause a fall in blood pressure, particularly the diastolic, in association with an increase in plasma 6 keto $PGF_{1\alpha}$ and a decrease in plasma TxB_2. These findings also suggest that local alterations in prostanoid production by the blood vessels play a role in control of blood pressure and that major changes are reflected in circulating levels.

The kidney is a principle target organ in gestational hypertension and a decrease in renal blood flow is

characteristic of the disease. Severe impairment of renal function is however a late manifestation of the disease, as the renal reserves are usually good. The levels of the primary prostanoid metabolites in urine are believed to mainly reflect local synthesis in the kidney. Although there were no significant relationships in the present study between maternal hypertension and the urinary prostanoid excretion, there was a tendency to deficiency of the vasodilator prostanoids. This tendency was more apparent in gestational hypertension than chronic hypertension which is consistent with the theory that a modest impairment of renal blood flow and function occurs in some patients with pre-eclampsia, whereas they are generally unimpaired in chronic essential hypertension.

The lack of correlation between plasma and urinary prostanoid levels illustrates that differences may occur in prostanoid production between different vascular beds. This is also illustrated by the finding of lower amniotic fluid levels of 6 keto $PGF_{1\alpha}$ in hypertensive pregnant women compared to normal women. Whether prostacyclin deficiency in the uteroplacental tissues is the cause or the result of the abnormal placentation in pre-eclampsia is not known. This study has however confirmed the findings of other workers

that there is a local intrauterine deficiency of the prostacyclin metabolite 6 keto $PGF_{1\alpha}$ in hypertensive pregnancy. Umbilical arterial blood levels of 6 keto $PGF_{1\alpha}$ and TxB_2 however demonstrated a marked suppression of the vasoconstrictor TxB_2. These findings are very similar to those seen in the maternal circulating prostanoid levels in association with hypertension. It is possible that this intrauterine vasodilator prostanoid deficiency stimulates a response in the fetus to minimise the effects, and is consistent with the theory advanced above in relation to the maternal response to a relative decrease of intrauterine vasodilatórs. Thus, the fetus may attempt to maintain the umbilical blood flow by suppression of the vasoconstrictor prostanoids and it could be envisaged that if this response is inadequate intrauterine growth retardation would ensue.

The pathophysiological changes of pre-eclampsia may result from a primary inability of the maternal tissues to synthesise adequate vasodilator prostanoids, resulting in abnormal placentation or the abnormal placentation may result in inadequate vasodilator prostanoid production by the intrauterine tissues. The placenta is central to pre-eclampsia as delivery

usually results in rapid resolution of the disease. A local imbalance of vasodilator and vasoconstrictor prostanoids in the intrauterine tissues appear to be of major importance in the initiation of the disease. Therefore, treatment of the mother with drugs to reverse this imbalance may prevent the development of overt disease. The effect of aspirin and aspirin and dipyridamole in combination on circulating prostanoid metabolites and in prevention of pre-eclampsia were studied. Although circulating 6 keto $PGF_{1\alpha}$ and TxB_2 levels were unaffected by low dose aspirin therapy in the present study, it is possible that any changes affected at tissue level were too small to be detectable in peripheral blood. Aspirin and dipyridamole therapy however was shown to have some suppressant effect on circulating TxB_2 levels with a resultant improvement in the 6 keto $PGF_{1\alpha}$ to TxB_2 ratio. Both treatments however were less effective than hoped in the prevention of pre-eclampsia. There were in addition significantly more perinatal problems (mainly perinatal death or IUGR) in patients treated with an aspirin and dipyridamole combination than in patients treated with aspirin alone or patients on no treatment. Although the numbers were small there was a suggestion that the use of low dose aspirin alone from the second trimester may improve fetal birthweight.

The role of vasoactive prostanoids in intrauterine growth retardation is less well documented than their involvement in pre-eclampsia but it is believed that a deficiency of vasodilator prostanoids in the fetoplacental tissues may be an aetiological factor in inadequate fetal growth. The present study was primarily intended to study the effects of maternal hypertension on prostanoid levels and the numbers were not sufficient to fully evaluate the effects of intrauterine growth retardation. Maternal plasma prostanoid levels were unaffected by the presence of intrauterine growth retardation. Mothers with IUGR babies however had a tendency to an excess of vasoconstrictor prostanoids in the urine. Although it is believed that urinary prostanoids reflect mainly local intrarenal prostanoid production, a possible contribution from other tissues cannot be excluded and it is possible that these differences reflect a generalised change of vasoactive prostanoids in these patients. However, the amniotic fluid and umbilical arterial blood prostanoid levels were not related to fetal growth. The findings therefore suggest that although there may be alterations in the vasoactive prostanoids in the intrauterine tissues in relation to IUGR, that they are of more importance in the hypertensive disorders of pregnancy.

The studies described in this thesis have advanced knowledge of vasoactive prostanoids in hypertension pregnancy by illustrating varying roles of the vasodilator and vasoconstrictor factors in different tissues. They have also illustrated that the balance of the vasodilator and vasoconstrictor effects in various organs may be of greater importance than the absolute level of an individual prostanoid. These studies suggest that alterations may occur in circulating maternal prostanoids in response to hypertension and furthermore that plasma prostanoid levels can be favourably altered by intervention.

A better understanding of the primary insult which causes pre-eclampsia and initiates the imbalance of the vasoactive prostanoids and pathophysiological changes, will hopefully lead to effective prevention of the disease.

APPENDIX 1

DEFINITIONS OF THE HYPERTENSIVE DISEASES IN PREGNANCY

The hypertensive disorders of pregnancy in clinical practice fall broadly into three groups. The classification of Davey and MacGillivray (1986) was used for the purposes of this study. This classifications is based on clinical criteria and makes no assumptions regarding the underlying pathology.

A. Gestational Hypertension

Gestational hypertension is defined as a DBP of 90mmHg or more with or without proteinuria arising during pregnancy in a previously normotensive woman, reverting to normal in the puerperium. The term pre-eclampsia is reserved for women who develop both hypertension and proteinuria during pregnancy.

B. Chronic Hypertension

Patients presenting with hypertension before 20 weeks gestation or who were known to have hypertension pre-dating the pregnancy were classified as chronic hypertensives. The diagnosis of chronic hypertension was therefore made on the finding of hypertension:

(a) Before 20 weeks gestation by menstrual data or ultrasound scan, or

(b) at any stage of pregnancy in women with known chronic hypertension, or

(c) persisting at more than 42 days after delivery.

As gestational hypertension is rare in the absence of trophoblastic disease before 20 weeks of pregnancy, the finding of hypertension at this stage is believed to justify a diagnosis of chronic hypertension.

C. Unclassified Hypertension

Patients presenting with hypertension after 20 weeks gestation where no information is available regarding the blood pressure before or earlier in the pregnancy, cannot be placed into categories A or B and are hence termed "unclassified". Measurement of the blood pressure more than 6 weeks after delivery may allow reclassification as either gestational hypertension or chronic hypertension.

For the purpose of this study all case records were reviewed after the six week postnatal visit to ensure that the diagnostic classification of each patient was correct. Hypertension was diagnosed if the average

diastolic blood pressure of 4 readings over a 24 hour period in hospital was 90mmHg or more. Patients with unclassified hypertension were excluded from this study.

APPENDIX 2

ABBREVIATIONS

AII	:	angiotensin II
Anova	:	analysis of variance
BGG	:	bovine gamma globulin
BP	:	blood pressure
CH	:	chronic hypertension
DBP	:	diastolic blood pressure
DCC	:	dextran coated charcoal
DIC	:	disseminated intravascular coagulation
dL	:	decilitre
GCMS	:	gas chromatography mass spectrometry
GC/NICIMS	:	gas chromatography negative ion chemical ionization mass spectrometry
GH	:	gestational hypertension
gm	:	grams
h	:	hour/s
Hb	:	haemoglobin
HETE	:	hydroxytetranoic acids
HPETE	:	hydroperoxytetranoic acids
hPL	:	human placental lactogen
IUD	:	intrauterine death
IUGR	:	intrauterine growth retardation

K-W	:	Kruskal Wallis test statistic
kg	:	kilogram
l	:	litre
LSCS	:	lower segment caesarean section
min.	:	minute/s
ml	:	millilitre
n	:	number of observations
N	:	normal/normotensive
ng	:	nanogram
NSB	:	non specific binding
pg	:	picogram
PG	:	prostaglandin
Q_3-Q_1	:	interquartile range (75th - 25th percentile)
RIA	:	radioimmunoassay
SD	:	standard deviation
Tx	:	thromboxane
vs	:	versus
X^2	:	Chi square test statistic

APPENDIX 3

ROUTINE MANAGEMENT OF HYPERTENSIVE PREGNANT PATIENTS

The antenatal Hypertension Clinic and Ward MC at Groote Schuur Hospital are the tertiary referral centre for hypertensive pregnant patients in the Peninsula Maternity and Neonatal Service (PNMS). Hypertensive patients less than 36 weeks gestation who do not require urgent delivery are referred for further management. Twenty-six thousand patients are delivered annually by the PNMS of whom approximately 15% are diagnosed antenatally as hypertensive (on the basis of a diastolic blood pressure exceeding 90mmHg on two occasions).

All the hypertensive patients in the study were admitted to the ward for assessment and were managed according to the ward protocol which briefly is as follows:

1. History and examination to assess any secondary cause of hypertension and fetal condition.

2. Six hourly blood pressure recording.
   Blood pressure is measured with the patient lying on the right side with a tilt of 30° to the horizontal.

The blood pressure is taken in the right arm (which is supported at the level of the heart) after at least 5 minutes rest. Care is taken to use a cuff of appropriate size. Korotkoff phase 4 sounds are used for the recording of diastolic blood pressure. For the purposes of the study all blood pressures were recorded by the same research sister.

3. Daily 24 hour urine collection for measurement of total protein.

4. Renal and liver function tests by standard biochemical profile (SMAC) weekly or more frequently if abnormal.

5. Full blood count by Coulter counter weekly or more frequently if necessary.

6. Ultrasound scan of the abdomen at initial admission and as required thereafter to assess gestational age and fetal growth.

7. Cardiotocography after 26 weeks gestation at a frequency indicated by clinical condition.

8. Antihypertensive therapy if the mean of the 4 readings of diastolic blood pressure in the second 24 hours after admission exceed 90mmHg. The choice of antihypertensive therapy varies according to current research protocols.

9. Patient activity is generally unrestricted.

If the diastolic blood pressure is less than 90mmHg after the initial 48 hour observation period, the patient is allowed home and managed as an outpatient unless complications arise. Patients in whom the blood pressure is stabilised by antihypertensive therapy are also managed as outpatients, if the condition of the mother and fetus are satisfactory in other respects. If the blood pressure is difficult to control or there is evidence of maternal or fetal compromise, the mother is kept under close observation in the ward to enable immediate delivery to be undertaken.

Delivery by the most appropriate means is carried out at 38 weeks gestation or earlier if the maternal or fetal condition are giving cause for concern.

UNIVERSITY OF CAPE TOWN

(WITH WHICH IS INCORPORATED THE SOUTH AFRICAN COLLEGE)

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DEPARTMENT OF OBSTETRICS & GYNAECOLOGY
MEDICAL SCHOOL,
OBSERVATORY,
7925 CAPE,
SOUTH AFRICA.

CONSENT FORM FOR INVESTIGATIONS

I, the undersigned, ..., Hospital no .....
hereby declare that the proposed investigations regarding .....
..... have been fully explained
to me by Dr .....and that I understand the explanations.
I am aware that some aspects of the investigations may be of a research nature.
Having been assured that I will be exposed to no unreasonable or unwarranted risk
or treatment, I agree to participate voluntarily in the proposed investigations.

SIGNATURE OF PATIENT: .....

SIGNATURE OF DOCTOR EXPLAINING AND
RESPONSIBLE FOR INVESTIGATIONS .....

WITNESSES: 1. ..... DATE: ☐☐☐☐☐☐

2. .....

.....

UNIVERSITEIT VAN KAAPSTAD

DEPARTEMENT VAN VERLOSKUNDE EN GINEKOLOGIE

TOESTEMMINGSVORM VIR ONDERSOEKE

Ek, die ondergetekende ....., Hospital nr .....,
verklaar hiermee dat die beoogde ondersoeke aangaande .....
..... volledig aan my verduidelik
is deur Dr ..... en dat ek die verduidelikings verstaan.
Ek is daarvan bewus dat sekere aspekte van die ondersoeke van 'n navorsingsaard
mag wees. Daar ek die versekering ontvang het dat ek aan geen onredelike of
ongeoorloofde risiko blootgestel sal word nie, neem ek vrywillinglik deel aan
die beoogde ondersoeke.

HANDTEKENING VAN PASIENT: .....

HANDTEKENING VAN DOKTER WAT VERDUIDELIK
EN VIR ONDERSOEKE VERANTWOORDELIK IS: .....

GETUIES: 1. .....

2. ..... DATE: ☐☐☐☐☐☐

APPENDIX 5

PATIENT DATA PROSTAGLANDIN STUDY

NAME	HOSPITAL NO.		
AGE	1 - 2		
GRAVIDA	3 - 4		
PARA	5 - 6		
RACE BLACK 1, COLOURED 2, WHITE 3	7		
GESTATION (COMPLETED WEEKS) BY DATES	8 - 9		
GESTATION (COMPLETED WEEKS) BY ULTRASOUND	10 - 11		
SYSTOLIC B.P. (AT TIME OF SAMPLING)	12 - 14		
DIASTOLIC B.P.	15 - 17		
DURATION OF HYPERTENSION	Y 18 - 19	W 20 - 21	
OEDEMA ABSENT 0 PRESENT 1	22		
PROTEINURIA ABSENT 0 PRESENT 1	23		
CREATININE	24 - 26		
URATE	27 - 29		
UREA	30 - 32		
SODIUM	33 - 35		
POTASSIUM	36 - 38		
Hb	39 - 42		
PLATELETS	43 - 45		
ANTIHYPERTENSIVE DRUGS YES 1 NO 0	46		
FETAL BIRTH WEIGHT (Gm)	47 - 50		
BIRTH PERCENTILE	51 - 52		

NAME		HOSPITAL NO.	
PGE2 (serum)		53 - 57	
PGE2 (urine)		58 - 62	
PGF1α (serum)		63 - 67	
PGF1α (urine)		68 - 72	
PGF2α (serum)		73 - 77	
PGF2α (urine)		78 - 82	
Thromboxane B2 (serum)		83 - 87	
Thromboxane B2 (urine)		88 - 92	
Estradiol		93 - 97	
HPL		98 - 101	
Progesterone		102 - 104	
20 week BP (mmHg)	systolic	105 - 107	
	diastolic	108 - 110	
20 week weight (Kg)		111 - 113	
32 week weight (Kg)		114 - 116	
Postpartum BP (6/52)	systolic	117 - 119	
	diastolic	120 - 122	

APPENDIX 5

EXPLANATORY NOTES

Appendix 5 : Note 1

Gestational age by menstrual dates and ultrasound scan was recorded. If these differed by less than two weeks the menstrual dates were considered to be correct. If they differed by more than two weeks the ultrasound estimate was used for statistical analysis. After delivery all case notes were reviewed and subsequent Dubowitz gestational age scoring of the baby taken into account to make the best estimate of gestational age at the time of sampling in the doubtful cases.

Appendix 5 : Note 2

Duration of hypertension was recorded in years (y) for chronic hypertensive patients and weeks (w) for gestational hypertensives. This information together with blood pressure at 20 weeks gestation and 6 weeks post partum were used for assignment of the patient to the correct diagnostic group.

Appendix 5 : Note 3

At birth the weight, sex and birth percentile of the infant were recorded. Initially the birthweight charts of Thomson et al (1968) were used as they give a precise birth percentile but this excluded all infants less than 32 weeks gestation and tended to overestimate the incidence of growth retardation. Subsequently the birthweight percentiles were recalculated from the charts of Lubchenco et al (1963) which corresponded better with the "normal" population in the study.

APPENDIX 6

RADIOIMMUNOASSAY PROCEDURE

A6.1 INTRODUCTION

All samples were immediately transferred into chilled tubes containing a freshly mixed EDTA, theophylline and aspirin solution, kept on ice until spun down within 1 hour at 4°C and prostanoids extracted within 48 hours from 2ml of sample. (As described in Part 4). After extraction samples were stored at -20°C for later batch assay. Each sample was thawed only once as thawing and refreezing has been shown to affect assay results (Morris et al 1981). After thawing at room temperature samples were reconstituted in 1 ml of 0.01M phosphate buffered saline containing 0.1% gelatin, at ph7 immediately prior to assay.

Commercial radioimmunoassay kits were used for assay of the prostanoids or their major metabolites. The following assays were performed:

1. Prostaglandin E_2 (^3H) RIA kit (Seragen Code No. SG.6001).
2. 13-14 dihydro-15-keto prostaglandin $F_{2\alpha}$ (^3H) RIA (Seragen Code No. 6006).
3. 6-keto-prostaglandin $F_{1\alpha}$ (^3H) RIA kit (Seragen Code No. 6004).
4. Thromboxane B_2 (^3H) RIA kit (Seragen Code No. 6003).

The factor influencing the choice of samples and metabolites for assay and the decision to use commercial RIA kits have been described in Part 2.3.3 and 4.3.3. All assays were performed according to the manufacturer's instruction which are documented below.

A6.2 PRINCIPLES OF RADIOIMMUNOASSAY

The principle of RIA is based upon competitive binding between the molecule to be measured and a radioactively labelled molecule for a limited number of antibody binding sites. The higher the concentration of the compound in the sample, the less labelled antigen is bound to the specific antibody. The amount of labelled compound is known and constant for all the tubes in the assay.

The binding of the antigen to antibody is not static but is an equilibrium reaction. After equilibration the free and antibody bound fractions are separated and the radioactivity of either or both may be determined. The proportion of labelled antigen that is antibody bound is inversely related to the amount of unlabelled antigen present. Varying known amounts of the unlabelled substance are added to a number of tubes to construct a standard curve against which the unknown tubes are compared to calculated the exact amount of the compound in the sample.

The prostanoid molecules are too small to be antigenic but must be coupled to a substance of larger molecular weight to evoke an immune response.

The immunogens used for this study are raised by the use of carbodiimides to conjugate the prostanoid to bovine serum albumin carrier molecules as described by Caldwell et al (1971). The solution of prostanoid-protein conjugates are then emulsified with Freund's adjuvant and administered subcutaneously in rabbits. Ten days after immunisation the rabbits are bled and the serum lyophylised (Jaffe and Behrman 1978). The antiserum is then used to compare the effects of the various biological samples on the binding of the radioactive label to the antiserum.

The carboxyl side chain of the prostaglandin molecule is usually used for the coupling as the ring structure has the strongest antigenic properties but this has the disadvantage that the ^{5} double bond in prostaglandins of the "2" series are not always well recognised so cross-reactions with the "1" series may be high (Granstrom and Kindahl 1978(b)). The coupling site may effect the properties of the resulting antibody and is reflected in the binding and the cross-reactivity of the assay with other prostanoids. The performance characteristics of the assays used have been calculated by the manufacturer and it is hoped that quality control ensures these are relatively constant to reduce

interassay variation.

In each assay samples containing no antibodies are included. As no antibody binding occurs the radioactivity counts obtained reflect the amount of isotope added and the measurement response to reagent and sample constituents exclusive of the desired analyte. This allows calculation of the non specific binding of the reagents alone (NSB-blank) and non specific binding by other substances in the sample (NSB sample). The reagent and sample blanks are then subtracted from the measured radioactivity counts. Ideally blank readings should be low but the stability and precision is more important to reliability of the assay.

Maximal binding (Bo) is the amount of antibody binding of the labelled ligand in the absence of unlabelled molecules. The most suitable working dilution of antibody varies in reported studies but lie in the range producing 25-50% binding of the labelled ligand in the absence of unlabelled molecules (Morris et al 1981, Mitchell 1978(b), Jaffe and Behrman 1978). The use of excess antibody decreases the sensitivity of the assay as all binding sites must be occupied before the unlabelled molecules can compete with and displace the labelled antigen (Granstrom and Kindahl 1978(b)).

The techniques of sampling, extraction and storage have been described in the Methods (Part 4.3.3).

A6.3 METHOD OF RADIOIMMUNOASSAY OF PROSTAGLANDIN AND THROMBOXANE METABOLITES

The assay procedure for each prostanoid metabolite was performed according to the manufacturers instructions and was identical for each substance. The procedure for 6 keto $PGF_{1\alpha}$ is described below.

Kits were stored at 4°C prior to assay and then used in their entirety so that storage of reconstituted reagents was not required.

A6.3.1 Reagent preparation.

A. Anti 6 keto $PGF_{1\alpha}$ prepared in rabbit as described above was reconstituted using 10ml BGG phosphate buffer (giving a final assay titre of 1:27000).

B. The lyophilised ^{3}H-6 keto $PGF_{1\alpha}$ tracer was reconstituted using 10ml of buffer solution (total radioactivity < 74 KBq).

C. A standard solution of 6 keto $PGF_{1\alpha}$ was prepared by dilution of the lyophylised standard to 1ml with buffer (100 ng/ml).

D. BGG phosphate buffer containing 0.01M phosphate at pH7 with 0.1% Bovine Gamma globulin and 0.1% sodium azide was supplied ready for use.

E. Dextran coated charcoal containing 0.4% Norit in water with 0.4% Dextran and 0.1% sodium azide was supplied ready for use.

Polypropylene tubes and disposable plastic pipettes were used throughout.

A6.3.2 Preparation of Standards

The 6 keto $PGF_{1\alpha}$ standard was diluted with buffer solution to obtain a dilution range from 2000 to 2.7 pg/0.1ml and 6 keto $PGF_{1\alpha}$ standards were assayed in duplicate (for calculation of the standard curve).

The dilutions used were :

	Concentration (pg/0.1ml)
Standard 1 (S1)	2.7
S2	8.2
S3	24.7
S4	74.0
S5	222
S6	667
S7	2000

A6.3.3 Assay procedure

Total radioactivity counts were obtained by the addition of 100ul ^{3}H 6 keto $PGF_{1\alpha}$ (tracer) to 900µl buffer. All samples were assayed in duplicate. 100µl standard or sample, 100µl tracer and 100µl antiserum were added in that order to each standard or sample tube. NSB (blank) tubes contained 200µl buffer and 100µl tracer. Sample NSB was estimated from tubes containing 100µl buffer, 100µl of a pooled sample from 5 patients and 100µl tracer. Tubes for maximum binding (Bo) contained 100µl buffer, 100µl tracer and 100µl of antiserum. A sample for calculation of intra-assay variation was included at intervals in each assay run. The reagents were then mixed on a vortex mixer and incubated for 18 hours at 4°C to allow the antibody-antigen reaction to reach equilibrium.

Prior to the separation step the dextran coated charcoal (DCC) was placed in an ice bath and mixed continuously with a magnetic mixing bar. The assay volume was then made up to 1ml by the addition of 700µl DCC to each tube followed by centrifugation at 1000 x g for 15 minutes at 4°C in a Sorrall Refrigerated centrifuge. The tubes were kept cold and centrifuged quickly (within 10 minutes of commencing addition of DCC) to avoid further binding of the radioactivity labelled molecules to the charcoal. The supernatant containing the antibody bound analyte was then transferred to glass counting vials containing 10ml Instagel scintillation fluid and the radioactivity counted in duplicate for 5 minutes in a Packard Tricarb Model 3390 Liquid Scintillation Spectrometer. The samples for total radioactivity counts were similarly added to 10ml of Instagel and counted.

A6.3.4 Calculations

The standard curve was plotted for the displacement of antibody-bound labelled ligand (% B/Bo) by known amounts of the unlabelled 6 keto $PGF_{1\alpha}$. This was calculated as follows:

$$\% \ B/Bo = \frac{\text{Counts per minute (cpm) standard} - \text{NSB (blank)} \times 100}{\text{cpm Bo} - \text{NSB (blank)}}$$

Semi logarithmic paper was used with % B/Bo on the abscissa and the concentration of 6 keto $PGF_{1\alpha}$ in pg/0.1 ml on the ordinate. A sigmoidal curve was constructed. A typical curve is illustrated in Figure A6.1.

Concentrations in samples were then determined by comparison with the standard curves. In each assay nonspecific binding of the sample was estimated but as these values showed only negligible differences from NSB (blank) values, the latter was used for calculation of % B/Bo in samples.

The results obtained were then corrected according to the percentage extraction for the samples.

Percentage binding for each assay was in the range 23% to 35% being fairly constant for each prostanoid (mean values 6 keto $PGF_{1\alpha}$ 30.1%, thromboxane B_2 34.1%, 13,14 dihydro 15 keto $PGF_{2\alpha}$ 23.3%, PGE_2 33.5%). NSB values were between 2.5% and 3.5% for all assays.

The specificity of the assays have been assessed by the manufacturers. The cross reactivities of each compound based on the amount that causes 50% displacement of labelled ligand from the antibody are summarised in Tables A6 I - IV.

These data must be interpreted with caution as the]displacement of the label by cross reacting substances may not be equal at different points of the standard curve.

6 KETO PGF1

HELIOS D26/43

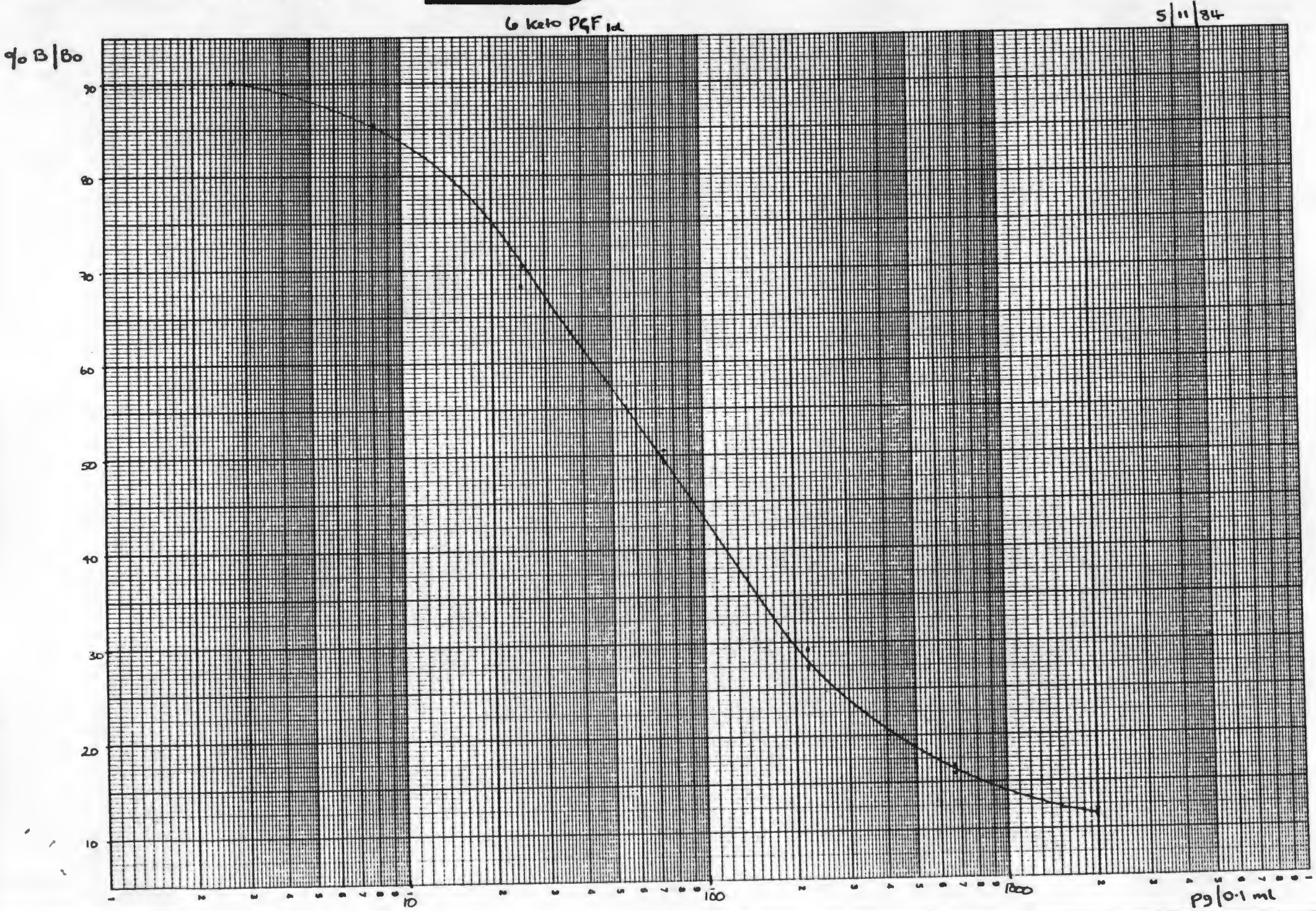


TABLE A6-I

RIA OF 6 KETO $PGF_{1\alpha}$

PERCENTAGE CROSS REACTIVITY OF PROSTANOIDS AT 50% B/Bo

COMPOUND	%
6 KETO $PGF_{1\alpha}$	100
$PGF_{1\alpha}$	7.8
6 KETO PGE_1	6.8
$PGF_{2\alpha}$	2.2
PGE_1	0.7
PGE_2	0.6
PGD_2	< 0.1
PGA_2	< 0.1
PGA_1	< 0.1
PGB_1	< 0.1
PGB_2	< 0.1
TxB_2	< 0.1
15-KETO $PGF_{2\alpha}$	< 0.1
15-KETO PGE_2	< 0.1
DIHYDROKETO PGE_2	< 0.1
DIHYDROKETO $PGF_{2\alpha}$	< 0.1

TABLE A6-II

RIA OF TxB_2

PERCENTAGE CROSS REACTIVITY OF PROSTANOIDS AT 50% B/Bo

COMPOUND	%
THROMBOXANE B_2	100
PGD_2	< 0.1
PGE_1	< 0.1
PGE_2	< 0.1
$PGF_{2\alpha}$	< 0.1
$PGF_{1\alpha}$	< 0.1
6 KETO $PGF_{1\alpha}$	< 0.1
PGA_2	< 0.1
PGA_1	< 0.1
PGB_1	< 0.1
PGB_2	< 0.1
DIHYDRO KETO PGE_1	< 0.1
DIHYDRO KETO PGF	< 0.1

TABLE A6-III

RIA OF 13, 14 DIHYDRO 15 KETO $PGF_{2\alpha}$

PERCENTAGE CROSS REACTIVITY OF PROSTANOIDS AT 50% B/Bo

COMPOUND	%
13,14,DIHYDRO-15-KETO $PGF_{2\alpha}$	100
PGF_2	1.7
DIHYDROKETO PGE_2	0.14
6 KETO $PGF_{1\alpha}$	0.02
TxB_2	0.02
PGA_2	0.02
PGB_2	0.02
PGE_2	0.02
PGE_1	0.02

TABLE A6-IV

RIA OF PGE_2

PERCENTAGE CROSS REACTIVITY OF PROSTANOIDS AT 50% B/Bo

COMPOUND	%
PGE_2	100
PGE_1	100
PGA_2	6.0
PGA_1	3.0
6 KETO PGE_1	1.0
$PGF_{2\alpha}$	1.3
DIHYDROKETO PGE_2	< 0.1
PGD_2	< 0.1
PGB_2	< 0.1
PGB_1	< 0.1
6 KETO $PGF_{1\alpha}$	< 0.1
TxB_2	< 0.1
DIHYDROKETO $PGF_{2\alpha}$	< 0.1
$PGF_{1\alpha}$	< 0.1

The PGE_2 assay has 100% crossreactivity with PGE_1 which reflects the inability of the antigen to differentiate between molecules differing only by a single double bond. Due to the lack of specificity the assay reflects the immunoreactivity of PGE rather than just PGE_2. This

similarly affects the assay for 6 keto $PGF_{1\alpha}$ which also cross reacts with structurally related compounds notably $PGF_{1\alpha}$ and 6 keto PGE_1.

Other aspects of reliability which may be important in interpretation of the RIA results have been considered in Part 4.3.3.3.

APPENDIX 7

PATIENT DATA

PROSTAGLANDIN STUDY AND PLASMA VOLUME EXPANSION

DATE

HOSPITAL NO.

NAME

AGE

GRAVIDA

PARA

RACE BLACK 1, COLOURED 2, WHITE 3

GESTATION (COMPLETED WEEKS) BY DATES

GESTATION (COMPLETED WEEKS) BY ULTRASOUND

SYSTOLIC B.P. (AT TIME OF SAMPLING)

DIASTOLIC B.P.

DURATION OF HYPERTENSION

PROTEINURIA ABSENT 0 PRESENT 1

Hb

PLATELETS

ANTIHYPERTENSIVE DRUGS YES 1 NO 0

FETAL BIRTH WEIGHT (GM)

BIRTH PERCENTILE

GESTATION AT DELIVERY

6 OXO PG F1 A

6 OXO PG F1 B

THROMBOXANE B2A

THROMBOXANE B2B

ESTRADIOL

HPL

AMNIOTIC FLUID

6 OXO PGF1

THROMBOXANE B2

UMBILICAL ARTERY

6 OXO PGF1

THROMBOXANE B2

PROSTAGLANDIN STUDY : EFFECT OF ASPIRIN AND DIPYRIDAMOLE

DATA SHEET	Sheet No. [1] 2 3 4 5	1
DATE		2 - 7
NAME		
HOSPITAL NO.		20 - 27
AGE		28 - 29
GRAVIDA		30 - 31
PARA		32 - 33

PAST HISTORY

Chronic Hypertension	34
Gestational Hypertension/Pre-eclampsia	35

No 0 Yes 1

Family History of Hypertension	36

No 0 Yes 1

PROSTAGLANDIN STUDY : EFFECT OF ASPIRIN AND DIPYRIDAMOLE

SHEET NO. 1 2 3 4 5	1	☐
DATE	2 - 7	☐☐☐☐☐☐
GESTATIONAL AGE	8 - 9	☐☐
TREATMENT Aspirin 1 Aspirin & Dipyridamole 2 Control 3 Not applicable 4	10	☐
COMPLIANT No 0 Yes 1 Not applicable 2	11	☐
DURATION OF TREATMENT (weeks) Not applicable 0	12	☐
SIDE EFFECTS No 0 Yes 1 Not applicable 2	13	☐
ANTIHYPERTENSIVE THERAPY No 0 Yes 1	14	☐

Specify .....

.....

SYSTOLIC BP	15 - 17	☐☐☐
DIASTOLIC BP	18 - 20	☐☐☐
PROTEINURIA gms/24 hr	21 - 23	☐☐☐
PRE-ECLAMPSIA No 0 Yes 1	24	☐
Hb	25 - 27	☐☐☐
PLATELETS	28 - 30	☐☐☐
UREA	31 - 34	☐☐☐☐
URATE	35 - 39	☐☐☐☐☐
CREATININE	40 - 42	☐☐☐
PGF1 A	43 - 47	☐☐☐☐☐
PGF1 B	48 - 52	☐☐☐☐☐
TxB2 A	53 - 57	☐☐☐☐☐
TxB2 B	58 - 62	☐☐☐☐☐

PROSTAGLANDIN STUDY : EFFECT OF ASPIRIN AND DIPYRIDAMOLE

SHEET NO 1 2 3 4 [5] 1 ☐

GESTATION AT DELIVERY 2 - 3 ☐☐

BIRTHWEIGHT 4 - 7 ☐☐☐☐

SEX 0 Male 1 Female 8 ☐

BIRTHWEIGHT PERCENTILE 9 - 10 ☐☐

NEONATAL PROBLEMS No 0 Yes 1 11 ☐

Specify .....

.....

POSTPARTUM HAEMORRHAGE (500 ml) No 0 Yes 1 12 ☐

BLOOD TRANSFUSION No 0 Yes 1 13 ☐

PRE-ECLAMPSIA No 0 Yes 1 14 ☐

APPENDIX 9

ADDITIONAL TABLES AND FIGURES

TABLE I

MATERNAL PLASMA CONCENTRATIONS (Ln pg/ml) OF PROSTANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCIES

MEAN (SD)

	n	NORMOTENSIVE	n	CHRONIC HYPERTENSION	n	GESTATIONAL HYPERTENSION
PGE	38	7.4 (1.2)	22	7.8 (1.7)	35	7.9 (1.1)
PGFM	35	5.7 (1.5)	21	5.5 (1.8)	38	5.4 (1.9)
6 KETO PGF_1	43	6.2 (0.6)	31	5.9 (0.95)	68	6.0 (0.8)
TxB_2	43	6.6 (1.1)	31	5.9 (1.5)	68	6.0 (1.1)

TABLE II

MATERNAL 4 HOUR URINARY EXCRETION RATE (Ln pg) OF PROSTANOIDS IN NORMAL AND HYPERTENSIVE PREGNANCIES

(MEAN (SD)

	n	NORMOTENSIVE	n	CHRONIC HYPERTENSION	n	GESTATIONAL HYPERTENSION
PGE	32	13.6 (1.0)	22	13.1 (1.3)	37	13.2 (1.2)
PGFM	32	8.7 (1.5)	22	8.2 (1.4)	37	8.7 (1.5)
6 KETO PGF_1	32	11.4 (1.1)	22	11.5 (1.5)	37	11.7 (1.0)
TxB_2	32	9.2 (1.7)	22	9.4 (1.8)	37	9.6 (1.6)

FIG. A.9.1: PLASMA PGE IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT CENTILE

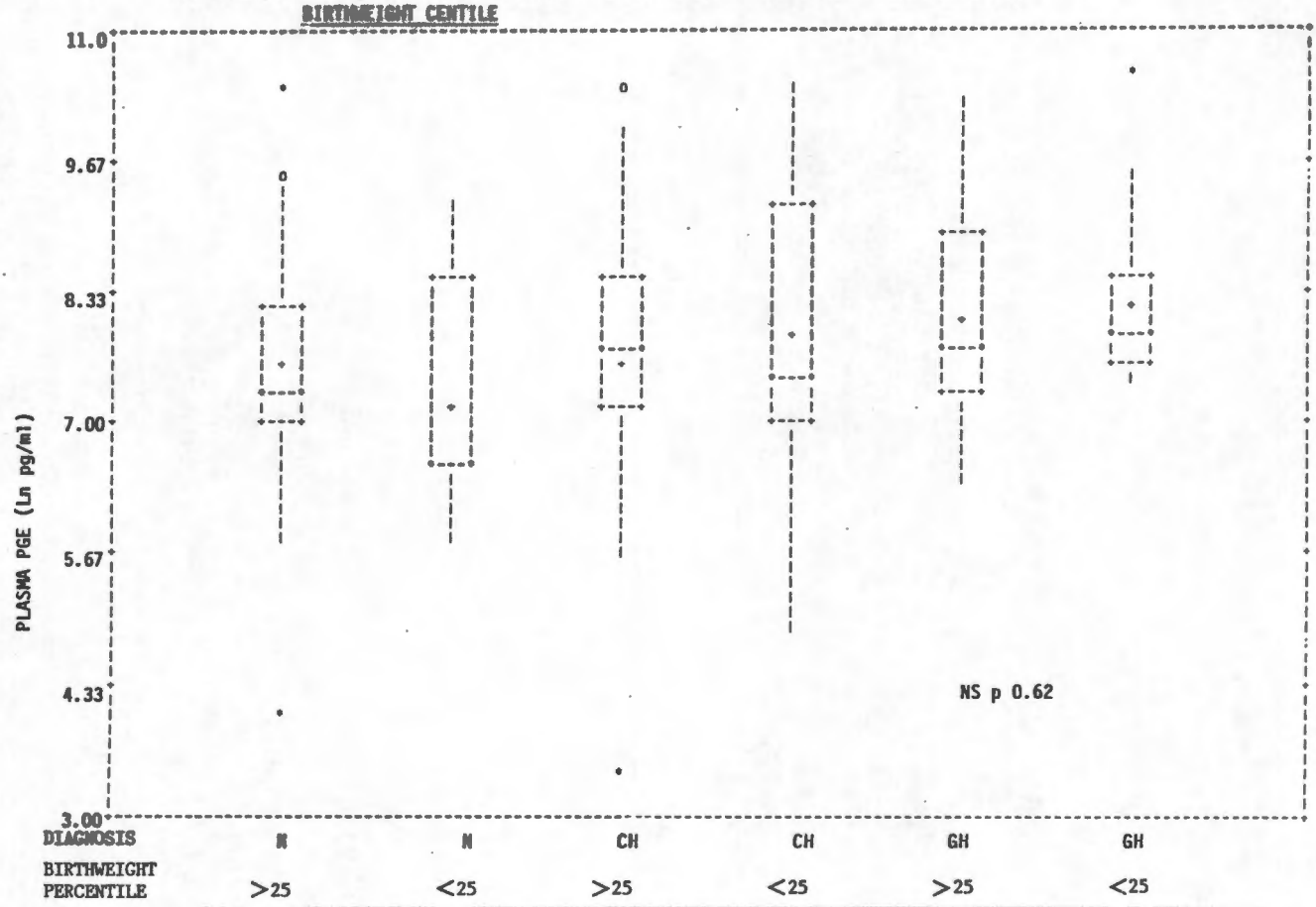


FIG. A.9.2: PLASMA PGFM IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT CENTILE

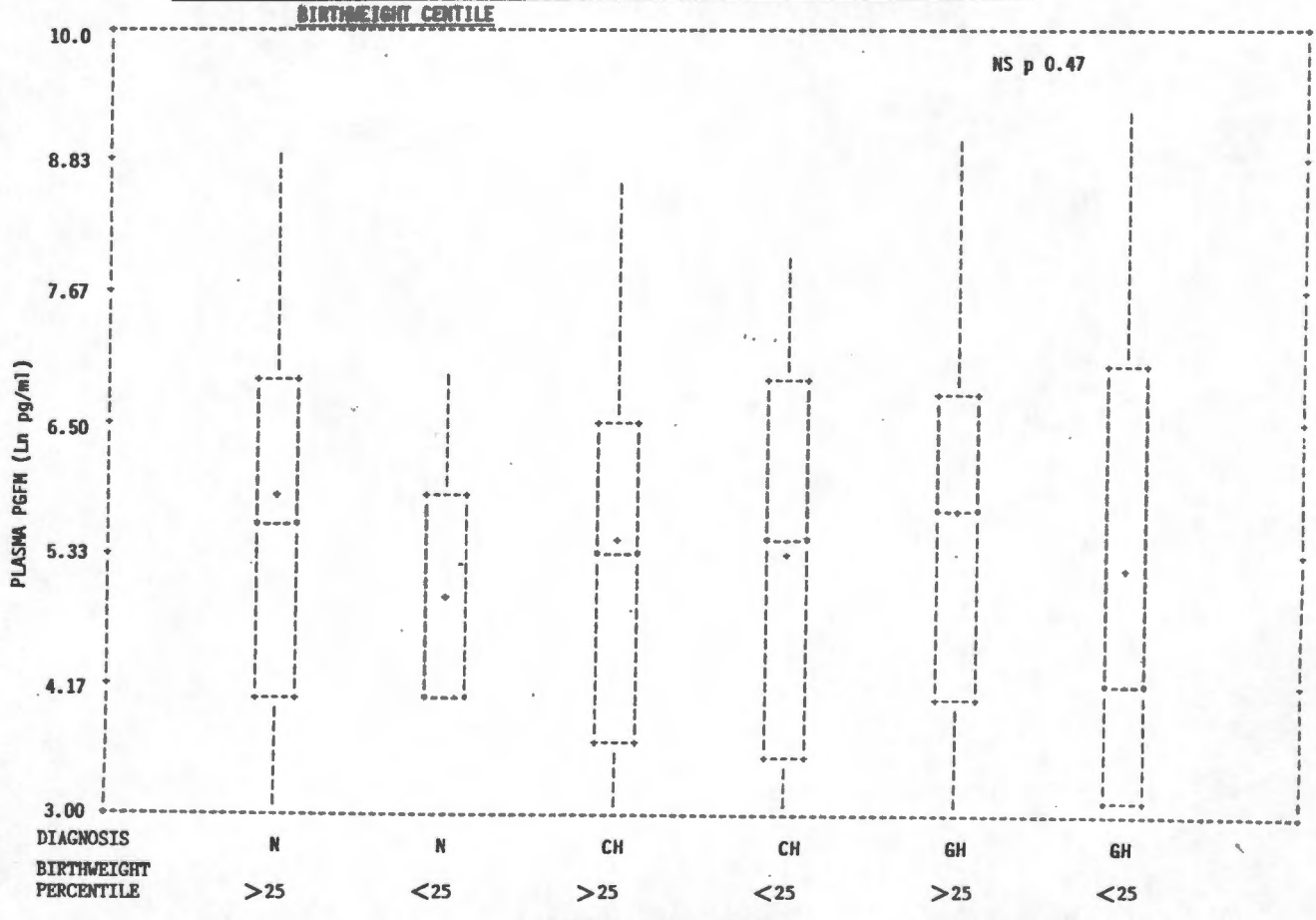


FIG. A.9.3: PLASMA 6 KETO PGF1α IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE

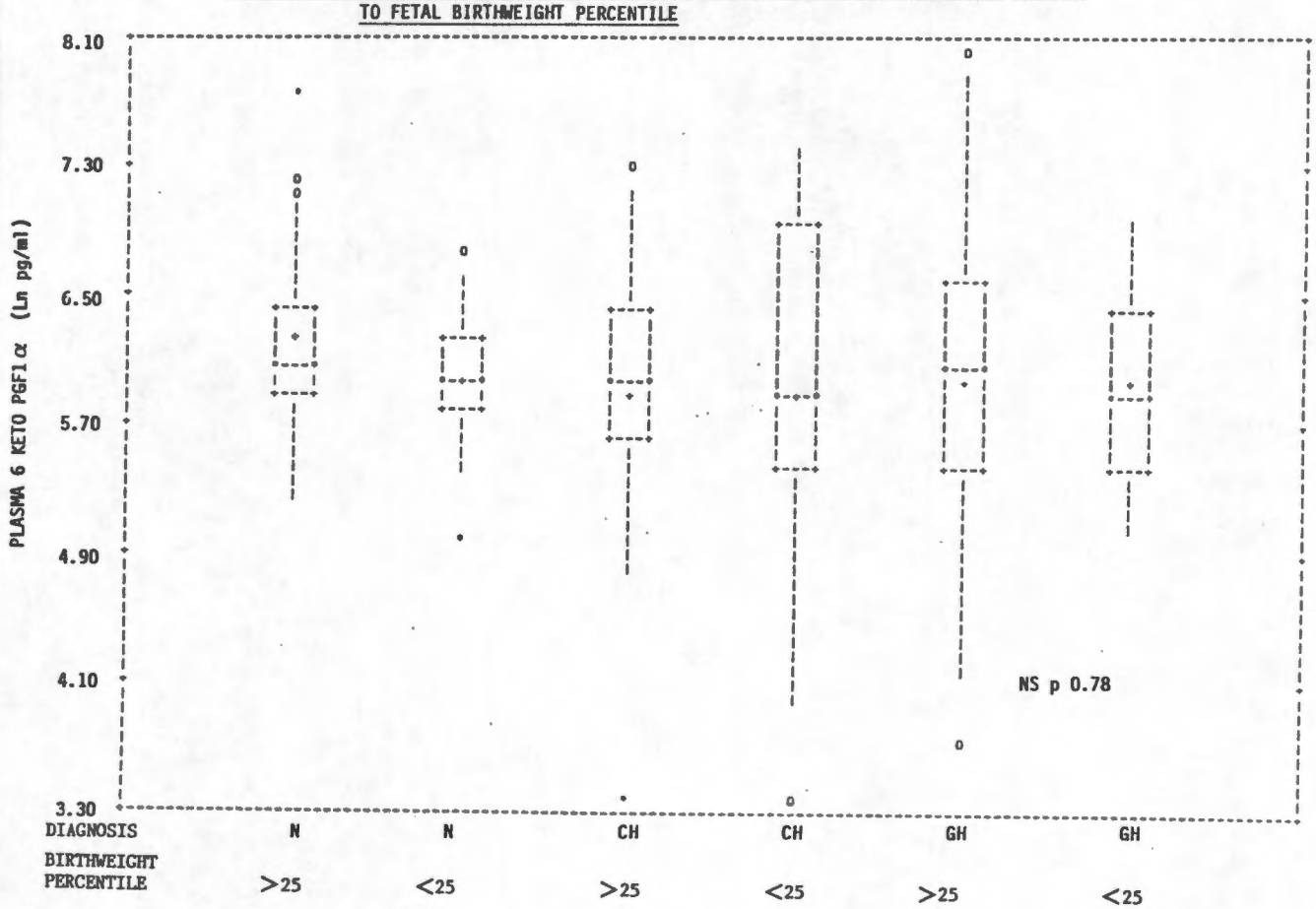


FIG. A.9.4: TOTAL 4 HOUR URINARY EXCRETION OF PGE IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT CENTILE

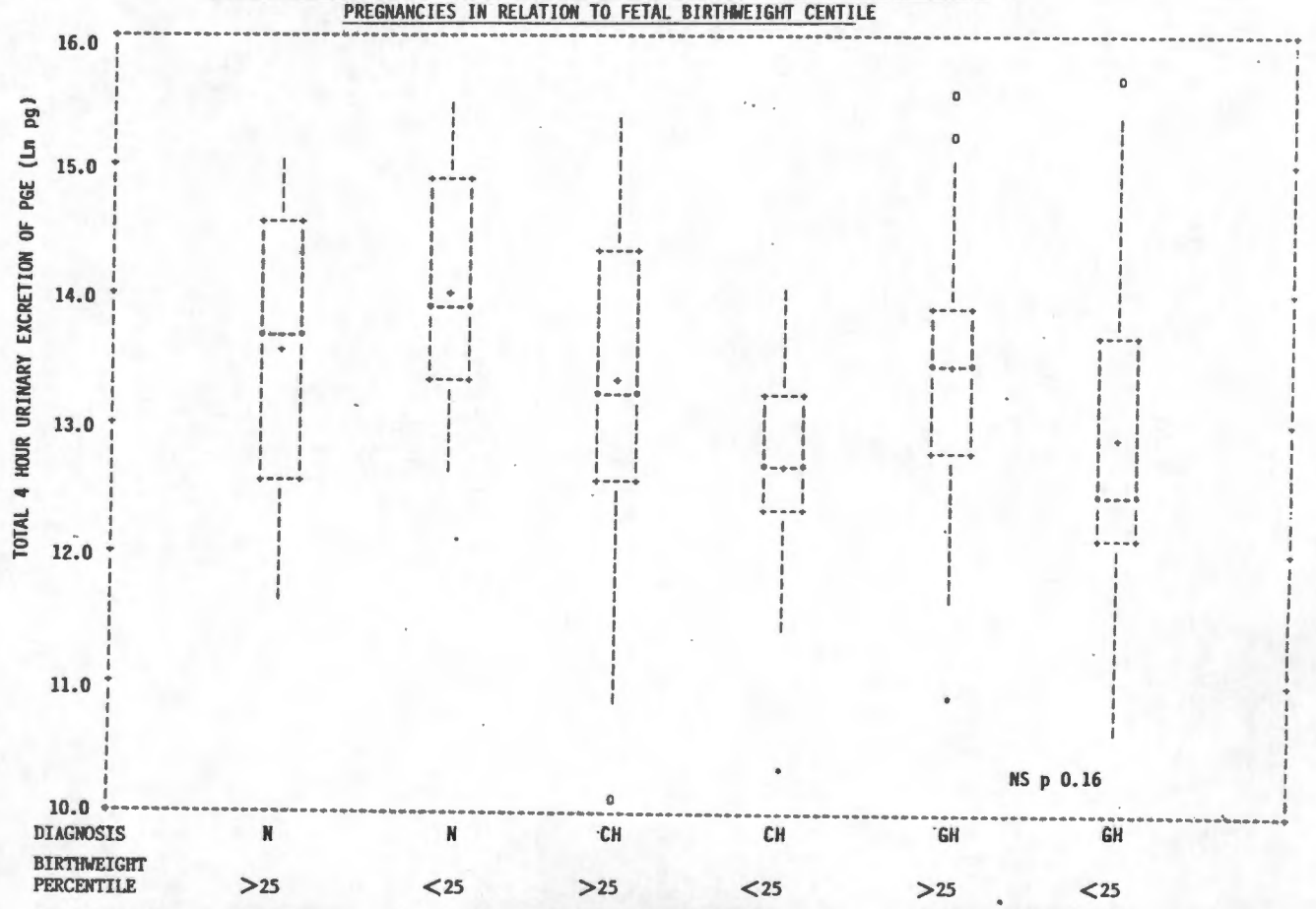


FIG. A.9.5: TOTAL 4 HOUR URINARY EXCRETION OF 6 KETO PGF1α IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE

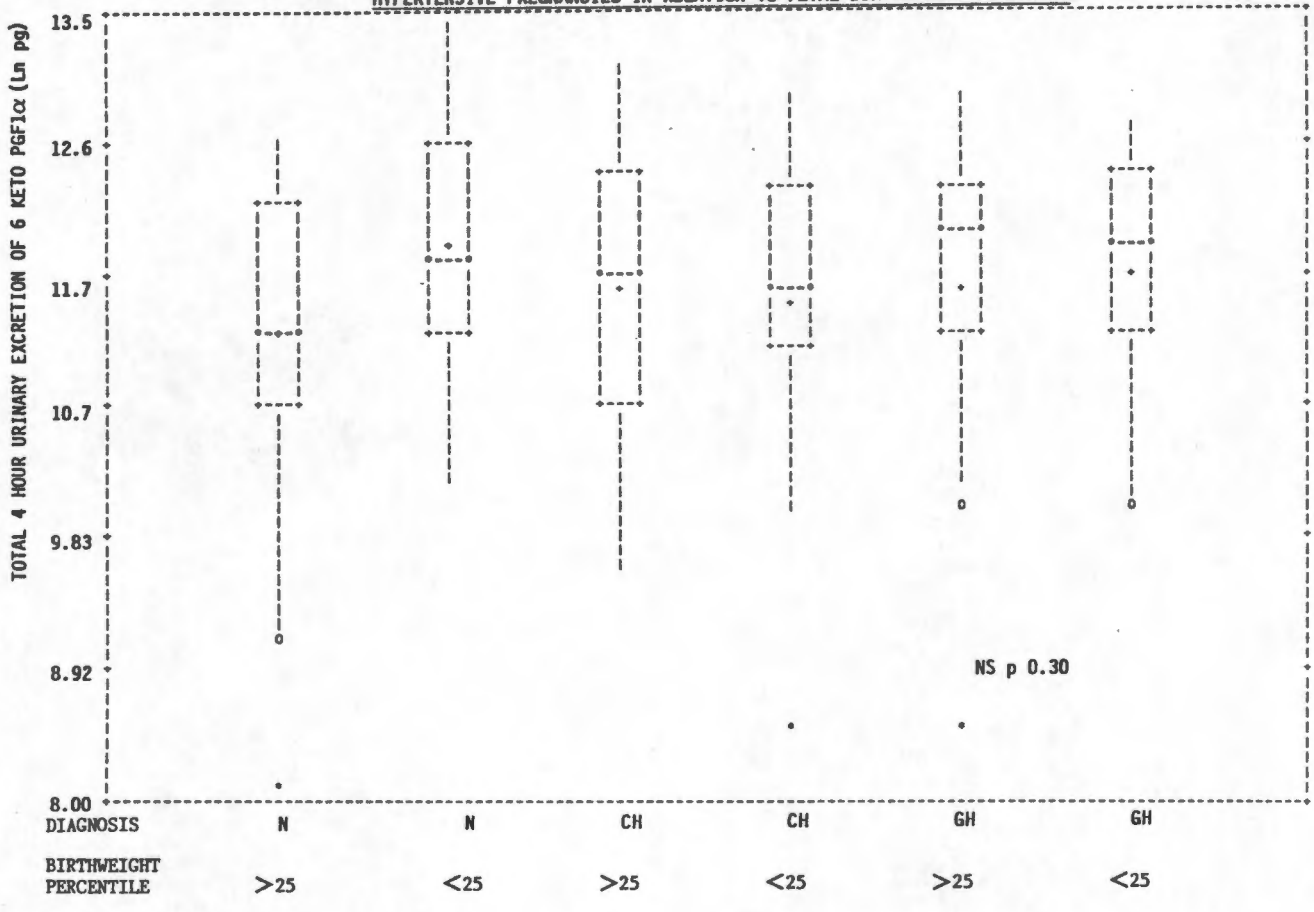


FIG. A.9.7: RATIO OF URINARY PGE TO PGFM IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT CENTILE

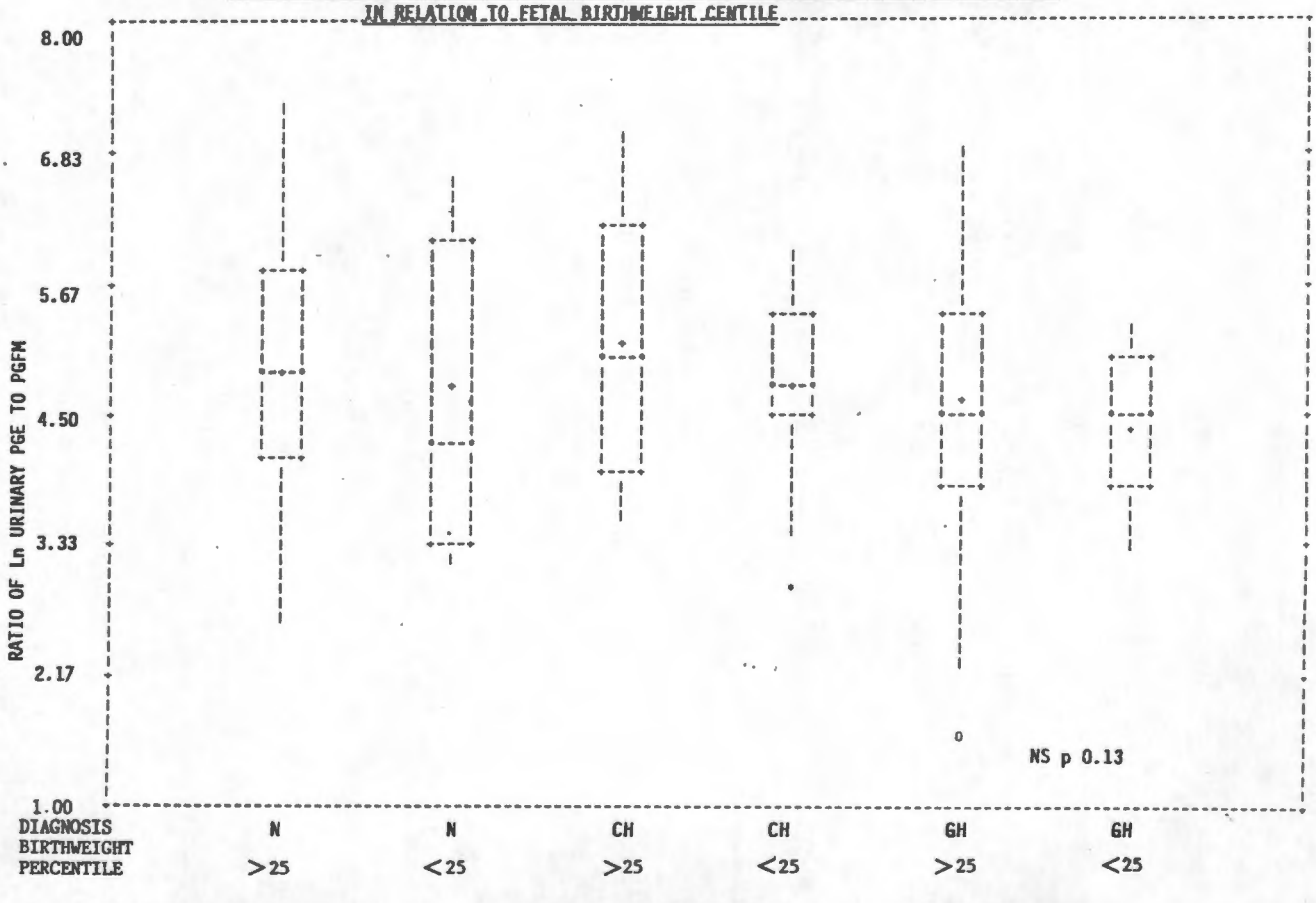
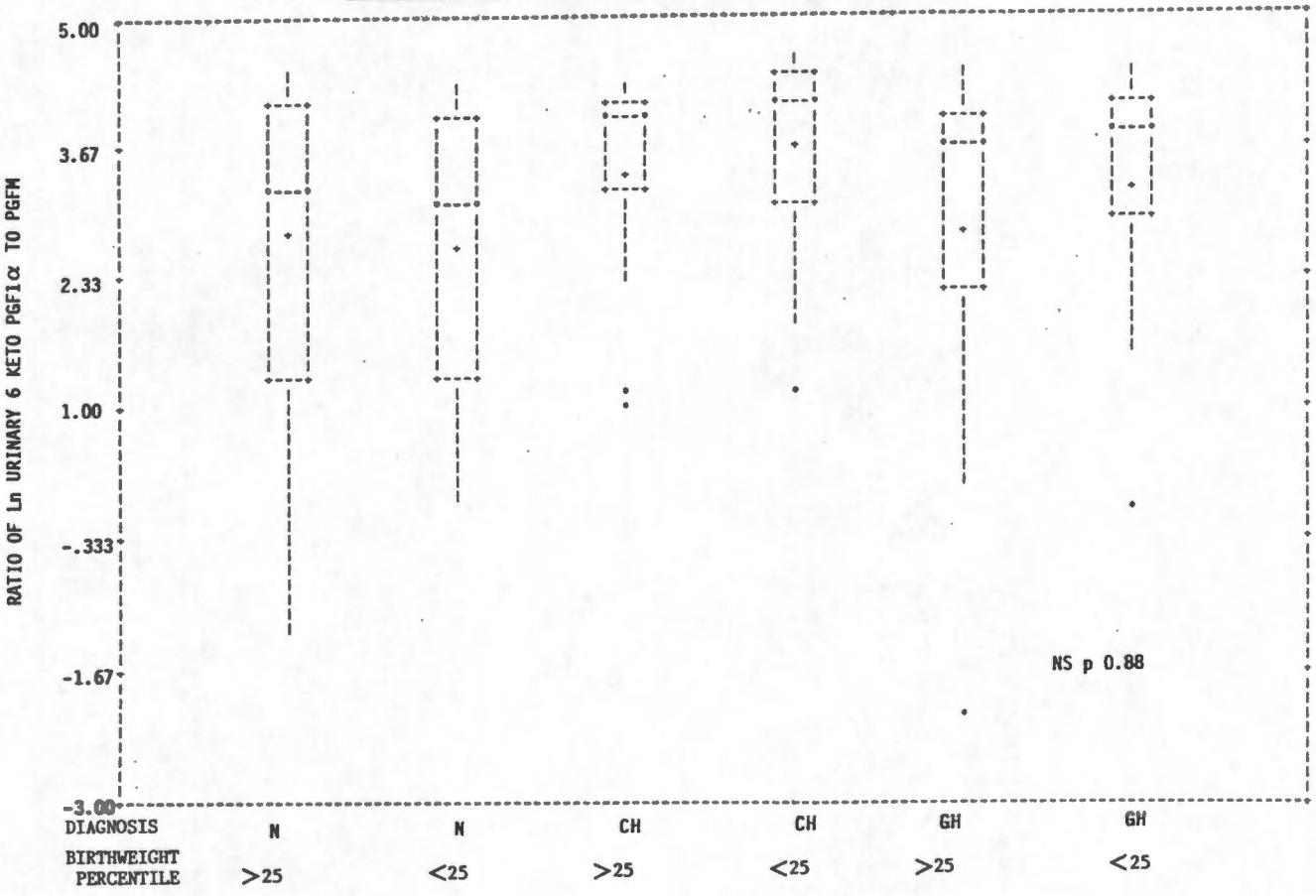


FIG. A.9.6: RATIO OF URINARY 6 KETO PGF1α TO PGFM IN NORMAL AND HYPERTENSIVE PREGNANCIES IN RELATION TO FETAL BIRTHWEIGHT PERCENTILE



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