PREDICTION AND PREVENTION OF PREGNANCY-INDUCED HYPERTENSIVE DISORDERS: A CLINICAL AND PATHOPHYSIOLOGIC STUDY

PREDICTIE EN PREVENTIE VAN ZWANGERSCHAPS-HYPERTENSIE: EEN KLINISCH EN PATHOFYSIOLOGISCH ONDERZOEK

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Let it be stated that this work is far from completion: some parts are quite clear in my mind; other things are still nebulous....

Goodall
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Hypertensive disorders constitute the most common medical complication of pregnancy. However, it is extremely difficult to provide figures for the true incidence of hypertension during pregnancy because of different diagnostic criteria and because of the fact that most reports are based on hospital studies rather than on general populations. In developed countries some form of hypertension is said to occur in approximately 15-20% of pregnancies (Turnbull 1987). At least two-thirds of cases concern pregnancy-induced hypertensive disease, an entity that is fundamentally different from chronic hypertension as a long-term problem coinciding with pregnancy (MacGillivray 1983; Ferris 1988a). Pregnancy-induced hypertensive disease develops during pregnancy, mainly but not exclusively in nulliparous women and resolves within a variable period of time after delivery.

In most countries hypertensive disease during pregnancy appears to be the largest single cause of maternal death (Zuspan 1984; Turnbull 1987). The impact of maternal hypertension on the fetus remains disputed, again due to differences in criteria of selection and diagnosis between various studies. According to the World Health Organization hypertensive disease during pregnancy is the main cause of perinatal mortality and morbidity (MacGillivray 1983). Indeed, there seems no doubt that pregnancy-induced hypertensive disease associated with proteinuria, that is preeclampsia, is accompanied by a perinatal mortality that is substantially higher than that in normotensive pregnancies. However, in the absence of proteinuria pregnancy-induced hypertensive disease has been shown to carry a perinatal mortality that is similar to or perhaps even lower than that in normotensive pregnancies (MacGillivray 1983).

No other disease in pregnancy has been surrounded for so long by so many uncertainties and controversies, concerning its etiology, pathophysiology and treatment (Chesley 1978). The etiology of pregnancy-induced hypertensive disease remains unknown, but in recent years evidence has been adduced to support the hypothesis that the eicosanoid system may play an important part in the pathophysiologic mechanisms involved in the development of its various signs and symptoms (Walsh 1985; Ylikorkala and Makila, 1985). Results of recent biochemical studies suggest that a pathophysiologic functional imbalance between vasodilator and vasoconstrictor eicosanoid products could be of pivotal importance in this respect (Walsh 1985).

The eicosanoid system has several particularities that make it hazardous to draw conclusions with regard to its physiologic and pathophysiologic effects from results of biochemical studies either in vivo or in vitro (Wallenburg 1981a).
The known possibility to manipulate eicosanoid synthesis by means of selective pharmacologic inhibition of certain key enzymes of the eicosanoid cascade, using e.g. a low dose of Aspirin (Moncada and Vane 1980), offers a potential alternative approach.

Based on the hypothesis that the pathophysiologic development of pregnancy-induced hypertensive disease depends on a functional imbalance between two eicosanoid substances with opposing physiologic effects, it may be attempted to correct the putative imbalance by means of pharmacologic manipulation. The demonstration that such a pharmacologic intervention would result in preventing, halting or retarding the clinical signs of the disease (secondary prevention) would strongly support the hypothesis; on the other hand, the absence of a preventive effect of such an approach would cast significant doubt on the validity of the hypothesis. The description of the design, the execution and the results of such a clinical pathophysiologic study, using low-dose Aspirin, forms the core of this thesis.

During the preparation of that study the need arose for a reliable method to define a population of pregnant women at risk. A survey of the pertinent literature revealed that a multitude of predictive tests for pregnancy-induced hypertensive disease has been proposed, but that their validity is ill-defined or controversial. On the basis of a critical search of the available literature we selected the two most promising predictive tests and determined their validity in an additional clinical study.

Based on the considerations presented above the objectives of this thesis can be summarized as follows:

1. to review the pertinent literature concerning the pathophysiology of pregnancy-induced hypertensive disease, in particular with regard to the role of the eicosanoid system.

2. to review the literature on predictive tests used for early diagnosis of pregnancy-induced hypertensive disease, in an attempt to assess their reported validity.

3. to assess the validity of the two most promising predictive tests, as selected on the basis of the literature, in a prospective clinical trial.

4. to investigate in a prospective clinical trial whether or not pharmacologic manipulation of the eicosanoid system by means of low-dose Aspirin prevents or reverts the clinical signs of pregnancy-induced hypertensive disease in pregnant women considered to be at risk as indicated by a positive predictive test, the angiotensin-II sensitivity test, in the early third trimester of pregnancy.

5. to evaluate the implications of the results of these studies for our understanding of the pathophysiology of pregnancy-induced hypertensive disorders.
THE CARDIOVASCULAR PATHOPHYSIOLOGY OF PREGNANCY-INDUCED HYPERTENSIVE DISORDERS: A REVIEW

The pathophysiology of pregnancy-induced hypertensive disorders can only be understood against the background of the changes in cardiovascular physiology that occur in all pregnant women. These changes involve blood volume, cardiac output, and peripheral vascular resistance. This chapter first describes the changes in cardiovascular physiology induced by normal pregnancy, which will subsequently be compared with those occurring in pregnancy-induced hypertensive disease.

2.1 Normotensive pregnancy

2.1.1 Volume of blood and water

The two major components that make up total blood volume, plasma and total red cell mass, are controlled separately. Total red cell mass is governed by the need to transport oxygen, and plasma volume expands and contracts in relation to the need to fill the vascular space and to maintain blood pressure (Hytten 1985).

Pregnancy is characterized by maternal physiologic hypervolemia, but the volume receptors appear to sense those large gains as normal; when hypervolemia is limited by salt restriction or diuretic therapy, the maternal response can be similar to that in salt-depleted non-pregnant subjects (Lindheimer and Katz, 1986). Total body water, increases by 6-9 L during pregnancy, and the increment is almost equally divided between the fetus and the mother (Lindheimer et al, 1986). Of the total increase in body water 4-6 L are extracellular; expansion of plasma volume accounts for 20-25% of the increase in extracellular space, the other 75-80% are increments in interstitial fluid (Hytten and Chamberlain, 1980; Hytten, 1985). In normal gravidae, with and without edema, the greatest increase in interstitial fluid occurs in the third trimester, contrasting with the increment in plasma volume, which takes place primarily in the first two trimesters (Longo 1984, Hytten 1985).

During the first trimester blood volume begins to increase, reaching a level of about 40% above non-pregnant values at the 30th week of gestation. Thereafter, blood volume remains stable or decreases slightly, until term (Hytten and Paintin, 1983; Hytten 1985). Part of the rise in blood volume results from an increase in the number of erythrocytes. Hytten and Leich (1971) showed that there is
a mean rise in red cell mass of 250 ml in women not having supplemental iron and 400-450 ml in iron supplemented women. If an average figure of 1400 ml is accepted as the red cell mass of an average non-pregnant woman, then the increase of 250 ml represents a rise of about 18%; the stimulated response following iron medication leads to an increase of about 30%. The mechanisms behind the changes in total red cell mass are not known. Longo (1983) hypothesized that human placental lactogen (HPL) and the effect of the low resistance shunt in the placental bed could be responsible, but there is also a marked increase in the circulating levels of erythropoietin, which must be the ultimate stimulus to the extra red cell production. Whether the action of erythropoietin is potentiated by other hormones, such as HPL is uncertain (Hytten 1985). As a result of the relatively greater increase in plasma volume, red cells in the blood are diluted and the venous hematocrit drops from a non-pregnant average of about 40% to about 33% during the last trimester of pregnancy.

A much larger portion of the increase in blood volume is caused by expansion of plasma volume, which rises to a level of about 50% above non-pregnant values by the 32nd week of pregnancy (Longo 1983). For example, a healthy woman bearing a normal sized fetus with an average birthweight of about 3.3 kg will increase her plasma volume by an average of about 1250 ml, a little under 50% of the average non-pregnant volume of about 2600 ml in European women.

The increase in plasma volume is believed to be subsequent to changes in the eicosanoid and renin-angiotensin-aldosterone system, possibly following the rise in blood progesterone and estrogen levels accompanying a normal pregnancy (de Swiet 1988; Broughton Pipkin 1988). Release of renin initiates a sequence that results in salt and water retention, thus expanding plasma volume. The formation of an arterio-venous shunt in the placental bed probably plays only a minor role in the causation of the increase in plasma volume (Hytten 1985).

The large increases in plasma volume and interstitial space are accompanied by a gradual retention of 800-1000 mEq of sodium. This gain, distributed between the products of conception (40%) and the maternal compartment (60%), accumulates gradually over a 9-month period (Barron and Lindheimer, 1984). During pregnancy both effective renal plasma flow and glomerular filtration rate (GFR) increase to levels of 30-50% above those measured in non-pregnant women. Increments in GFR are observed already during the initial weeks following conception; they peak by the early second trimester, and are sustained through the 36th gestational week, after which a small decrease may occur. The increment in GFR means that the filtered load of sodium will also increase by 30-50% as compared with non-pregnant levels, that is from about 20,000 mEq/day to as much as 30,000 mEq of sodium/day. Such increments in filtered load must obviously be accompanied by parallel increments in tubular reabsorption. Not only does the adaptive incremental tubular reabsorption of sodium accomodate the large increase in filtered load in pregnancy, but an additional 2 to 6 mEq of sodium are reabsorbed daily for fetal and maternal stores. This increase in tubular reabsorption represents the largest renal adjustment during pregnancy.
The concentrations of several potentially natriuretic hormones rise during pregnancy. Progesterone inhibits the sodium-retaining effects of the mineralocorticoids (competitive inhibitor of aldosterone) and may also have a direct inhibitory effect on the reabsorption in the proximal tubule (Burg 1986). Progesterone also produces renal vasodilatation, an effect that would promote natriuresis (Burg 1986; Ferris 1988a). Also the vasodilator prostaglandins, the levels of which are said to increase during gestation, are natriuretic (Lindheimer and Katz, 1986). The physiologic role of natriuretic hormone concerning sodium homeostasis in pregnancy is still controversial (Lindheimer and Katz, 1986). The so-called atrial natriuretic peptide (ANP) is dissimilar to natriuretic hormone; according to some investigators the concentration of ANP does not increase during pregnancy, according to others it increases slightly (Grace et al, 1987; Visser et al, 1987; Thomsen et al, 1987). ANP levels rise during the early puerperium, this increase is associated with marked natriuresis. The postpartum shift in fluid from the extravascular space to the circulation probably leads to increased atrial wall tension and in this way to increased release of ANP (Steegers et al, 1987; Grace et al, 1987; Genest and Cantin, 1987).

Other hormonal alterations promote salt reabsorption. The secretory rate, plasma concentration, and urinary excretion of aldosterone are elevated in normotensive pregnancy, often to levels exceeding those measured in patients with primary hyperaldosteronism. Presently consensus has been reached that changes in aldosterone production during pregnancy are not excessive but serve a homeostatic function as they do in non-pregnant individuals (Barron and Lindheimer, 1984). The view that aldosterone secretion increases in order to balance the increments in GFR and natriuretic hormonal changes is probably too simplistic. In non-pregnant individuals, aldosterone-dependent sodium reabsorption amounts to less than 2% of the filtered sodium load. By contrast, the daily increment in filtered sodium in human pregnancy ranges from 5,000 to 10,000 mEq. Aldosterone-stimulated sodium retention alone is clearly inadequate to balance this load appreciably (Barron and Lindheimer, 1984; Burg 1986).

Plasma desoxycorticosterone levels are also increased in pregnancy. Sequential measurements of this potent mineralocorticoid have demonstrated a significant increase in the first trimester, after which levels continue to rise throughout gestation reaching levels that are 10-20 times higher than those measured in non-pregnant women (Parker et al, 1980). Most of the desoxycorticosterone in the maternal compartment is produced by extra-adrenal 21-hydroxylation of circulating progesterone (Lauritzen and Klopper, 1983).

Plasma osmolality decreases during gestation to values that average 8 to 10 mOsm/kg below those of non-pregnant women. This decrement starts shortly after conception; it becomes significant during the 5th gestational week and reaches a nadir by the 10th week of pregnancy, after which the decreased osmolality is sustained until term. If similar decreases in the tonicity of body
fluids were to occur in a non-pregnant subject, secretion of vasopressin would cease and a large and continuous water diuresis, similar to that in patients with diabetes insipidus, would ensue. This does not occur, which shows that osmoregulation in pregnancy is altered; the osmotic thresholds for both vasopressin secretion and thirst are decreased by approximately 10 mOs/kg during gestation (Barron and Lindheimer, 1984). It should be stressed that parallel declines in the osmotic thresholds for vasopressin release and thirst are required in order to maintain the new steady state within a narrow range (Burg 1986). The product of the fetoplacental unit that is responsible for the osmoregulatory changes of pregnancy is as yet unidentified (Lindheimer and Katz, 1986).

Other factors may influence a woman’s ability to concentrate and dilute her urine during gestation. These include posture, changes in the production of vasodilator prostaglandins, and placental production of cystine aminopeptidase (vasopressinase). These influences were recently reviewed by Lindheimer and Katz (1986).

2.1.2 Hemodynamics

The term hemodynamics refers to the relationship between the motion of the blood and the forces affecting the motion, the results of which can be expressed as "flow" = "pressure" divided by "resistance to flow". Flow may be cardiac output or any organ flow, and the driving pressure is practically equal to the mean arterial pressure. These hemodynamic variables will be considered separately.

**Systemic blood pressure** - There are few large longitudinal studies of blood pressure in pregnancy, and there are none in which blood pressure before pregnancy is compared with blood pressure during pregnancy. In 226 primigravid women on their first antenatal visit MacGillivray et al. (1969) found a blood pressure of 103 +/- 11 mmHg systolic and 56 +/- mmHg diastolic in sitting position, and 113 +/- 10 mmHg systolic and 57 +/- 10 mmHg diastolic supine. Although a rise in both systolic and diastolic pressure occurs after the 28th week of gestation, blood pressure remains low. Data from a collaborative study of over 24,000 pregnancies has demonstrated that a blood pressure in excess of 125/75 mmHg prior to 32 weeks and of 125/85 mmHg thereafter is associated with a significant increase in fetal risk and on this basis can be considered abnormal in pregnancy (Friedman and Neff, 1977). Because the upper limit of normal blood pressure, 140/90 mmHg, is based on actuarial statistics in non-pregnant individuals and has no significance in pregnancy, the American Obstetrical Committee has suggested a blood pressure of 130/80 mmHg as the upper limit of normal at any time during pregnancy (Ferris 1988a; Redman and Jefferies, 1988).

**Cardiac output** - Cardiac output begins to rise during the first 10 weeks of
pregnancy, reaching a peak of 30-45% above resting, non-pregnant levels at around the 20th week. For an average pregnant woman this means an increase in cardiac output from about 4.5 to about 6.5 L/min (Wallenburg 1988). The weight of current evidence indicates that cardiac output remains elevated until delivery, if not measured in supine position. As the uterus enlarges, it interferes with venous return of blood from the legs by compressing the inferior vena cava, thus reducing cardiac preload, cardiac output, and blood pressure (Hytten and Leitch, 1971). This effect is most pronounced in the supine position and is least marked in the left lateral position (Vorys et al, 1961).

Heart rate increases from about 65 to 80 beats/min (de Swiet 1988). Since the mean cardiac output in pregnancy rises by about 1.5-2 L/min, an increase of about one third, and the heart rate rises by only about one fifth, it is clear that stroke volume must increase (Hytten and Leitch, 1971; Wallenburg 1988).

Peripheral vascular resistance – Since in pregnancy blood pressure falls and cardiac output increases, peripheral vascular resistance must decrease. This is mainly due to the vasodilatation, although the decrease in viscosity due to the fall in hematocrit also plays a role (Thornburn et al, 1982; Sullivan 1986). Maternal organ systems such as the kidney and the skin are affected by the generalized vasodilatation in pregnancy; peripheral vasodilatation is clinically evident in palmar erythema and spider telangiectases that frequently develop during pregnancy. The slow return of blood pressure towards non-gravid levels after a midtrimester nadir demonstrates that increasing vasoconstrictor tone is a feature of late pregnancy in normal pregnant women as well as in those who develop pregnancy-induced hypertensive disease (Broughton Pipkin 1985).

Some pertinent data concerning the changes in the physiologic balance between vasoconstrictor and vasodilator systems that occur during pregnancy will be discussed in the following paragraphs.

2.1.3 Vasoconstrictor and vasodilator systems

This paragraph begins with a brief overview of changes occurring in the vasoconstrictor systems, especially the renin-angiotensin-aldosterone system, in normotensive pregnancy. Next the changes occurring in vasodilator systems will be discussed, with emphasis on the eicosanoid system.

2.1.3.1 Vasoconstrictor systems

The renin-angiotensin-aldosterone system

The renin-angiotensin-aldosterone system (RAAS) is activated during each menstrual cycle, and is one of the first hormone systems to 'recognize' pregnancy (Broughton Pipkin 1988). Helmer and Judson (1967) first demonstrated that plasma renin activity (PRA) is markedly increased in normal pregnancy. At
this moment there is general agreement that the plasma concentration of angiotensinogen, PRA, and plasma renin concentration (PRC) are all increased during pregnancy. There is a 2-3 fold increase in PRC; this increase occurs within the first 14 weeks of pregnancy. Similar changes occur in PRA and seem to be largely determined by the enzyme concentration as the major increase in angiotensinogen occurs in the second half of pregnancy (Symonds 1987). The increase in circulating angiotensinogen levels is a consequence of the stimulating effect of estrogen on angiotensinogen production by the liver; in the last month of normal pregnancy angiotensinogen levels show a five-to-six fold increase (Symonds 1983; Alhenc-Gelas et al, 1986; Derkx 1987). Of interest is the observation that oral contraceptives, progestins and estrogens, enhance circulating angiotensin-II levels, but they decrease the PRC, presumably because of the negative feedback by angiotensin-II on renin secretion (Davis and Freeman, 1976). This phenomenon does not occur during pregnancy and the increase in angiotensinogen and angiotensin-II production is accompanied by an increase, rather than a decrease, in PRC (Derkx 1987). This suggests that other factors, possibly vasodilator prostaglandins, overrule the negative feedback of angiotensin-II on renin secretion (Jackson et al, 1985; Alhenc-Gelas et al, 1986). Feedback suppression of renin release by angiotensin-II also exists in human pregnancy, but is less sensitive or has another setpoint (Broughton Pipkin et al, 1983).

A large proportion of renin circulates in plasma as an enzymatically inactive form (prorenin). In many of the published studies it is often not clear whether only naturally occurring active renin or both active and prorenin were measured, and to what extent activation of prorenin had occurred during storage and handling of the plasma samples (Skinner et al, 1972; Skinner et al, 1979; Derkx et al, 1979; Schalekamp and Derkx, 1981). The fraction of total renin in plasma that is in the active form is about 5% in pregnant women. The fact that prorenin is much (about tenfold) higher during pregnancy than in other situations with a stimulated renin release from the kidney may point to a smaller proportion of prorenin-renin conversion intrarenally before release into the circulation and / or extrarenal production of prorenin (Derkx 1987), which has been shown to exist in the uteroplacental-fetal complex (Symonds 1983), and in the ovary (Sealey et al, 1985; Derkx 1987). Recently it was suggested that the ovary may be the main source of elevated plasma prorenin in pregnant women (Derkx 1987; Itskovitz and Sealey, 1987).

In pregnancy, plasma aldosterone levels increase, reaching a maximum during the third trimester. The enhanced secretion of aldosterone by the adrenals can probably be explained by the increased circulating level of angiotensin-II (Alhenc-Gelas et al, 1986).

Guyton et al. (1967; 1983) developed the concept that the long term level of arterial blood pressure, that is the regulation of vascular tone and cardiac output, is determined by two major factors:

a. the rate of sodium intake
b. the slope and position of the renal pressure-natriuresis curve.
According to this concept the long term level of arterial blood pressure can never be controlled to any other pressure than the equilibrium point where sodium intake and urinary sodium output are equal. These considerations would lead one to conclude that large alterations in sodium intake should markedly alter steady state levels of mean arterial blood pressure, but apparently this is not the case in healthy individuals. The reason this does not occur is that as sodium intake increases, the rates of renin secretion and angiotensin-II formation decrease (Guyton et al, 1983). The decrease in angiotensin-II has a profound effect on the urinary sodium output curve. Thus a major importance of the RAAS in the control of arterial blood pressure is that it allows human beings to consume very minute or extreme amounts of salt and still have a reasonably normal arterial blood pressure. Because of the existence of the kidney-volume mechanism for pressure control, hypertension does not occur unless there is some abnormality in the function of this mechanism. As long as the mechanism functions normally, any hypertensive state will cause pressure natriuresis with progressive loss of extracellular sodium and extracellular fluid until arterial blood pressure returns to normal (Jackson et al, 1985; Cowley and Roman, 1986). The kidney-volume mechanism adjusts blood volume and cardiac output until the cardiac output is a mirror image of the change in total peripheral resistance (Guyton et al, 1983; Cowley and Roman, 1986; Guyton 1987). Despite a marked increase in the activity of the RAAS, sodium excretion does not differ significantly between non-pregnant and normotensive pregnant women (Brown et al, 1988). Factors which influence plasma concentrations of the various components of the RAAS outside of pregnancy continue to do so during gestation. Normal stimuli like standing or sodium deprivation are operative, but act on a system which is being driven by some other factor. The difficulty in understanding renin secretion in pregnancy is that high secretion occurs during expansion of the extracellular volume, increased renal blood flow, increased GFR, and increased delivery of sodium to the macula densa. Recent studies showed that this increase is probably mediated via an increase in renal biosynthesis of prostacyclin (Ferris 1982; Pedersen et al, 1982; Jackson et al, 1985). In normotensive pregnancy a direct correlation exists between urinary metabolites of prostacyclin and PRC (Broughton Pipkin et al, 1983). The increased prostacyclin levels themselves as well as the dilated intravascular volume (baroreceptor mechanism in juxtaglomerular apparatus) lead to a marked stimulation of the RAAS. This explains why renin, angiotensin-II and aldosterone levels are increased despite the absolute hypervolemia of gestation, the "effective volume" is still sensed as suboptimal (Ferris 1982). The increase in renal biosynthesis of vasodilator prostaglandins may be the cause of the normal functioning of the kidney-volume mechanism in normotensive pregnancy, because vasodilator prostaglandins antagonize the intrarenal effects of angiotensin-II (Kaplan 1985; Zanchetti et al, 1985; de Leeuw and Birkenhager, 1986).

In normotensive pregnancy the uteroplacental unit seems to contribute only slightly to both maternal active renin and angiotensin-II levels (Symonds 1981; Broughton Pipkin 1988).
The adrenergic system

Assali and his group (Assali et al, 1952; Assali and Brinkman, 1972) showed that, in contrast to non-pregnant subjects, pregnant women exhibit a significant hypotensive response to blockade of the autonomic nervous system; this response increases with gestational age. These results obtained with short term inhibition of the autonomic nervous system, have been confirmed in recent years in animal studies in which the adrenergic system was chronically ablated (Tabsh et al, 1986). Basal plasma catecholamine levels are reported to be similar in pregnant and non-pregnant subjects, but responses to physiologic stress such as upright posture and exercise are significantly altered in late gestation (Pedersen et al, 1982; Barron et al, 1986). However, a study of plasma norepinephrine levels during normal pregnancy, using the more sensitive radioenzymatic assay for catecholamines, has described a progressive decrease in plasma norepinephrine levels throughout normal pregnancy (Tunbridge and Donnai, 1981). Vasodilator prostaglandins, especially prostaglandin-E₂, inhibit the neuronal release of norepinephrine (Spokas et al, 1983; McGiff et al, 1985). The decrease in plasma norepinephrine levels in normotensive pregnancy could be a result of an increased production of vasodilator prostaglandins (Petersen 1988). Most recent studies show a blunted noradrenergic response (heart rate, norepinephrine levels) in late pregnancy (Nisell and Lunell, 1984; Nisell et al, 1985a; Nisell et al, 1985b; Barron et al, 1986) to standing, upright tilt and isometric exercise. The attenuated pressor response seen in normotensive women, during norepinephrine infusion, is not caused by vasoconstriction but by an increase in cardiac output (Nisell et al, 1987).

It should be stressed that sympatho-adrenal activity is not uniform; determination of forearm venous norepinephrine concentration will overemphasize the activity of the adrenergic nervous system because about half of the measured norepinephrine has been released from nerves in the forearm, and reflects local sympathetic activity. Since epinephrine is extracted across the vascular bed, 30-50% of epinephrine is removed during one passage through the forearm, arterial levels give a more accurate measure of adreno-medullary activity and determination of forearm venous epinephrine concentration will underestimate adreno-medullary activity (Folkow 1982; Kuchel 1983; Bohr and Welb, 1986).

Recently neuropeptide Y, a potent vasoconstrictor and cardiodepressant 36-amino acid peptide, has been discovered (Tatemoto et al, 1982; Allen and Bloom, 1986). Neuropeptide Y is released together with norepinephrine (Zukowska-Grojec et al, 1987). As yet there is no data on the importance of neuropeptide Y in cardiovascular physiology in human pregnancy.

Natriuretic hormone

Natriuretic hormone, a small (MW 200) polar, neutral, non-peptidic compound (Blaustein 1977; Blaustein 1984; de Wardener and MacGregor, 1986), is a circulating inhibitor of Na⁺/K⁺ ATPase. Natriuretic hormone is said to increase
urinary sodium excretion and to cause a rise in intracellular sodium and calcium (de Wardener and Clarkson, 1985). Because the pharmacologic effect of digitalis is to inhibit Na\(^+\)/K\(^+\) ATPase it has been postulated that the so-called digitalis-like substance (DLS) is this circulating inhibitor of Na\(^+\)/K\(^+\) ATPase.

It is conceivable that in pregnancy the overall vasodilatation by diminishing the intrathoracic volume lowers intrathoracic vascular pressure so that, although total blood volume is increased, the volume receptors in the thorax signal a fall in blood volume. This will then depress the natriuretic and sodium transport inhibiting properties of the blood (Blaustein 1977; Blaustein and Hamlyn, 1984; Buckalew and Gruber, 1984). A reduction in the plasma concentration of DLS, the putative sodium transport inhibitor, could account for the resistance to the pressor effects of infused angiotensin-II in normotensive pregnancy (de Wardener 1985; de Wardener and Clarkson, 1985; de Wardener and MacGregor, 1986). As yet this hypothesis is entirely speculative, but most known hormonal changes concerning cardiovascular functioning fit in.

The vascular responsiveness to vasoconstrictor substances

The concentration of angiotensin-II in plasma of normotensive, non-pregnant subjects is just below the level required to exert an effect on basal systemic arterial blood pressure (Chinn and Dusterdieck, 1972). Plasma concentrations of angiotensin-II are known to increase 2-3 fold in normal pregnancy (Broughton Pipkin 1988), and yet the majority of pregnant women do not become hypertensive. A marked reduction in pressor responsiveness to angiotensin-II was reported for the first time more than 25 years ago by Abdul-Karim and Assali (1961). Subsequently several investigators have confirmed that, during normotensive human pregnancy, a greater amount of angiotensin-II is required than in non-pregnant women to produce the same rise in blood pressure (Chesley et al, 1965; Gant et al, 1973).

There are at least four possible mechanisms by which pregnancy might alter the vascular response to angiotensin-II (Paller 1984; Goodfriend 1986; Ferris 1988):

1. High circulating levels of endogenous angiotensin-II may prevent receptor binding of exogenous angiotensin-II. Experiments in sheep and humans have shown that acute volume expansion with a demonstrated fall in PRA is not associated with any change in vascular reactivity to angiotensin-II (Gant et al, 1975; Gant et al, 1983). A potential problem with these studies is that volume expansion also suppresses activity of the adrenergic and vasopressin systems and might thereby indirectly affect the response to angiotensin-II (Paller 1984). However Matsura et al., (1981) reported that refractoriness to angiotensin-II was not affected by volume expansion in the pregnant ewe, whereas vascular reactivity was increased in the non-pregnant ewe by a similar treatment. To circumvent these potential problems studies were performed in which circulating angiotensin-II levels were lowered by captopril, a converting enzyme inhibitor. Following
administration of captopril pregnant rats and sheep have a decreased pressor response to exogenous angiotensin-II at a time when fewer vascular receptors for angiotensin-II are occupied (Siddiqi et al, 1983; Paller 1984). Only Siddiqi et al. (1983) reported an increased pressor response to exogenous angiotensin-II in pregnant sheep after prolonged administration of captopril.

2. The number of vascular angiotensin-II receptors could be decreased in pregnancy (down-regulation) because of the high circulating levels of endogenous angiotensin-II. Indeed, the number of vascular angiotensin-II receptors appears to be somewhat less in pregnancy, but this insignificant change cannot be held responsible for the changes in angiotensin-II responsiveness (Paller 1984; Broughton Pipkin 1988).

3. Vascular receptors may have a decreased affinity for angiotensin-II in pregnancy, but this has not been demonstrated (Paller 1984; Broughton Pipkin 1988).

4. Endogenous vasodilators attenuate the pressor response to angiotensin-II. This possible mechanism is supported by the observation that vascular hyporesponsiveness in pregnancy is not unique for angiotensin-II but affects the response to norepinephrine and vasopressin as well; this observation strongly suggests a postreceptor phenomenon (Paller 1984). The physiologic loss of angiotensin-II sensitivity in pregnancy appears to involve prostaglandins since prostaglandin synthesis inhibitors restore angiotensin-II sensitivity to a non-pregnant level in normotensive pregnant women (Everett et al, 1978a) and in several experiments with pregnant animals (Paller 1984). Prostaglandin synthesis inhibitors also normalize the attenuated pressor response to norepinephrine and vasopressin in pregnant animals (Paller 1984; Venuto et al, 1984; Robertson and Berl, 1986).

Broughton Pipkin et al. (1982) showed that infusion of prostaglandin-E2, at doses which do not affect basal blood pressure, significantly attenuates the pressor response to angiotensin-II in pregnant, but not in non-pregnant subjects. It is important to note that the inhibitory effect of vasodilator prostaglandins on the pressor response to angiotensin-II, norepinephrine and vasopressin is observed even when the dose of prostaglandin used in itself does not cause vasodilatation. If the prostaglandins do cause vasodilatation, the inhibitory effects on the pressor response persist long after the direct vasodilator effects of the prostaglandins have faded away (Paller 1984).

Probably vasodilator prostaglandins induce vascular refractoriness to vasoconstrictor hormones by stimulating adenyl cyclase, in this way increasing cyclic adenosine monophosphate (cAMP) in the myocyte. An increase in cAMP results in a decrease in free intracellular calcium, especially by sequestration of calcium in cellular membranes (Bohr 1963; Somlyo and Somlyo, 1983; Ballermann et al, 1986).

Although some experiments on animals or humans suggest that also some progesterone metabolites (e.g. 5-alpha-dihydroprogesterone) and 17 beta-estradiol could be involved in the development of vascular refactoriness to pressor substances during pregnancy (Everett et al, 1978b; Tamai et al, 1984), the vasodilator prostaglandins probably mediate the greatest part of vascular hyporesponsiveness in pregnancy.
2.1.3.2 Vasodilator systems

The Eicosanoid System

The eicosanoids are among the most prevalent autacoids and have been detected in almost every tissue and body fluid. Their production increases in response to a great variety of stimuli; they produce a multitude of physiologic and pharmacologic effects that embrace practically every biologic function. Inhibition of their biosynthesis is now recognized as a mechanism of some of the most widely used drugs, the nonsteroidal anti-inflammatory drugs such as Aspirin. Samuelsson and his group (1980), elucidated eicosanoid biosynthesis and metabolism and established methods for the quantification of eicosanoids and their metabolites. It became clear that under basal conditions eicosanoids do not occur in biologically relevant amounts. They are synthesized and metabolized locally and the small amounts escaping local metabolism are removed by the lungs, preventing recirculation (Moncada and Vane, 1980; Blair et al, 1982; Dusting et al, 1986). They can be thought of as local mediators of functions such as tissue perfusion and tissue metabolism. Thromboxane is an exception, because it is synthesized primarily in the platelets, which enjoy the full extent of the cardiovascular system, abnormalities in platelet thromboxane synthesis can therefore effect the entire cardiovascular system and the organs which the vasculature supplies (Granström et al, 1982; Greenberg 1982).

Biochemistry: The term eicosanoid covers oxygenated 20-carbon fatty acids, including the prostaglandins, thromboxanes, leukotrienes, and epoxy, mono-, and dihydroxy eicosenoic acids (Moncada et al, 1985; Smith 1986). Because this paragraph deals mainly with prostaglandins and thromboxane these terms will be used here. Prostaglandins fall into several main classes, designated by letters and distinguished by substitutions on the cyclopentane ring. Prostaglandins of the E- and F-alpha series are sometimes referred to as the "primary prostaglandins", even though they are products of the metabolism of prostaglandins of the G and H series. The A, B, and C prostaglandins are all derivatives of the corresponding E prostaglandins; they either have little biologic activity, or do not exist in significant concentrations in human tissues (Bakhle 1983; Speroff 1983). A compound with a structure different from that of the "primary prostaglandins" is thromboxane-A2 (TXA2), formed by the enzyme thromboxane-A2 synthetase, first isolated in platelets (Hamberg et al, 1975; Hamberg et al, 1976). Thromboxanes contain a six-membered oxane ring instead of the cyclopentane ring of the prostaglandins and result also from the metabolism of the prostaglandins of the G and H series. Another compound with a structure that differs from those of the "primary prostaglandins" is prostacyclin (PGI2), formed by the enzyme prostacyclin synthetase, first discovered in vascular tissue (Moncada et al, 1976; Moncada et al, 1977). PGI2 has a double-ring structure, closed by an oxygen bridge between carbons 6 and 9.

The main classes of prostaglandins are further subdivided according to the
number of double bonds in the side chains. This is indicated by the subscript 1, 2, or 3, and reflects the fatty acid precursor. Prostaglandins (PG) with one, two, and three double bonds belong to the monoenoic, dienoic, and trienoic series respectively, e.g. PGE1, TXA2 and PGI3. Most attention has been paid to the normally prevalent dienoic prostaglandins, which are synthesized from arachidonic acid (Blackwell 1983; Schlondorff 1986). Only a very small amount of the monoenoic prostaglandins is present and little if any of the trienoic series of prostaglandins is normally found in the human body (Kadowitz et al, 1982).

Once liberated from the membrane lipids by activation of phospholipase A2 and/or sequential activation of phospholipase C and diacylglycerol kinase (Spokas et al, 1983) brought about by various stimuli, free arachidonic acid is rapidly metabolized. Synthesis of the primary prostaglandins is accomplished in stepwise manner by an ubiquitous complex of microsomal enzymes, primarily located in the endoplasmatic reticulum, the first of which is referred to as fatty acid cyclo-oxygenase (Bakhle 1983). Incorporation of 2 atoms of molecular oxygen leads to formation of unstable endoperoxide intermediates, the first two true prostaglandin compounds, PGG2 and PGH2 (half-life of about 5 minutes), the mothers of all prostaglandins. The cyclic endoperoxides are further converted to prostaglandins by isomerases or by spontaneous breakdown. The prostaglandin isomerases determine the result of prostaglandin synthesis; e.g. lung, kidney and spleen possess a wide range of isomerases and are able to synthesize a variety of prostaglandins, but other tissues cannot. Platelets synthesize mainly TXA2, whereas the blood vessel wall primarily produces PGI2.

Until 1976, PGE2, PGF2-alpha and PGD2 were regarded as the prostaglandins with the greatest biologic significance, but the discovery of TXA2 and PGI2 has modified this view, in particular because of their extensive effects on the cardiovascular system and platelet function.

Prostacyclin and Thromboxane: In the past most interest with regard to blood pressure control has centered on the potential role of renal synthesis of PGE2. With the finding that endothelial and vascular smooth muscle cells synthesize PGI2, and platelets synthesize TXA2, these two arachidonic acid metabolites, rather than PGE2 became the eicosanoids thought mostly likely to control vascular resistance (Hamberg et al, 1976; Moncada and Vane, 1977; Moncada et al, 1985). PGI2 (half-life about 2-3 minutes) and TXA2 (half-life also about 2-3 minutes) (Kadowitz et al, 1982; Bakhle 1983) can be viewed as opponents, each having powerful biologic activity which counters or balances the other (Moncada and Vane, 1979; Moncada et al, 1985). TXA2 is the most powerful vasoconstrictor known, while PGI2 is a potent vasodilator. These two agents also have opposing effects on platelet function. PGI2 is the most potent inhibitor of platelet aggregation known, and TXA2 a potent stimulator of platelet aggregation (Moncada 1982; Whittle and Moncada, 1983; Moncada 1985). Platelets predominantly synthesize TXA2, while the heart, stomach, and particularly the blood vessels throughout the body, including the uterine artery synthesize PGI2 (Wallenburg et al, 1981; Spokas et al, 1983).
Several pregnancy-associated tissues such as the human myometrium (Moonen et al., 1986; Bamford et al., 1980), decidua, chorion, amnion (Mitchell et al., 1978) and trophoblast (Rakoczi et al., 1983) produce prostacyclin (PGI2) in vitro. The trophoblast lining the uteroplacental arteries also produces PGI2 (Keirse et al., 1986).

Several studies have described the changes in PGI2 levels that occur throughout normotensive pregnancies. Maternal PGI2 formation in vivo has been measured in terms of the PGI2 hydrolysis product 6-keto-PGF1alpha by radioimmunoassay or with gaschromatography-mass spectrometry. Despite the controversy concerning methodology and the absolute levels of 6-keto-PGF1alpha the earlier findings of increased peripheral plasma levels of 6-keto-PGF1alpha in pregnancy (Lewis et al., 1980; Bolton et al., 1981; Mitchell 1981) have been subsequently confirmed (Barrow et al., 1983; Greer et al., 1985). The plasma levels found at term (8 pg/ml) are greatly increased compared to non-pregnant values (<1.0 pg/ml) but still are not high enough for PGI2 to exert any effect in the systemic circulation (Barrow et al., 1983). Rather the elevated 6-keto-PGF1alpha levels may reflect much greater release from a particular vascular bed or tissue during pregnancy (Myatt 1987). In some studies (Bolton et al., 1981; Spitz et al., 1983; Greer et al., 1985) the maternal plasma 6-keto-PGF1alpha level increased during the midtrimester, but this rise disappeared in late pregnancy. In this regard it is noteworthy that diastolic blood pressure in normal human pregnancy shows a midpregnancy drop, thereafter blood pressure gradually almost rises to non-pregnant level near term. Also vascular angiotensin-II sensitivity increases in the last ten weeks of pregnancy (Broughton Pipkin 1985). The parallelism between the levels of PGI2 found in these studies and the changes in blood pressure and vascular angiotensin-II refractoriness during normotensive pregnancy is striking (Bolton et al., 1981; Broughton Pipkin 1985).

The presence of PGI2 in amniotic fluid has been shown by bioassay of prostacyclin-like activity (Bodzenta et al., 1981; Wilcox et al., 1983), and by radioimmunoassay of 6-keto-PGF1alpha (Mitchell et al., 1979; Ylikorkala et al., 1981). The amniotic fluid 6-keto-PGF1alpha level increases with advancing gestational age, and during labor. It may originate from various fetoplacental tissues and/or fetal urine (Ylikorkala and Makila, 1985). In contrast to the decrease in plasma 6-keto-PGF1alpha levels in late pregnancy, found in some studies, the renal excretion of urinary metabolites of PGI2 (2,3-dinor-6-keto-PGF1alpha) in normotensive pregnancy is enhanced already early in pregnancy and eventually increases to levels 5-10 fold above the levels found in non-pregnant women (Goodman et al., 1982; Fitzgerald et al., 1987). 2,3-dinor-6-keto-PGF1alpha is a major oxidative metabolite of PGI2 in human urine, and excretion of this metabolite is linearly related to infused prostacyclin (Fitzgerald et al., 1987). These findings give strong support to the concept that PGI2 formation increases markedly in normotensive human pregnancy. It is not known however whether this rise comes solely from the maternal and uteroplacental vasculature or whether the fetoplacental unit also contributes to it.
Renal synthesis of PGE2 also increases during normotensive pregnancy (Bay and Ferris, 1979; Pedersen et al, 1984). Urinary PGE2 represents renal synthesis of PGE2, the urinary PGE2 excretion increases in normal pregnancy to 200-300% of the non-pregnant values (Bay and Ferris, 1979). The marked increase in renal renin synthesis in normal pregnancy may be due to the increased production of vasodilator prostaglandins in the renal and utero-placental vascular systems (Myatt 1987; Friedman 1988; Ferris 1988b).

Prostaglandins have several particularities that make it hazardous to draw conclusions with regard to their physiologic effects from results of biochemical studies either in vitro or in vivo (Wallenburg 1981a). Therefore, evidence for the physiologic importance of prostaglandins in the regulation of maternal and uteroplacental vascular reactivity has been obtained by using prostaglandin synthesis inhibitors. In experiments in pregnant animals prostaglandin synthesis inhibitors increased uteroplacental and systemic vascular resistance (Venuto et al, 1975; Terragno et al, 1980; Katz and Creasy 1981; Naden and Rosenfeld 1985). The vasoconstrictor response in these studies was short-lived (McLaughlin et al, 1978; Naden and Rosenfeld 1985), suppression of prostaglandin synthesis persisted well beyond the time when all hemodynamic evidence of vasoconstriction had disappeared, the effect on the uteroplacental circulation was briefer than that seen in the overall systemic circulation. These animal studies give support to the concept that in the unstressed situation a low basal level of vasodilator prostaglandins is enough for the maintenance of uteroplacental and systemic blood flows (Clark et al, 1982; Clark and Brody, 1982; Ferris 1988b). Another explanation for the quick recovery of uteroplacental and systemic blood flows after administration of prostaglandin synthesis inhibitors, is the fact that these inhibitors also decrease circulating angiotensin-II levels (Keeton and Campbell 1981; Churchill 1985).

Studies on uteroplacental circulatory effects of intra-arterial infusion of PGI2 showed a dissociated response; intra-arterial injection of a large dose of PGI2 caused vasodilation with a rise in blood flow in the uterine vasculature, but also an increase in cotyledonary vascular resistance. The observed cotyledonary vasoconstriction was probably secondary to the maternal hypotension and release of catecholamines (Rankin et al, 1979; Rankin et al, 1981). Infusion of PGI2 in the uterine artery in the chronically instrumented unanesthetized sheep, thus preventing effects on systemic arterial pressure, led to dose-related increases in uterine blood flow (Clark et al, 1982).

The vasodilator prostaglandins modulate the action of vasoconstrictor hormones on the uteroplacental vasculature. Angiotensin-II stimulates PGI2 release from various tissues including the vascular endothelium of the uterine (Magness et al, 1985), renal (Mullane and Moncada, 1980) and feto-placental circulations (Glance et al, 1985). These effects are mediated via specific angiotensin-II-receptors and are not simply a response to vasoconstriction (Goodfriend 1983). As these sites are rich sources of renin and angiotensin-II production, locally produced angiotensin-II may be more important than circulating angiotensin-II in determining the vascular response there (Glance et al, 1986; Broughton
Pipkin 1988; Ferris 1988a). The finding that prostaglandin synthesis inhibitors significantly increase the uterine vasoconstrictor response to angiotensin-II in the near-term pregnant ewe (McLaughlin et al., 1978), and when administered to pregnant women revert the physiologic diminished vascular sensitivity to angiotensin-II, supports the importance of this mechanism in man (Everett et al., 1978a).

PGI2 only partially attenuates the placental vasoconstrictor response to norepinephrine; in this regard PGE2 is more effective (Wallenburg 1981b; Broughton Pipkin 1985). Both PGI2 and PGE2 depress the response of the uteroplacental vasculature to norepinephrine in near-term pregnant sheep (Wallenburg 1981b).

Among all pregnancy-associated tissues the umbilical and fetal-placental vessels have the greatest relative PGI2 production (Ylikorkala and Makila, 1985). PGI2 is the major product of prostaglandin biosynthesis in vascular tissues of fetal animals, and may be of major importance in the maintenance of the low peripheral resistance typical of the fetal circulation (Kaäpa et al., 1982; Ylikorkala and Makila, 1985). Glance et al. (1985; 1986) studied isolated human placental cotyledons perfused in vitro. Injection of angiotensin-II into the fetal circulation stimulated the release of PGE2 and 6-keto-PGF1alpha into the fetal circulation. In combination with angiotensin-II PGI2 caused significant dose-related attenuations of the angiotensin-II-induced vasoconstrictive response, but no change occurred in fetal perfusion pressure when endogenous production of prostaglandins in the fetal-placental circulation was decreased by indomethacin treatment (Glance et al., 1986). This suggests that, in the isolated perfused human placental cotyledon, fetal vascular tone is not maintained by prostaglandins and that the mechanism by which they exert their actions on fetal-placental vessels may be by modulating the vasoconstrictive actions of angiotensin-II. This mechanism assumes even greater importance in the preservation of fetal placental blood flow when one considers the fact that the placenta may be the major site of angiotensin-II formation in the fetal-placental unit (Glance et al., 1984).

Another important physiologic effect of vasodilator prostaglandins, especially PGE2 may be to maintain the ductus arteriosus in a dilated state (Coceani et al., 1980; Rudolph 1981). PGI2, the main product in the fetal pulmonary circulation, is the major factor in maintaining vasodilatation in the fetal pulmonary bed (Coceani 1980; Rudolph 1981; Kaäpa et al., 1982).

TXA2, a vasoconstrictor and a potent stimulator of platelet aggregation, is mainly synthesized by platelets, but also all pregnancy-associated tissues (myometrium, decidua, placenta, fetal vessels and membranes) are known to produce TXA2 in vitro (Ylikorkala and Makila, 1985). Circulating levels of TXB2 and concentrations of TXB2 in amniotic fluid have been shown to increase during pregnancy (Ylikorkala and Viinikka, 1980; Ylikorkala et al., 1981). The capacity of maternal platelets to synthesize TXA2 increases during pregnancy (Ylikorkala and Viinikka, 1980; Spitz et al., 1983).
TXA2 is a powerful vasoconstrictor in the uteroplacental and fetal-placental vascular bed (Clark et al, 1982; Walsh 1985). The local action of TXA2 on the uteroplacental vasculature may be due to activation of maternal platelets passing through the uteroplacental vessels (Wallenburg 1981a; Wallenburg 1987), but the placenta itself may also be of importance in TXA2 production (Walsh 1985).

Striking physiologic alterations occur in the distal parts of the uteroplacental arteries during human pregnancy, due to the invading trophoblast (non-villous cytotrophoblast) (Brosens et al, 1967; Brosens et al, 1972; Pijnenborg et al, 1980). The endovascular trophoblast produces a remarkable series of morphologic alterations in the walls of spiral arteries. The spiral arteries show dilated coils, replacement of musculoelastc tissue in their walls by fibrinoid, and an interrupted endothelial lining. These pregnancy-induced physiologic changes produce a significant reduction in peripheral vascular resistance in the uteroplacental bed allowing substantial blood flow under low pressure through the intervillous spaces of the placenta. Prostaglandins are involved in blastocyst implantation (Kimball 1983; Snabes and Harper, 1984). The production in vitro of PGI2 by trophoblast obtained from early pregnancy shows a progressive increase throughout the first trimester (Rakoczi et al, 1983). Colonization of the spiral arteries by trophoblast is probably facilitated by PGI2. PGI2 production by the uteroplacental endovascular trophoblast may be important in preventing platelet aggregation and thrombosis in spiral arteries during endovascular trophoblast invasion in the first twenty weeks of pregnancy, but also in the second half of pregnancy. Endothelial production of PGI2 by proximal uterine and radial arteries may be of importance in preventing platelet aggregation, uteroplacental arterial thrombosis, and subsequent placental infarction (Wallenburg et al, 1973; Wallenburg 1981a, Wallenburg 1987; Rakoczi et al, 1983).

Trophoblast also contains an adenosine diphosphatase distinct from heat-stable alkaline phosphatase (O'Brien et al, 1987). Adenosine diphosphatase degrades adenosine diphosphate, a potent proaggregatory compound, to the inactive adenosine monophosphate and adenosine, an antiaggregatory and vasodilatory agent (Hutton et al, 1980; Colman and Walsh, 1985). The degradation of adenosine diphosphate, as a mechanism of limiting thrombosis in the uteroplacental vessels may, just like PGI2, be of physiologic importance to maintain smooth and unimpeded flow of maternal blood through the uteroplacental arteries and in the intervillous space. Further studies are required to elucidate the relative importance of these two mechanisms (Wallenburg 1987).

Why is prostacyclin synthesis increased in pregnancy?

During early pregnancy plasmatic prostacyclin stimulating activity is said to be similar to that in non-pregnant women, in late pregnancy the plasmatic activity is significantly depressed (Remuzzi et al, 1981). This finding makes it unlikely that plasmatic prostacyclin stimulating activity is primarily involved in the increase of PGI2 production during pregnancy.
It has been suggested that estrogens could be involved in the increase in PGI2 synthesis occurring in pregnancy. In pregnant rats Ali and Williams (1983) showed a progressive increase in uterine and aortic PGI2 production to a maximum in the final week of gestation; in the same period the blood levels of estrogens increased, whereas progesterone concentrations declined. Because estrogens are known stimulators of phospholipases and progesterone inhibits phospholipases, these findings suggest the possibility of a link between PGI2 formation and circulating levels of the various sex steroids, at least in this animal. The concentration of vasodilator prostaglandins in utero-ovarian venous plasma in the non-pregnant ewe is increased by the administration of 17-beta-estradiol (Meldrum et al, 1976). Also, estrogens and progesterone have been shown to increase uterine tissue prostaglandin levels (Clark and Brody, 1982). On the other hand Clark et al. (1980) suggested that 17-beta-estradiol could stimulate an increase in the level of another vasodilator substance, e.g. endothelium derived relaxing factor (Furchgott 1983) or vasoactive intestinal polypeptide (Ottesen et al, 1982; Ottesen 1983), or might interfere directly with a basic mechanism of vasoconstriction, e.g., by inhibition of calcium transport in vascular smooth muscle (Resnick et al, 1976; Naden et al, 1985).

In conclusion there is good evidence that prostaglandins play an important role in maintaining resting uteroplacental and maternal vasomotor tone, and in modulating resistance to flow in response to vasoconstrictor substances (Wallenburg 1981a; Clark 1982; Clark and Brody, 1982; Myatt 1987; Ferris 1988a; Ferris 1988b). The overall physiologic effect of prostaglandin synthesis in the uteroplacental vascular bed is vasodilatation. This vasodilator effect can be attributed mainly to PGI2 and perhaps to a lesser extent to PGE2 (Wallenburg 1981a; Broughton Pipkin 1985). Vasodilator prostaglandin synthesis is enhanced in pregnancy, but the mechanisms of this increased synthesis remain unknown.

**Kallikrein-Kinin System**

Kallikrein may promote vasodilatation as well as the increase of RAAS activity that occurs in normotensive pregnancy. During normotensive pregnancy urinary kallikrein excretion shows a consistent and significant rise (Elebute and Mills, 1976; Valdes et al, 1981). The most likely source of the high levels of urinary kallikrein is the maternal kidney, subjected to multiple humoral stimuli, especially of adrenal origin. It is not impossible that the fetus, the placenta, or the uterus contributes to the raised kallikrein levels (Campbell et al, 1987). Recently Campbell et al. (1987) demonstrated a striking difference between active and inactive urinary kallikrein levels in normal pregnancy. Active urinary kallikrein levels rise above non-pregnant levels in the first trimester, and decline thereafter. Inactive urinary kallikrein rises to a much greater extent and remains about ten times above the level seen in non-pregnant women.

Kinin-generating enzymes have been found in the uterus. Kinins are produced
locally in the uteroplacental circulation by a glandular kallikrein or by plasma 
prekallikrein that may be activated continuously within the uteroplacental 
circulation because of physiologic processes of local hemostasis with activation 
of Hageman factor (Seino et al, 1982; Derkx 1987). The vascular effects of 
the locally released kinins may in part be mediated through release of vasodilator 
prostaglandins (Nasjletti and Malik, 1981). The endothelium-derived relaxing 
factor (Furchgott 1983; Furchgott 1984) may be involved in mediating the non­ 
prostaglandin dependent part of the uteroplacental vasodilator response to kinins.

**Atrial Natriuretic Peptide**

ANP concentrations probably increase slightly during normotensive pregnancy 
(see paragraph 2.1.1). Because ANP antagonizes angiotensin-II (Genest and 
Cantin, 1987), ANP may be involved in the physiologic refractoriness to 
angiotensin-II during normotensive pregnancy. Niesert et al, (1987) showed a 
significant increase in plasma ANP levels in normotensive pregnant women after 
infusion of angiotensin-II.

**Calcitonin Gene Related Peptide**

Calcitonin gene related peptide (CGRP) is an extremely potent vasoactive 
peptide that causes profound vasodilatation. Its distribution in perivascular nerves, 
which seem to be a major source of the circulating peptide, suggests that one 
of its functions may be the regulation of peripheral vascular tone (Struthers 
sectional study throughout normotensive pregnancy, and at 5 to 7 days post 
partum. CGRP concentrations were significantly increased throughout pregnancy 
but fell sharply after delivery. It is unknown if the increased plasma concentration 
of CGRP during pregnancy is derived from the perivascular nerves. As CGRP 
is also found in the thyroidal C-cells, the increased circulating concentrations 
might be derived from hyperplasia or increased activity of these C-cells during 
pregnancy (Stevenson et al, 1986). Finally, CGRP is also found throughout the 
urogenital tract (Ghatei et al, 1985), and uterus and placenta or both may thus 
be a source of the increase in circulating levels (Stevenson et al, 1986; Samuelsson 
et al, 1985). The contribution, if any, of CGRP to the physiologic balance between 
vasoconstrictor and vasodilator influences during pregnancy remains to be 
determined.

2.1.4 Conclusion

Impressive physiologic changes take place in pregnancy in the maternal 
organism in general and in the cardiovascular system in particular, most likely 
induced by the interaction of the fetal (paternal) allograft with maternal tissue.
The development of mutual immunologic tolerance (Stirrat 1987) in the first trimester is thought to lead to important morphologic and biochemical changes in the systemic and uteroplacental maternal circulation. In recent years a number of these adaptational changes have been elucidated (fig. 2.1).

Increased plasma volume  
Trophoblast invasion in spiral arteries  
Increased cardiac output  
Reduced vascular sensitivity  
Reduced systemic resistance  
Reduced resistance to flow  

Fig. 2.1. Concept of physiologic adaptational circulatory changes in pregnancy.

Morphologic changes due to invasion of migratory trophoblast into the walls of the spiral arteries transform the uteroplacental arterial bed into a low resistance, low pressure, high flow system.

Biochemical adaptations in the maternal vasculature include changes in the prostaglandin system, leading to an increasing dominance of the vasodilator and platelet-aggregation inhibiting effects of PGI₂, produced by vascular walls, over the vasoconstrictor and platelet-aggregation promoting effects of platelet-derived TXA₂. Other adaptational changes involve the RAAS and the kallikrein-kinin system.

The physiologic inhibition of platelet aggregation in the uteroplacental vascular bed, as well as the vasodilatation and low vascular resistance to flow, and the vascular refractoriness to vasoconstrictors, such as angiotensin-II and norepinephrine, may depend on production of biologically balanced amounts of vasodilator prostaglandins and vasoconstrictor TXA₂ (fig. 2.2).
Fig. 2.2. In pregnancy, dominance of the effects of vascular prostacyclin over those of thromboxane-A$_2$ - mainly produced by platelets - leads to vasodilatation and inhibition of platelet aggregation.

The mechanisms underlying the increase in production of vasodilator prostaglandins, the interrelationships with adaptational changes in vasoconstrictor systems and the possible links to fetal-maternal immunologic interaction are still obscure.
2.2 Hypertensive pregnancy

Hypertensive disorders in pregnancy constitute a syndrome, i.e. a collection of signs and, to a lesser extent, symptoms which have been clinically observed to occur together and to form a characteristic pattern, but are not necessarily due to the same pathologic cause. The central signs by which the hypertensive syndrome in pregnancy is recognized are an elevated arterial blood pressure and a pregnant or early postpartum state. Other classic signs of the syndrome are edema, proteinuria and convulsions.

The terminology and definitions used to describe the hypertensive disorders of pregnancy have been – and still are – inconsistent and confusing (Chesley 1978). More than 60 names in English and over 40 in German have been applied to the condition (Rippman 1969) and terms like "toxemia", "toxicosis" and "gestosis" are still used in many countries. In different ways most definitions of the syndrome emphasize four features, occurring alone or in combination: hypertension, edema, proteinuria, and convulsions (Chesley 1978). Of these an elevation of blood pressure is essential to be able to define the disorder as hypertensive. However, the threshold between normotension and hypertension has been defined using various and largely arbitrarily chosen levels, and the same is true for the definitions of pathologic proteinuria. The diagnosis of convulsions will cause little difficulty, but the recognition and assessment of edema is very subjective.

Another and perhaps even more important source of confusion is the fact that the hypertensive syndrome in pregnancy comprises two etiologically completely different entities. One is pregnancy-induced hypertensive disease, a disorder induced by pregnancy, the cause of which lies within the gravid uterus. It is a disease mainly but not exclusively of the nullipara, it appears in the course of pregnancy and is reversed by delivery. The other condition is preexisting chronic hypertension and/or proteinuria, unrelated to but coinciding with pregnancy, which may be detected for the first time in pregnancy and will not regress after delivery. To complicate matters further, the two conditions may occur together in one patient.

This review of the cardiovascular pathophysiology of hypertensive pregnancy will focus on pregnancy-induced hypertensive disorders which, as a group will be designated PIH.

2.2.1 Terminology and defining signs

Hypertension

Hypertension is considered to be the clinical defining feature of PIH. Phase I of the Korotkoff sounds defines the systolic blood pressure, the diastolic blood pressure should be defined by Korotkoff phase IV (Rubin 1988). The known variability of blood pressure (Chesley 1978) means that any reading, however carefully and accurately taken, may deviate significantly from what is repre-
sentative of an individual. For that reason, some definitions require that a hypertensive reading is confirmed after a certain time interval, for example of 4 (Davey and MacGillivray, 1986), 6 (Hughes 1972) or 24 hours (Nelson 1955).

Hypertension is categorized by either an absolute threshold or by an increment from a baseline in the first half of pregnancy. The conventional dividing line between normotensive and hypertensive is 140/90 mmHg (Hughes 1972), but some use only the diastolic pressure limit of 90 mmHg or higher (Nelson 1955; Butler and Bonham, 1963); even a level as high as 110 mmHg has been advocated (Redman 1987). Lower thresholds have also been advocated, including 135/85 mmHg (Rippman 1968), 130/70 mmHg (Browne and Dodds, 1942) or even 125/75 mmHg (Chesley 1976), based on the finding that an increase in perinatal mortality can be discerned in women with higher blood pressure levels (Chesley 1976). In an attempt to distinguish between women with transient pregnancy-induced hypertension and those with permanent forms of hypertensive disease increments in blood pressure from an early baseline have been defined. Increments in diastolic pressure of 20 mmHg (Hughes 1972) to 25-30 mmHg (Redman 1987) have been defined as being abnormal and denoting PIH.

There are two fundamental problems in using hypertension as the defining sign of PIH. The first is that an arbitrary threshold is used to divide a continuously distributed variable – arterial pressure – into the two artefactual categories of normotension and hypertension (Pickering 1968; Redman 1987). The second problem is that a rise in peripheral vascular resistance is the more fundamental feature of the disorder. It is only because cardiac output is usually maintained that the change in peripheral resistance can be discerned by measuring the blood pressure. Although hypertension is an artificial concept which depends on a variable which is difficult to measure with some degree of accuracy and precision, there is as yet no alternative to define the circulatory disturbances of pregnancy.

Proteinuria

Renal protein excretion increases in normal pregnancy; the amount of protein in urine increases as pregnancy progresses from approximately 5 mg/100 ml in the first and second trimester to 15 mg/100 ml in late pregnancy (Lopez­Espinoza et al, 1986), and proteinuria is not considered abnormal until it exceeds 300 mg in 24 hours (McEwan 1968; McEwan 1987; Lindheimer et al, 1987). Like hypertension, proteinuria may be a temporary phenomenon due to pregnancy-induced renal lesions (McEwan 1987), or it may be an expression of preexisting renal disease coinciding with pregnancy (Lindheimer and Katz, 1983; Eden et al, 1983). In the first case it should disappear at some time after delivery, in the latter case it will remain present. Proteinuria is a late sign of pregnancy-induced hypertensive disease and, when present, the risk of perinatal death, as well as that of the occurrence of eclampsia, increases about two-fold (Friedman and Neff, 1976; Page and Christianson, 1976; MacGillivray 1983). For that reason the combination of pregnancy-induced hypertension and proteinuria is
usually termed preeclampsia (Davey and MacGillivray 1986). The close association between the magnitude of the proteinuria, the perinatal risks, and the severity of the renal lesions (MacGillivray 1983; Lindheimer et al., 1987) illustrates that it is the underlying circulatory disturbance that determines the signs of pregnancy-induced hypertensive disease, not the raised blood pressure as such.

Edema

Moderate edema can be detected in 60-80% of normotensive pregnancies, which is so common that it must be regarded as normal (Robertson 1971; Chesley 1978). Among women with no edema and among those with early or late onset edema the incidence of hypertension is similar (Redman 1987). The combination of excess weight gain or edema and hypertension does not produce an appreciable change in fetal outcome associated with hypertension alone (Friedman and Neff, 1976). Preeclampsia can occur without fluid retention; this so-called "dry" preeclampsia has long been recognized as a particularly dangerous variant of the disorder (Eden 1922; Vosburgh 1976). It is now generally accepted that the development of edema in pregnancy is very common and does not define a group of women at particular risk, as do hypertension and proteinuria. For that reason edema or an accelerated weight gain should not be used as defining signs of pregnancy-induced hypertensive disease.

In conclusion, as long as the cause or causes of pregnancy-induced hypertensive disease are not understood, a simple symptomatic definition is needed, based on the classic signs of hypertension and proteinuria, that can be used by clinicians and epidemiologists everywhere. Of the many definitions that have been proposed the recommendations of the International Society for the Study of Hypertension in Pregnancy fit in best with the general considerations presented above (Davey and MacGillivray, 1986). Table 2.1 gives a summary of these definitions. In clinical research or practice these definitions may be subdivided, and other diagnostic criteria may be added as long as the basic definitions can be recognized by others.

2.2.2 Volume of blood and water

Many investigators agree that pregnancies complicated by a pregnancy-induced hypertensive disorder, at least in its more severe forms, are associated with a reduction in plasma volume, roughly in proportion to the severity of the disease. Chesley (1972) reported that the average plasma volume in women with preeclampsia was 9% below expected values and was as much as 30% to 40% below normal in those with severe disease. It is not clear whether the reduction in maternal plasma volume is a cause or a result of the vasoconstriction. Some investigators have demonstrated that inadequate plasma volume expansion occurs
Table 2.1. Summary of the definitions and classification of hypertensive disorders in pregnancy, as recommended by the International Society for the Study of Hypertension in Pregnancy (Davey & MacGillivray, 1986).

<table>
<thead>
<tr>
<th>DEFINITIONS</th>
<th>CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypertension</strong></td>
<td>1. One indirect measurement of diastolic blood pressure (Korotkoff 4) of 110 mm Hg or more, or &lt;br&gt;2. Two consecutive indirect measurements of DBP of 90 mm Hg or more, 4 hours or more apart.</td>
</tr>
<tr>
<td><strong>Proteinuria (1)</strong></td>
<td>Hypertension or proteinuria developing after the 20th week of pregnancy in a previously normotensive nonproteinuric woman.</td>
</tr>
<tr>
<td>1. Total protein excretion of 300 mg or more per 24 hours. &lt;br&gt;2. Two random clean-catch or catheter urine samples collected 4 hours or more apart, with &lt;br&gt;a. ++ (1 g/L) or more on reagent strip if SG is more than 1030 or &lt;br&gt;b. + (0.3 g/L) or more if SG is less than 1030.</td>
<td></td>
</tr>
<tr>
<td><strong>Gestational hypertension or proteinuria (2)</strong></td>
<td>Hypertension in combination with proteinuria developing after the 20th week of pregnancy in a previously normotensive nonproteinuric woman.</td>
</tr>
<tr>
<td><strong>Gestational proteinuric hypertension (preeclampsia) (2)</strong></td>
<td>Hypertension and/or proteinuria in pregnancy in a woman with chronic hypertension or chronic renal disease diagnosed before or during, or persisting after pregnancy.</td>
</tr>
<tr>
<td><strong>Chronic hypertension or chronic renal disease (2)</strong></td>
<td>Proteinuria developing for the first time during pregnancy in a woman with chronic hypertension.</td>
</tr>
<tr>
<td><strong>Chronic hypertension with superimposed preeclampsia</strong></td>
<td>Hypertension and/or proteinuria found at first antenatal examination after the 20th week of pregnancy. May be classified after delivery.</td>
</tr>
<tr>
<td><strong>Unclassified hypertension and/or proteinuria</strong></td>
<td>1) Definition based on protein/creatinine index omitted. &lt;br&gt;2) Further subdivided according to development during pregnancy, labor, or within 48 hours after delivery.</td>
</tr>
</tbody>
</table>

1) Definition based on protein/creatinine index omitted.  
2) Further subdivided according to development during pregnancy, labor, or within 48 hours after delivery.
before any clinical signs of PIH are present (see paragraph 3.3.1). On the other hand Sibai et al. (1983) found no difference in mean plasma volumes between normotensive pregnant women and women with mild pregnancy-induced hypertension. Plasma volume in this study was reduced only in pregnancies with mild pregnancy-induced hypertension with delivery of small-for-gestational age infants. In this study no patient showed a reduction of plasma volume antedating the development of hypertension.

Even if a reduction in plasma volume antedates the development of hypertension (Gallery et al, 1979), that is no proof that the contraction of plasma volume causes the vasoconstriction. In normotensive pregnancy vasodilatation precedes any alteration in circulating blood volume (Phippard et al, 1984). Gant et al. (1973) showed that the increase in pressor response to angiotensin-II, that precedes the clinical development of PIH, antedates all other cardiovascular changes, including the decrease in circulating blood volume. These observations are consistent with the concept that the development of PIH begins with a loss of vascular refractoriness to vasoactive agents, followed by vasoconstriction (Assali 1977). This results in a decrease in intravascular volume, and intravascular water is shunted to extravascular spaces.

The decrease in plasma volume results in hemoconcentration. With increasing hemoconcentration the circulatory properties of the blood will change (Harkness 1981). In preeclampsia Buchan (1982) found a mean increase of 30% in whole blood viscosity. This increased viscosity is caused by increased hematocrit, reduced red cell deformability and increased plasma viscosity (Heilman et al, 1977; Heilman 1984). Hyperviscosity reduces the oxygenation of the tissues with a fall in the local pH, which in turn causes an increasing rigidity of the erythrocyte wall (Stratton 1985). The rigid red cell in PIH may fragment during capillary passage, thus causing the hemolysis that is a clinical sign of severe preeclampsia (Stratton 1985; Weinstein 1982; Weinstein 1985). The reduced flow qualities of the blood during severe PIH may also be of pathophysiologic importance in causing, in concert with a disturbance in platelet-vessel wall interaction, thrombosis in the uteroplacental arteries (Sagen et al, 1982; Thornburn et al, 1982). The frequency of large placental infarcts, leading to fetal growth retardation and perinatal death, is positively correlated with the hematocrit of the mother (Koller 1982; Sagen et al, 1984), and in preeclampsia maternal hemoglobin concentration is inversely correlated with the weight percentile of the newborn (Sagen et al, 1984).

In preeclamptic gravidae GFR and effective renal plasma flow are, on the average, 30% and 20% lower, respectively, than in normotensive pregnant women, and usually fall in ranges that would be normal in non-pregnant women (Lindheimer et al, 1987). Preeclamptic women have lower urinary sodium concentration and/or excretory rates, and they also excrete a smaller percentage of an infused sodium load as compared with normotensive pregnant women (Brown et al, 1988). A "salt tolerance test" was once utilized as a means of
distinguishing PIH from other hypertensive disorders during pregnancy (Chesley 1972). However, the degree of impairment in the ability to excrete sodium varies considerably (Lindheimer et al, 1987).

In contrast to what might be expected plasma ANP levels in PIH are raised, the highest values are found in severe preeclampsia (Hirai et al, 1986; Fournier et al, 1986; Visser et al, 1987; Thomsen et al, 1987). ANP levels are also elevated in patients with essential hypertension who usually have an increased central venous pressure and pulmonary capillary wedge pressure (Tarazi 1983; Safar et al, 1983). This may explain the increased production of ANP in this condition. In contrast, preeclamptic patients have a low central venous pressure and a low pulmonary capillary wedge pressure (Groenendijk et al, 1984; Wallenburg 1988). Hence, it remains unexplained at this moment why preeclampsia is associated with increased plasma ANP concentrations.

2.2.3 Hemodynamics

*Systemic blood pressure*

By definition arterial blood pressure is elevated in hypertensive pregnant women, whether they have pregnancy-induced hypertensive disease or chronic hypertension of whatever cause. Also blood pressure behavior appears to be altered in hypertensive pregnant women. Women with PIH may have a reversal of the normal diurnal blood-pressure rhythm, so that the highest levels occur during the night (Chesley 1978). Hypertension in PIH is characteristically labile, probably reflecting the intense sensitivity of the vasculature to the endogenous vasoconstrictor substances, angiotensin-II, TXA2 and catecholamines (Lindheimer and Katz, 1985). In women with PIH blood pressure usually returns rapidly to normal following parturition, but in 10-20% of cases hypertension may continue or even increase during the puerperium. This may be followed by a slow fall, with blood pressure returning to normal only after weeks of observation (Lindheimer and Katz, 1983; MacGillivray 1983).

*Cardiac output*

The literature, dealing with cardiac output measurements in PIH, was recently reviewed by Wallenburg (1988). Most reports in the literature concern uncontrolled studies and describe only a few patients studied during childbirth, lying on their back, and/or receiving parenteral fluids, anti-hypertensive treatment or magnesium sulphate. Important exceptions are the studies by Groenendijk et al. (1984) and Wallenburg (1988). In these studies, performed in untreated preeclamptic patients who had received no parenteral fluids, and were not in labor, cardiac index was found to be markedly lower than in normotensive pregnancy (mean 4.5 L/min/m²); in patients with severe preeclampsia cardiac index was reduced to values (mean 2.75 L/min/m²) that are normal for non-pregnant women (Wallenburg 1988).
Peripheral vascular resistance

Since systemic arterial blood pressure is elevated in PIH and cardiac output is decreased in the untreated patient, the calculated peripheral vascular resistance must be elevated (Wallenburg 1988). The calculated increase in peripheral vascular resistance may be expected because vasoconstriction is fundamental to the disease process of PIH (Ferris 1988a; Wallenburg 1988). This was already noted by Volhard (1918), based upon his direct observation of small blood vessels in the nail beds, ocular fundi, and bulbar conjunctivae in preeclamptic women. The vasoconstriction imposes a resistance to blood flow and accounts for the development of arterial hypertension.

There is evidence that the vasoconstriction that occurs in pregnant women who develop PIH results from a breakdown of the normally benign interaction between vasoconstrictor and vasodilator systems.

2.2.4 Vasoconstrictor and vasodilator systems

This paragraph presents a brief overview of the changes that have been reported to occur in the vasoconstrictor and vasodilator systems of pregnant women with a pregnancy-induced hypertensive disorder. Of the vasoconstrictor systems most emphasis will be placed on the renin-angiotensin-aldosterone system; of the vasodilator systems we will focus on the eicosanoid system.

2.2.4.1 Vasoconstrictor systems

Renin-angiotensin-aldosterone system

The majority of studies on plasma renin activity (PRA) and plasma renin concentration (PRC) found lower levels in patients with PIH in comparison with normotensive pregnancy (Chesley 1978; Pedersen et al, 1982; Broughton Pipkin 1988); levels of active renin may be even lower than those of non-pregnant women (Derksen et al, 1986).

The levels of angiotensin-II in plasma of preeclamptic women have been reported as severely depressed, normal and considerably elevated in comparison with those in normal pregnancy (Chesley 1978; Worley 1984). However, more recent studies show a similar pattern to that of PRC and PRA, with suppression of plasma angiotensin-II levels in severe preeclampsia (Pedersen et al, 1984; Broughton Pipkin 1988).

The plasma aldosterone concentration is also suppressed in PIH, although not to the extent which might be expected from the suppressed PRA (Pedersen et al, 1984; Broughton Pipkin 1988).

Proposed causes of the decreased activity of the RAAS in pregnancy-induced hypertensive disorders include:

1. A loss of vascular refractoriness to angiotensin-II and hence an increased
effectiveness of angiotensin-II negative feedback on the juxtaglomerular apparatus (Davis and Freeman, 1976; Peach 1977).


As discussed in paragraph 2.1.3.1 prostacyclin could be the main determinant of the stimulation of the RAAS that is physiologic in pregnancy. For that reason a deficient production of prostacyclin may be the central mechanism in the reduction in RAAS activity in women with pregnancy-induced hypertensive disorders (Friedman 1988).

The adrenergic system

Different results and opinions about the role of the sympatho-adrenal system in PIH have been published. Differences in methodology and varying criteria for patient selection might explain some of the contradictions (Nisell and Lunell, 1984). Venous plasma norepinephrine concentrations have been observed to be reduced (Tunbridge and Donnai, 1981; Natrajan et al, 1982), normal (Pedersen et al, 1982) or elevated (Davey and MacNab, 1981; Coevoet et al, 1982). Results from venous plasma epinephrine measurements have likewise shown reduced (Natrajan et al, 1982), unaltered (Pedersen et al, 1982) or elevated levels (Coevoet et al, 1982; Abboud et al, 1982). In urine, increased excretion of both norepinephrine and epinephrine in PIH has been observed (Zuspan and Kawada, 1976; Zuspan et al, 1981). Arterial levels of norepinephrine have been observed to be similar (Nisell et al, 1987) or elevated (Oian et al, 1986) in PIH as compared to the levels found in normotensive pregnancy. Results from arterial plasma epinephrine measurements have shown elevated levels (Oian et al, 1986; Nisell et al, 1987).

Nisell (1987) concluded that the normal adreno-medullary suppression during pregnancy is absent in PIH, but that there are no indications of an altered sympathetic nerve activity in the body as a whole in PIH. This, however, does not exclude the possibility of a neurogenic contribution to blood pressure elevation in PIH since sympathetic nerve activity occurs in a differentiated fashion (Folkow 1982; Kuchel 1983; Zanchetti et al, 1985).

Several vascular and sympatho-adrenal responses in preeclamptic patients are similar to those observed in the non-pregnant state, implying an inadequate adaptation of the autonomic nervous system in PIH (Nisell et al, 1987; Pedersen 1988).

The reduction in vasodilator prostaglandin production said to be present in PIH could be one of the mechanisms involved in the increased production of
catecholamines and the increase in pressor responses to their exogenous administration (Broughton Pipkin 1985; Oian et al, 1986; Pedersen 1988).

**Natriuretic hormone**

Levels of digitalis-like substance (DLS) in amniotic fluid are said to be elevated in PIH as compared with normotensive pregnancy (Graves 1984). Beyers et al. (1984) found higher levels of DLS in cord blood of infants born to preeclamptic mothers than in cord blood of infants born to normotensive women. Recently Graves et al. (1986; 1987) and Gusdon et al. (1984) found higher levels of DLS in serum of preeclamptic women. In addition Graves (1987) found a significant correlation between DLS levels and diastolic blood pressure. Postpartum the increased levels of DLS in preeclamptic women were found to normalize rapidly. Endogenous DLS could contribute to the increased vascular tone and vascular sensitivity to angiotensin-II in PIH (de Wardener 1985). However, these findings require further study, in particular concerning the exact molecular structure of this still hypothetical circulating vasoconstrictor. DLS may represent a previously unrecognized vasoactive compound, but identification is needed to resolve its exact pathophysiologic role in the development of PIH.

**The vascular responsiveness to vasoconstrictor substances**

A considerable body of evidence confirms the view, that women with PIH have a markedly greater pressor response to several vasoconstrictor hormones than normotensive pregnant women.

As first shown by Chesley (1966) and later confirmed in another study by the same group (Talledo et al, 1968), preeclamptic women have lost the physiologic vascular refractoriness to angiotensin-II. The work of Gant et al. (1973) demonstrated that pressor responsiveness to angiotensin-II can already be significantly increased by 22 weeks gestation in women who are destined to develop PIH, well before the onset of hypertension and other abnormalities such as a reduction in plasma volume. It is this observation, probably more than any other, that led to the concept of PIH as a chronic disease, albeit one confined by definition to pregnancy.

In view of the considerations presented in paragraph 2.1.3.1 the progressive loss of angiotensin-II refractoriness that develops even before PIH is clinically manifest may be a consequence of a vasodilator prostaglandin deficiency (Myatt 1987).

In comparison to the blunted pressor response in normotensive pregnant women, norepinephrine infusion in hypertensive pregnant women causes an increased pressor response comparable to that in normotensive non-pregnant women (Raab et al, 1956; Zuspan et al, 1981; Paller 1984; Nisell et al, 1987); the pressor response during infusion of norepinephrine in PIH is mainly caused by vaso-
constriction and essentially similar to the degree of vasoconstriction and pressor response in the non-pregnant state. Again, an insufficient action of vasodilator prostaglandins may be the main mechanism involved in the increase in vascular responsiveness to catecholamines in PIH.

Already 40 years ago it was found that the vascular reactivity to the pressor effects of vasopressin is enhanced in preeclamptic women in comparison to normotensive pregnant women (Dieckman and Michel, 1937; Schockaert and Lambillon, 1937). The increased pressor response to vasopressin in PIH may also be due to a deficiency in vasodilator prostaglandins (Paller 1984; Ballermann et al, 1986; Robertson and Berl, 1986; Pedersen 1988).

Tulenko et al (1987) recently developed an in vitro model to identify the serum factors responsible for the changes in vascular sensitivity to vasoconstrictor substances that occur in PIH. Sensitivity of the isolated segments of the rabbit carotid artery to vasoconstrictor substances and endothelium-mediated relaxation was studied. Tulenko and his coworkers found that arteries exposed to serum from preeclamptic women developed a 2.9-fold increase in sensitivity to angiotensin-II, and a 1.6-fold increase in sensitivity to norepinephrine. Endothelium-mediated relaxation was not affected by serum from preeclamptic women. This study supports the concept that a mechanism proximal to the intracellular vasoconstrictor pathways is altered in PIH; a likely site would be the smooth muscle cell membrane and its angiotensin-II and norepinephrine receptor excitation-coupling mechanisms (Bohr and Webb, 1986; Tulenko et al, 1987).

2.2.4.2 Vasodilator systems

The eicosanoid system

Changes in prostaglandin production and/or catabolism in uteroplacental and fetal tissues have been reported to be associated with PIH, although the reports are often conflicting (Myatt 1987). There have been reports of decreased PGE2 metabolism (Alam et al, 1973; Moutquin and Leblanc, 1982), decreased PGE but increased PGF concentrations (Demers and Gabbe, 1976), decreased placental PGF2alpha but normal PGE2 catabolism (Valenzuela and Bodhke, 1980), no change in placental concentrations of PGE2 and PGF2alpha (Hillier and Smith, 1981) or decreased TX and PGF2alpha metabolites in tissues from preeclamptic women (Robinson et al, 1979). These discrepancies may reflect some of the known problems inherent in the assessment of prostaglandin action (Wallenburg 1981a; Myatt 1987). Of late, evidence has accrued for PIH being a state of relative PGI2 deficiency and TXA2 dominance (Ylikorkala and Makila, 1985).

Prostacyclin and Thromboxane: Several groups have supplied evidence for a deficiency of PGI2 production at the tissue level by measuring reduced plasma levels (Lewis et al, 1981; Ylikorkala et al, 1981b; Koullapis et al, 1982) and urinary PGI2 metabolites (Goodman et al, 1982; Fitzgerald et al, 1987).
Moodley et al. (1984) found significantly lower levels of 6-keto-PGF1alpha and PGE2 in central venous blood in 21 primigravid women with eclampsia.

The normal gestational increment in urinary excretion of PGI2 metabolites is diminished in PIH; Fitzgerald and his group (Goodman et al., 1982; Brash et al., 1983 Fitzgerald et al., 1987) found a 5-10 fold increase in urinary metabolites of PGI2 during normotensive pregnancy, by contrast in patients who subsequently developed PIH the increase in urinary metabolites of PGI2 was only 2-3 fold. In PIH reduction in the urinary excretion of 2,3-dinor-6-keto-PGF1alpha precedes the development of clinical disease and is detectable already in the first trimester (Fitzgerald et al., 1987).

As PGI2 is a local rather than a circulating hormone and all tissues studied in PIH show reduced PGI2 production, PIH may be considered a state of generalized PGI2 deficiency, a deficiency already occurring at an early stage in the development of PIH (Pedersen et al., 1983; Fitzgerald et al., 1987).

In addition to a PGI2 deficiency, a relatively or absolutely increased TXA2 generation could cause the hemodynamic changes and changes in platelet behavior occurring in PIH (Wallenburg, 1987). Maternal plasma levels of TXB2 in PIH were increased in one study (Koullapis et al., 1982), but normal in another (Mitchell et al., 1978). The production of malondialdehyde by platelets, a stable by-product of platelet TXA2 synthesis, is enhanced in hypertensive pregnancies complicated by fetal growth retardation (Wallenburg and Rotmans, 1982). Also the production of TXA2 in the maternal placental circulation may be increased as shown by Martensson and Wallenburg (1984), who found TXB2 levels in uterine venous blood in preeclamptic patients to be almost twice those in normotensive pregnant women.

Placental production of PGI2 is significantly decreased in PIH (Walsh et al., 1985). Remuzzi et al. (1980) found umbilical PGI2 production more impaired than that of the maternal vasculature in PIH. Bjorø et al. (1986) showed that human umbilical arteries collected from preeclamptic women produce significantly less PGI2 after perfusion with angiotensin-II than umbilical arteries derived from normotensive pregnant women.

Walsh (1985) found the production of TXA2 by placentas from preeclamptic patients to be three times as high as TXA2 production in placentas from normotensive women; this author suggests that the increased placental production of TXA2 is not only secreted into the fetal circulation, but also into the maternal circulation. This suggestion seems to be supported by the study of Martensson and Wallenburg (1984), that was already mentioned, in which higher levels of TXB2 were found in uterine venous blood from preeclamptic patients than in that from normotensive pregnant women. Because prostaglandins and thromboxanes do not readily cross the placenta a more logical explanation for this finding is an increase in maternal placental TXA2 production, caused by an increased uteroplacental platelet activation and consumption (Wallenburg 1987).

A physiologic balance between PGI2 and TXA2 and other vasoconstrictor substances rather than either agent alone may be of major importance in
maintaining a vasodilated state in pregnancy. The impairment in uteroplacental blood flow that is known to occur in PIH (Lunell et al, 1982) may be caused by a relative dominance of vasoconstrictor TXA2 leading to vasoconstriction. Martensson and Wallenburg (1984) studied the ratio between the two eicosanoids in uterine venous blood in preeclamptic patients and found increased TXA2 and decreased PGI2 levels in uterine venous blood with a TXB2/6-keto-PGF1alpha ratio of 5.6 as compared to a ratio of 2.0 in normotensive pregnancy. In this study umbilical cord values showed a TXB2/6-keto-PGF1alpha ratio of 6.9 in PIH and 2.0 in normotensive pregnancy.

The decrease in uteroplacental blood flow in PIH may not only be caused by uteroplacental vasoconstriction but also by more permanent changes in the uteroplacental arteries. The elevated uteroplacental levels of PGI2 in normotensive pregnancy may be of importance for an unimpaired development of the trophoblast-induced physiologic changes in the spiral arteries (see paragraph 2.1.3.2). In women with PIH the physiologic changes in the uteroplacental arteries are confined to the decidual portions of the arteries, the myometrial segments remain anatomically intact and do not dilate; adrenergic nerve supply remains intact (Brosens 1972; Brosens 1977; Gerretsen 1979; Jones and Fox, 1980; Robertson and Khong, 1987). This observation implies failure or inhibition of the second wave of endovascular trophoblast migration into the myometrial segments of the uteroplacental arteries. This may have the effect of curtailing the increased blood supply required by the fetoplacental unit in the later stages of pregnancy. The imbalance between PGI2 and TXA2 that may exist in PIH may be of importance in causing this impairment of normal development of uteroplacental arteries. Also secondary lesions involving the spiral arteries in PIH, such as acute atherosis and thrombosis (Sexton et al, 1950; Robertson and Khong, 1987), may be caused by an imbalance between PGI2 and TXA2. On the virtually non-endothelialized surface of the spiral arteries in the absence of an adequate production of anti-aggregatory PGI2 by the uteroplacental vasculature and/or endovascular trophoblast, surface mediated platelet activation may be expected to occur (Wallenburg 1981a; Walsh 1985). Platelets will adhere and release their alpha- and dense granule constituents. TXA2 will be generated and more circulating platelets will be recruited. Coagulation will be triggered and thrombin will be locally generated, contributing to platelet aggregation and inducing the formation of fibrin to stabilize the platelet thrombus that may occlude maternal blood flow to a placental cotyledon, thus leading to placental infarction (Wallenburg 1973). Thrombin will be inactivated by ATIII, in this reaction ATIII is consumed. This concept of surface-mediated platelet activation and consumption in the uteroplacental vasculature fully explains the platelet and coagulation changes observed in PIH (fig. 2.3) (Wallenburg 1987).

Because the platelet is the principal source of circulating serotonin, the increased platelet aggregation in PIH (Wallenburg 1987) may be the cause of the higher levels of serotonin reported in blood and placentas of women with hypertensive as compared to women with normotensive pregnancies (Weiner 1985). The
increased level of free circulating, platelet-derived, serotonin facilitates further platelet aggregation, but may also amplify the vasoconstrictor action of certain neurohumoral mediators, in particular catecholamines and angiotensin-II, and may cause direct contraction of vascular smooth muscle itself (Van Nueten et al., 1985; Vanhoutte and Lusscher, 1986; Weiner 1987).

Defective PGI2 generation in the uteroplacental vascular bed and elsewhere in the maternal vasculature could also explain the micro-angiopathy that is associated with PIH (Weinstein 1982; Wallenburg 1987). Such a micro-angiopathy is also observed in other syndromes in which a defective PGI2 generation is said to exist, including the hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and the lupus anticoagulant syndrome (Harlan and Harker, 1981; Harlan 1983; Speroff 1983; Myatt 1987). The pathologic pictures of TTP, HUS and PIH are remarkable similar, and a strong argument can be made for a common pathogenesis that results in disease localized in the renal circulation in HUS or manifest in multiple organs in TTP (Eisenstaedt et al., 1987) and PIH (Weinstein 1982; Aarnoudse et al., 1986).

In PIH platelet count may show marked day-to-day variations, but thrombocytopenia with counts of less than $100 \times 10^9/L$ is relatively rare. This may be interpreted as a compensated thrombocytolytic state, a condition that has been demonstrated in TTP and HUS, disorders with an increased peripheral
platelet consumption due to non-immune, surface-mediated destruction in non-pregnant patients (Harlan 1983; Wallenburg 1987). The lupus anticoagulant, which is associated with habitual abortion, fetal death, and a tendency to early severe preeclampsia, is known to be an inhibitor of PGI2 synthesis (Carreras et al, 1981).

In pregnancy-induced hypertensive disease reduced vascular PGI2 synthesis may be linked, via a decrease in platelet cAMP and a redirection of prostaglandin endoperoxides (Wallenburg and Rotmans, 1982), to increased platelet TXA2 production and the increased peripheral plasma levels of its stable hydrolysis product TXB2 found in PIH (Koullapis et al, 1982; Wallenburg 1987). The production of MDA, a stable by-product of platelet TXA2 synthesis, is enhanced in PIH complicated by fetal growth retardation (Wallenburg and Rotmans, 1982).

Platelets from normotensive pregnant women are less sensitive to the inhibitory effect on platelet aggregation induced by PGI2 than platelets from non-pregnant women (Shepherd et al, 1983); in patients with fetal growth retardation and PIH, platelet sensitivity to PGI2 is still further decreased (Briel and Lippert, 1983; Baker et al, 1987), probably caused by a post-receptor defect, because platelet PGI2 receptors have the same affinity and binding capacity in normotensive and hypertensive pregnancies (Shepherd et al, 1983).

There is ongoing debate whether the kidney is the culprit or victim in many of the hypertensive disorders in non-pregnant individuals (Peart 1983; Zanchetti 1985; Cowley and Roman, 1986). The same holds true for the increase in vascular tone and hypertension seen in PIH. The primary pathology in PIH is in the spiral arteries. However, it is impossible to induce sustained hypertension without some change in renal function (Guyton and Coleman, 1967; Guyton et al, 1983; Guyton 1987). Although the changes in kidney function and morphology in PIH are part of the so-called secondary pathology of PIH (Redman 1987), these changes are essential in the pathogenesis of the rise in blood pressure.

In PIH the intrarenal production of PGE2 and PGI2 is decreased (Pedersen et al, 1982; Pedersen et al, 1984; Moutquin and Leblanc, 1982; Ferris 1982). Renal failure to produce vasodilator prostaglandins in this condition may be the cause of the decrease in effective renal plasma flow, GFR, urate clearance and the development of proteinuria (Grantham and Chonko, 1986; McFadyen et al, 1986; Lindheimer et al, 1987; Ferris 1988b).

The deficiency in intrarenal vasodilator prostaglandins may result in unopposed intrarenal vascular effects of angiotensin-II, in this way causing an impaired ability to excrete sodium. The impaired ability to excrete sodium causes a shift to the right in the renal-pressure-natriurese curve and in this way an increase in vascular tone and blood pressure (Guyton and Coleman, 1967; Guyton et al, 1983; Guyton 1987).

Why is prostacyclin synthesis decreased in pregnancy-induced hypertensive disease?
Steroids may affect prostaglandin production in reproductive tissues (Ramwell et al, 1977). Placental estradiol production is similar in placentas from pre-eclamptic or normotensive pregnant women (Walsh 1988). Progesterone production is higher in placentas from preeclamptic women than in placentas from normotensive pregnant women (Walsh 1988). Elevated progesterone concentrations in the placentas of preeclamptic women might be one of the factors that suppresses placental PGI2 production because progesterone is known to inhibit placental PGI2 production (Myatt et al, 1983; Jogee et al, 1983; Walsh and Parisi, 1986). The suppressive effect of progesterone on PGI2 production in PIH should be considered an autocrine or paracrine action exerted locally within the placenta, because maternal circulating levels and urinary metabolites of progesterone do not differ between normotensive and preeclamptic pregnancies (Lindheimer and Katz, 1981; Dennis et al, 1982; Walsh 1988). The stimulus for increased progesterone production by the placenta in PIH is not known, but it may relate to the elevated uterine content of norepinephrine in PIH (O'Shaugnessy et al, 1983). Norepinephrine binds to beta-adrenergic receptors in the human placenta (Whitsett et al, 1980), and stimulation of these receptors increases placental progesterone production by 36-49% (Caritis et al, 1983). This is consistent with the percentage increase for placental progesterone production reported to occur in PIH (Walsh 1988). However, because vasodilator prostaglandins decrease uterine adrenergic activity, the primary change in the development of PIH remains unresolved (Thorbert 1979; Zuspan 1984; Broughton Pipkin 1985).

Remuzzi et al (1981) found that in PIH concentrations of a maternal plasma prostacyclin synthesis stimulating factor were increased above values found in normotensive pregnancy; it therefore seems likely that the increase in plasma prostacyclin synthesis stimulating factor is a compensatory and not a causative feature.

Evidence for a dietary cause of the insufficient production of PGI2 in PIH is meagre. Less arachidonic acid has been found in the maternal and fetal circulations of a small group of patients with fetal growth retardation, often associated with severe PIH, than in normotensive pregnant women (Crawford et al, 1982; Crawford 1983). Ogburn et al (1984) found reductions in placental and fetal arachidonic acid and a concomitant increase in maternal concentrations (free and esterified) in PIH as compared with normotensive pregnancy; these authors suggested that in PIH a net shift could occur in arachidonic acid from the non-esterified and the companion triglyceride components in the fetus to the phospholipids and cholesterol esters in the maternal circulation. They formulated a hypothesis that, because the fetal circulation favors the production of PGI2, decreased availability or arachidonic acid for the fetus may result in a decrease in the total PGI2 produced in the feto-placental unit in PIH. Further suggestive evidence for an essential fatty acid deficiency in women with PIH are the increased ratios of 5,8,11-eicosatrienoic acid (Mead’s acid) to arachidonic acid found in umbilical cords. Prostacyclin production by human umbilical arteries
has been shown to correlate inversely with the free Mead's acid/arachidonic acid ratio, an indicator of essential fatty acid deficiency (Orchard et al., 1983). Thus it has been suggested that an essential fatty acid deficiency may contribute to the generalized PGI2 deficiency of PIH (Ongari et al., 1984; Myatt 1987).

However, Mead's acid is a substrate for lipoxygenase but not for cyclooxygenase enzymes. Lipoxygenase products include hydroxy fatty acids and leukotrienes, which may have physiologic effects on vascular smooth muscle and endothelial cell membrane permeability and may be important in causing edema. Recently it was shown that several lipoxygenase products inhibit renin release (Antonipillai et al., 1987). Saeed and Mitchell (1983) found 12-lipoxygenase activity to be mainly present in intra-uterine tissues, whereas in myometrium and uterine cervix both 5- and 12-lipoxygenases were present. Levels of maternal lipid peroxides, including 12-HPETE and 15-HPETE, are increased in PIH (Maseki et al., 1981; Ogburn et al., 1984; Fenner and Walsh, 1985). Since 12-HPETE and 15-HPETE, the labile precursors of 12-HETE and 15-HETE respectively, are potent inhibitors of PGI2 synthesis (Samuelsson 1982; Moncada et al., 1985), the presence of lipoxygenases in uterine and intra-uterine tissues may act to modulate PGI2 production in vascular tissues and may be involved in the inhibition of PGI2 production, the decreased plasma renin concentration, and the increased endothelial cell membrane permeability that occurs in women with PIH (Oian et al., 1986; Oian and Maltan, 1987; Friedman 1988).

Free radical activity increases during normal pregnancy. This may be due to increased cell turn-over or due to decreased antioxidant free-radical scavenging mechanisms (Wickens et al., 1981). Plasma concentrations of free-radical oxidation products are said to be higher in PIH. Wickens et al. (1981) found a correlation between rising blood pressure and increased free-radical activity. Because of the effects of free-radical oxidation products on vascular PGI2 synthesis, platelet aggregation and clotting (Barrowcliffe et al., 1975; Donati et al., 1980; Dormandy 1980; Remuzzi et al., 1980) it is possible that in the pathogenesis of PIH, free radical activity contributes to the prostacyclin deficiency. Erskine et al. (1985) found a significant higher ratio of 18:2(9,11) to 18:2(9,12) linoleic acid at 28 weeks' gestation in 6 women who subsequently developed PIH, compared with the ratio in normotensive pregnant women. Erskine and colleagues postulated that these findings reflect increased free-radical activity occurring before the onset of symptoms and signs of PIH.

**Kallikrein-Kinin system**

In normotensive pregnancy maximum levels of active urinary kallikrein are found around the end of the first trimester, with progressively lower levels later on in pregnancy (Campbell et al., 1987). The same pattern was found in studies of women who later in pregnancy developed hypertension, but the active urinary kallikrein levels in these hypertensive pregnant women were significantly lower as compared with those in a normotensive group (Valdes et al., 1981; Nukala
et al, 1984; Kovatz et al, 1985; Campbell et al, 1987). Inactive urinary kallikrein levels are lower in preeclamptic women as compared to normotensive pregnant women; this difference is already present in the first trimester, long before hypertension develops (Campbell et al, 1987). Amniotic fluid from PIH patients has significantly lower kallikrein levels than that from normotensive pregnant women (Bodzenta et al, 1981).

A direct link between kallikrein and the prostaglandins is formed by bradykinin, the peptide which stimulates blood vessels to synthesize vasodilator prostaglandins, as has been demonstrated to occur e.g. in the kidney (Smith and Dunn, 1981), and in umbilical cord vessels (Hong 1980). Bradykinin reduces vasoconstrictor responses to norepinephrine, sympathetic nerve stimulation, and angiotensin-II by an action that is prostaglandin-dependent and abolished by indomethacin (Margolius 1983). Thus a decrease in the activity of the kallikrein-kinin system in PIH may contribute to prostacyclin deficiency and vasoconstriction.

Atrial Natriuretic Peptide

ANP levels in PIH are elevated (see paragraph 2.2.2); the highest values are found in severe PIH. The cause of the increase in plasma ANP levels in PIH has yet to be clarified, but the phenomenon may contribute to some of the abnormalities occurring in PIH, such as reduced plasma volume, increased capillary permeability and suppression of the RAAS (Visser et al, 1988).

Calcitonin Gene Related Peptide

No data about Calcitonin Gene Related Peptide (CGRP) in PIH are available. Taufield et al (1987) recently demonstrated the occurrence of hypocalciuria in PIH. Theoretically, if CGRP concentrations in PIH were lower, and this was associated with lower plasma concentrations of calcitonin, these lower levels of calcitonin could be a cause of the hypocalciuria in PIH, because calcitonin inhibits tubular reabsorption of calcium (Sutton and Dirks, 1986). In PIH hypocalciuria is probably caused by increased distal tubular reabsorption of calcium (Taufield et al, 1987).

2.2.5 Conclusion

Some healthy and usually nulliparous women apparently fail to exhibit or maintain proper adaptational responses to the presence of fetal trophoblast. Trophoblast invasion into the walls of the uteroplacental arteries is incomplete or even absent; the spiral arteries are left with a non-pregnant architecture and fail to dilate (Robertson and Khong, 1987). Prostacyclin dominance fails to develop, leading to an increase in TXA2 action. Abnormal adaptational changes have also been demonstrated in various other systems, e.g. the RAAS.
Recently Wallenburg (1988) developed the concept of circulatory "maladaptation disease" ("MAD"-disease) of pregnancy of which PIH may be regarded as one of the clinical expressions. Fetal growth retardation, abruptio placentae and, perhaps, also premature labor could be other clinical signs of a MAD-disease, occurring alone or in combination with PIH (fig. 2.4).

**FUNDAMENTAL PROBLEM**

<table>
<thead>
<tr>
<th>MALADAPTATION</th>
</tr>
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<tr>
<td>Abnormal trophoblast</td>
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<tr>
<td>Abnormal prostacyclin</td>
</tr>
</tbody>
</table>

**PATHOPHYSIOLOGIC RESPONSE**

- Reduced plasma volume
- Elevated vascular sensitivity
- Elevated systemic resistance
- Reduced systemic perfusion

- Reduced uteroplacental circulatory adaptation
- Reduced placental perfusion
- Elevated platelet activation
- Spiral arterial thrombosis
- Placental infarction

**CLINICAL EXPRESSION**

| HYPERTENSIVE DISORDERS |

FETAL GROWTH RETARDATION
ABRUPTIO PLACENTAE
LABOR

Fig. 2.4. Concept of circulatory maladaptation in pregnancy, leading to "MAD-disease", of which PIH is one of the clinical expressions.

The causative factor or factors of a failure to respond adequately to fetal-maternal immunologic interaction remain unknown, although a lack of, or a defective, gene product has been postulated (Cooper 1980).

The data presented in the foregoing paragraphs suggest that the pathogenesis of pregnancy-induced hypertensive disorders could be largely a consequence of a deficiency in vasodilator prostaglandins, especially prostacyclin, resulting from a deficiency of precursors, reduced synthetic capacity or defective action. The balance between vasodilator prostaglandins and vasoconstrictors such as TXA2 and angiotensin-II, especially in the uteroplacental circulation and the kidney, may be pivotal in the development of pregnancy-induced hypertensive disease. The absence of the normal stimulation of the renin-angiotensin system, despite significant hypovolemia, and the increased vascular sensitivity to angiotensin-II and norepinephrine can be explained by a single mechanism: a deficiency in production and/or activity of vasodilator prostaglandins, in particular prostacyclin. The increased thromboxane-A2 to prostacyclin ratio, observed by various investigators in many maternal and fetal tissues, may be the cause of selective platelet destruction, sometimes accompanied by micro-angiopathic hemolysis, and the reduced uteroplacental blood flow with arterial thrombosis and placental infarction (fig. 2.5).

It should be noted that although the concept of a prostacyclin-thromboxane imbalance allows an explanation for many of the clinical manifestations of pregnancy-induced hypertensive disorders and provides a framework for further
investigations, the hypothesis remains unproven. In addition this hypothesis explains the pathogenesis, not the etiology of PIH. The question as to why some women, in particular primigravidae, would fail to respond to the pregnant state with an adequate immune response, and the proper adaptational reactions including an increase in vascular prostacyclin production, and thus develop a pregnancy-induced hypertensive disorder, or another expression of MAD-disease, remains as yet unanswered.
The signs of pregnancy-induced hypertensive disease become apparent at a relatively late stage in pregnancy, usually in the course of the late-second or third trimester; maternal symptoms may occur even later or not at all. However, the fundamental problem of a misalliance of fetal trophoblast with maternal tissue that is thought to be the underlying cause of the disturbance of the physiologic mechanisms of the maternal circulatory adaptation appears to occur much earlier in pregnancy, between 8 and 18 weeks' gestation (Robertson and Khong, 1987). For that reason it seems logical to search for earlier indicators of pregnancy-induced hypertensive disease than hypertension and proteinuria, and indeed, a multitude of tests have been proposed as a means of predicting the later development of the disease (Chesley 1978). In this chapter we will first assess methods of antenatal care in the prediction of pregnancy-induced hypertensive disorders. Then several biochemical and biophysical markers and various tests that have been proposed to predict the development of hypertensive disease in pregnancy will be reviewed, and their relative importance will be evaluated.

3.1 Diagnosis and prediction of pregnancy-induced hypertensive disorders by standard methods of antenatal care

Antenatal visits are conventionally arranged monthly between 20 and 28 weeks, 2-3 weekly up to 36 weeks, and weekly thereafter until delivery. These visits are dominated by a check of blood pressure and urine, and by the determination of weight gain. How do these routines contribute to the prediction of pregnancy-induced hypertensive disorders?

3.1.1 Blood pressure

The issue whether the development of pregnancy-induced hypertensive disorders may be predicted on the basis of the standard variables that are determined in antenatal care – blood pressure, proteinuria, and weight gain – has been addressed in several retrospective and some prospective studies. The use of the term "prediction" in this context is debatable. If it is accepted that the signs of pregnancy-induced hypertensive disorders are a relatively late expression of
a fundamental problem of circulatory maladaptation that begins early in pregnancy, then the search is for an early diagnosis, rather than for ways to predict the development of the disorder.

Of the prospective, controlled investigations, Gallery's (Gallery et al, 1977) study deals with 42 nulliparous and 40 parous, initially normotensive, pregnant women. Standardized blood pressure readings were obtained monthly from 16 weeks' gestation onwards, using a mercury random-zero sphygomanometer. In this group 15 women, 9 nulliparae and 6 parae, developed hypertension, defined as blood pressures above 135/85 mm Hg, in late pregnancy, presumably without proteinuria. The blood pressure values in this group of patients did not show the fall in diastolic blood pressure that occurred between 17 and 20 weeks' gestation in women who remained normotensive. Of the 15 women who became hypertensive later in pregnancy, 10 had diastolic blood pressure values of 75 mm Hg or above at 17-20 weeks' gestation, as compared with 6 of 67 women who remained normotensive. These results give a sensitivity of 66% and a specificity of 91% of predicting pregnancy-induced hypertension. However, the numbers are small and the 95% confidence limits wide. The authors do not state whether or not the results were withheld from the physicians in care of the patients.

A larger prospective study in 808 nulliparous and 175 parous pregnant women was reported by Moutquin et al. (1985). The investigators used an automatic oscillometric sphygomanometer and started their blood pressure readings between 9-12 weeks' gestation. A total of 7800 blood pressure readings were obtained at intervals of 4 weeks. The results were not withheld from physicians or patients, no intervention was suggested for any blood pressure reading. Of 734 initially normotensive nulliparae 46 (6.3%) developed preeclampsia (blood pressure above 135/85 mm Hg, edema and/or proteinuria after 20 weeks). The sensitivity for predicting preeclampsia early in pregnancy (9-20 weeks) on the basis of the occurrence of a diastolic blood pressure of 80 mm Hg or more was 32-46%, on the basis of 85 mm Hg or more it was 16-31%, with specificities of 84-91% and 93-97%, respectively. The data shows that a majority of pregnant women who remain normotensive during pregnancy have a diastolic blood pressure of 75 mm Hg or less before 20 weeks' gestation. The sensitivity of using an 80-85 mm Hg diastolic blood pressure level in the first half of pregnancy as a test for the later occurrence of preeclampsia is poor. The predictive value of a positive test will be in the order of magnitude of 20-30%, but the predictive value of a negative test will be approximately 95%.

Also values of mean arterial pressure in the second trimester (18-26 weeks) have been used in the prediction of the development of preeclampsia later in pregnancy. Page and Christianson (1976) presented data on the outcome of pregnancy based on a prospective study of 14833 single births. They found that when the averaged mean arterial pressure in the middle trimester (MAP-2) was 90 mm Hg or greater, there was a significant increase in the frequency of pregnancy-induced hypertension, proteinuria, fetal growth retardation and
stillbirths. On this basis an averaged MAP-2 value of 85 mm Hg or 90 mm Hg has been used by several investigators to predict the later occurrence of pregnancy-induced hypertension and preeclampsia. Moutquin et al. (1985) do not present their raw data, but they calculate for the average MAP-2 of 85 mm Hg or more in a population of 983 patients with mixed parity a sensitivity of about 85% and a specificity of about 50% in the prediction of pregnancy-induced hypertension (BP 140/90 mmHg or more) and preeclampsia (+ edema and/or proteinuria). These values are about 65% and 75%, respectively, when >90 mm Hg is taken as the threshold. In this group of patients the prevalence of hypertensive disorders was 11%; the predictive value of a negative MAP-2, for the >85 mm Hg as well as for the >90 mm Hg, was about 98%, the predictive value of a positive MAP-2 was around 10%.

Two reports from which sufficient information could be obtained to calculate statistics for the test results are summarized in Table 3.1. Only one group of authors (Quaas et al, 1982) states that the results of MAP-2 measurements were withheld from the physicians and the patients. The results indicate reasonable agreement about the definition of pregnancy-induced hypertension; the prevalence given by Öney & Kaulhausen (1983) includes hypertension during labor and within 24 hours post partum. The predictive value of a negative test (average MAP-2 below 90 mm Hg) is good, even when the prevalence of pregnancy-induced disorders is as high as 29%.

Reviewing the reports on standard blood pressure measurements as a means to predict or detect pregnancy-induced hypertensive disorders at an early stage, one is struck by the fact that very few investigators state whether or not the results of these measurements were revealed to the physicians making management decisions. It is not mentioned in any of the articles whether the outcome of pregnancy was assessed without knowledge of the test results. In fact, because measurements were usually part of routine antenatal care and will therefore have been recorded in the patients' charts, it is unlikely that the diagnosis of pregnancy-induced hypertensive disorders was made without knowledge of the test results in most cases. This can lead to serious biases in the assessment of test results and makes the estimates of sensitivity and specificity of questionable value. The general impression is that a pregnant woman who has a diastolic blood pressure of about 70 mm Hg or lower, or a mean arterial pressure of about 80 mm Hg or lower, in the second trimester, runs a small risk of developing pregnancy-induced hypertensive disorders later in pregnancy. The consequences of this rather obvious conclusion for antenatal care are minimal.

3.1.2 Proteinuria

In a recent investigation in 100 pregnant women with mixed parity attending a high-risk antenatal clinic Lopes-Espinoza et al. (1986) studied microalbuminuria using a sensitive radioimmunoassay to determine whether an increasing loss of albumin would predict the occurrence of proteinuric preeclampsia. No evidence
Table 3.1. Validity of test of mean arterial pressure in second trimester of pregnancy (MAP-2) in reported studies.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Map¹</th>
<th>Number of patients</th>
<th>Parity</th>
<th>Pregnancy-induced hypertension Definition²</th>
<th>Prevalence %</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>p.v.⁺³</th>
<th>p.v.⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Öney &amp; Kaulhausen (1983)</td>
<td>≥90</td>
<td>200</td>
<td>0</td>
<td>≥140 /90</td>
<td>29</td>
<td>93</td>
<td>66</td>
<td>32</td>
<td>98</td>
</tr>
<tr>
<td>Quaas et al. (1982)</td>
<td>≥85</td>
<td>275</td>
<td>?</td>
<td>≥140 /90</td>
<td>20</td>
<td>89</td>
<td>68</td>
<td>41</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>≥90</td>
<td>285</td>
<td>?</td>
<td>DBP † ≥20</td>
<td>20</td>
<td>62</td>
<td>89</td>
<td>58</td>
<td>91</td>
</tr>
</tbody>
</table>

¹Criteria of positive test
²Main blood pressure criteria for diagnosis of pregnancy-induced hypertensive disorders. DBP † ≥20 = increase in diastolic blood pressure of 20 mm Hg or more.
³Predictive value of a positive/negative test.
was found that gross proteinuria is preceded by a gradual increase in microalbuminuria, and the authors conclude that there is no value in using precise techniques of detecting proteinuria to predict preeclampsia (Clark et al., 1984; McEwan 1968; McEwan 1987).

3.1.3 Weight gain

In 1952 Hamlin (Hamlin 1952) made the rather dogmatic statement that "if a young primipara with a low initial blood pressure increases her weight by more than 8 lb between the 20th and 30th week, she will proceed almost inexorably towards eclampsia". This led to renewed and vigorous manipulation of the diets of millions of pregnant women. Hamlin's statements were checked in a careful retrospective study in 1457 selected primigravidae in Aberdeen (Nelson 1955). About two-thirds of the normotensives and three-quarters of the women who developed preeclampsia gained more than 8 lb in the 20-30 weeks period of pregnancy. The authors conclude that the relationship of weight gain between 20-30 weeks of pregnancy to preeclampsia is too indefinite to be of any practical value in predicting the development of preeclampsia. This conclusion was supported by the results of another retrospective study, also from Aberdeen (Thomson and Billewicz, 1957). In a prospective study of only 62 unselected primigravidae, Gardiner et al. (1957) determined a sensitivity of 90% of a weight gain of 8 lb or more in the 20-30 weeks period of pregnancy in 40 (!) patients who later developed hypertension (diastolic blood pressure 90 mm Hg or above), and a specificity of 23%. In the prospective Collaborative Perinatal Project, the largest study available on the influence of weight gain on the outcome of pregnancy, the relationship between weight gain and the development of hypertensive disorders in pregnancy was not investigated (Naeye 1979). However, very high (and very low) pregnancy weight gains were shown to have only modest influence on perinatal mortality rates.

In conclusion, there appears to be such an important variability in physiologic weight gain in pregnancy, and such an overlap between weight gains of normotensive and hypertensive pregnant populations, that weight gain cannot be used as an early sign or to predict hypertensive disorders in pregnancy (Robertson 1971; Vosburgh 1976).

3.2 Diagnosis and prediction of pregnancy-induced hypertensive disorders by biochemical and biophysical tests of vasoconstriction

Pregnancy-induced hypertensive disorders are characterized by an abnormal vascular reactivity leading to vasoconstriction (Wallenburg 1988). A variety of tests has been devised to demonstrate the presence or absence of an abnormal vascular responsiveness before the clinical onset of the disorder. Most of these tests have only historical value and will just be briefly mentioned. Only the angiotensin II sensitivity test and the roll-over test will be discussed in more detail.
3.2.1 Cold pressor test

The cold pressor test was devised more than 50 years ago (Hines and Brown, 1933). One hand is deeply immersed in ice-cold water for 1-2 minutes and the blood pressure response is measured. The ice water increases systemic blood pressure by an increase in systemic vascular resistance due to sympatho-adrenal activation (Nisell et al, 1985). The hypothesis that an exaggerated pressure response in pregnancy could predict the later development of hypertension or preeclampsia has been tested by various workers (Dieckmann et al, 1938; Reid and Teel, 1938; Chesley and Chesley, 1939; Chesley and Valenti, 1958). The general conclusion of these studies is that the test is unreliable and cannot be used to predict the onset of pregnancy-induced hypertensive disorders or to differentiate them from chronic hypertension.

3.2.2 Flicker fusion test

The nitroglycerine flicker fusion threshold test was first described by Krasno and Ivy (1950) and almost immediately applied as a predictive or prognostic test of hypertensive disorders in pregnancy (Brill et al, 1952). The test can be defined as the minimum number of cycles per second at which the separate flashes of a flickering light source appear to fuse into a steady light. Optimal results occur under conditions of normal retinal vascular tone, the results are adversely affected by retinal vasospasm. When the flicker fusion threshold increases following the administration of the vasodilator nitroglycerine, this is considered to indicate a state of retinal vasospasm being relieved by the vasodilator, and the test is considered to be positive. Following some enthusiastic reports on the reliability with which the test performed in early pregnancy could predict the development of preeclampsia later in pregnancy (Brill et al, 1952; Marty and Hardy, 1952), subsequent investigators have found the test to be utterly worthless (Gillim 1954; Rugart 1953; Gardiner and Herdan, 1957).

3.2.3 Isometric exercise test

Isometric handgrip exercise is known to increase systemic arterial blood pressure, presumably resulting from increased systemic vascular resistance (Lind and McNicol, 1967). For that reason Degani et al (1985) proposed that the diastolic blood pressure response to an isometric handgrip exercise test might reflect vascular reactivity in pregnant women, and might thus be used to detect vascular hyperreactivity and to predict pregnancy-induced hypertensive disorders. Hundred healthy primigravidae were subjected to an isometric handgrip exercise test between 28 and 32 weeks' gestation (Degani et al, 1985). Each woman was placed in left lateral position and blood pressure was recorded at regular intervals until it remained stable. The woman was then instructed to press an inflated cuff of a calibrated sphygmomanometer to maximal voluntary
contraction for 30 seconds for a three-minute period of sustained isometric handgrip exercise. The woman then compressed the inflated sphygmomanometer at a tension level of 50% of her previously determined maximal voluntary contraction. Blood pressure measurements were taken on the passive arm, and an increase in the diastolic pressure of at least 20 mm Hg was taken as a positive isometric exercise pressor response. The results of the test were not made available to the physicians taking care of the women and making the clinical diagnosis. Of the 100 primigravidae 84 had a negative test, and of these 3 developed pregnancy-induced hypertension (false negatives). Of the 16 patients with a positive test, only 3 did not develop pregnancy-induced hypertension (false positives). These results give a sensitivity of 81% and, a specificity of 96%. The positive predictive value is 81%, the negative predictive value 93%, with a prevalence of pregnancy-induced hypertensive disorders of 16% in this sample.

Although this data suggests that the test could be useful, the results are very suspect because of roll-over test conducted at the same time on the same women reported a prevalence of hypertensive disorders of 9%, which is incompatible with the 16% observed with the other test.

In conclusion, judgement on the isometric exercise test as a predictor of pregnancy-induced hypertensive disorders awaits the results of further studies to support or refuse its validity.

3.2.4 Roll-over test

During their angiotensin infusion studies Gant et al. (1973) noted that many nulliparous normotensive pregnant women who were sensitive to angiotensin II demonstrated an increase in diastolic blood pressure of 20 mm Hg or more when they turned from their left sides onto their backs. Thus the roll-over (ROT) or supine pressor test came into being. The test is performed between 28 and 32 weeks of pregnancy with the woman lying on her left side until the diastolic blood pressure, measured by Gant et al. (1974) at Korotkoff phase 5 on the uppermost right arm, is stabilized. The woman is then turned on her back, and diastolic blood pressure is again recorded. A rise in diastolic blood pressure of 20 mm Hg or more defines a positive test. The first study comprised 50 randomly selected healthy primigravid women. Of 20 women sensitive to angiotensin II, 19 also had a positive ROT. Of the 30 women resistant to angiotensin II, all gave a normal response in the ROT but one, and that one developed eclampsia. Of 16 women with a positive ROT 15 developed pregnancy-induced hypertension, of 22 women with a negative ROT 20 remained normotensive. Pregnancy outcome is given for only 38 of the 50 women, apparently 12 had not completed their pregnancies when the paper was written. The prevalence of pregnancy-induced hypertension (blood pressure 140/90 mm Hg or above and a rise in diastolic blood pressure of 20 mm Hg or more) in this unselected sample of primigravidae is 44.7%, which must be one of the highest frequencies of occurrence in the world.
Since this original report many other investigators have studied the validity of the ROT in their own institutions, and statistics of a number of these studies, together covering more than 1500 patients, are summarized in Table 3.2. Studies in which a positive test result on purpose led to some form of intervention are not included (Thompson and Mueller-Heubach, 1978; Spinapolice et al, 1983). The sensitivity and specificity values of about 90% that can be calculated from Gant's results are not approached by the majority of the other studies. Various factors may explain the marked disparity in results of a relatively simple test as the ROT. Although all investigators define an increase in diastolic blood pressure of at least 20 mm Hg as a positive test, there is considerable variation among investigators in the methodology of the test (Poland et al, 1980), and either Korotkoff phase 4 or phase 5, or doppler-shift signals are used to determine diastolic blood pressure. The definition of pregnancy-induced hypertensive disorders shows the usual inconsistencies, it is usually not stated whether or not elevations of blood pressure occurring for the first time during labor or in the early postpartum period are included; in most studies proteinuric pre-eclampsia is not distinguished from nonproteinuric hypertension. All authors, with only a few exceptions (Campbell 1978; Kuntz 1980) fail to identify the arm used to record blood pressure. This is of great importance, since turning from the left side on the back with blood pressure recorded from the right (superior) arm will result in a predictable increase in diastolic pressure of approximately 10-12 mm Hg due to the increase in hydrostatic pressure relative to the level of the heart (Webster et al, 1984; Wichman et al, 1984).

The same criticism expressed with regard to blood pressure studies in pregnancy must be made here: on the basis of all reports we remain uncertain whether or not the tests were assessed in a double-blind fashion. It is stated in a few publications (Gusdon et al, 1977; Phelan et al, 1977; Kassar 1980; Verma 1980) that the results of the tests were not revealed to the physicians in charge or to the patients themselves, but it is not mentioned if the diagnosis of pregnancy-induced hypertensive disorders was made without knowledge of the test results. In conclusion, the roll-over test has gained some popularity because it is easy to perform, but its validity remains questionable.

3.2.5 Infusion of catecholamines

The pressor effects of adrenaline infused in physiologic (Nisell et al, 1985) and pharmacologic (Zuspan et al, 1964; Raab et al, 1956) doses appear to be more outspoken in women with (mild) pregnancy-induced hypertension than in normotensive pregnant women. Raab et al. (1956) investigated the pressor effects of two doses of adrenaline in 163 normotensive pregnant women between 28 and 34 weeks' gestation. They noted an increase in systolic blood pressure that was higher in women who became hypertensive later in pregnancy than in women who remained normotensive. However, there was extreme variability in blood pressure responses and considerable overlap between responses of normal
Table 3.2. Validity of the roll-over test in reported studies.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of patients</th>
<th>Parity</th>
<th>Pregnancy-induced Hypertension Definition</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>p.v.+</th>
<th>p.v.-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥140/90 DBP ≥20 Prevalence %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gant et al. 1974</td>
<td>38</td>
<td>0</td>
<td>45</td>
<td>88</td>
<td>95</td>
<td>93</td>
<td>91</td>
</tr>
<tr>
<td>Phelan et al. 1977</td>
<td>207</td>
<td>?</td>
<td>13</td>
<td>78</td>
<td>82</td>
<td>39</td>
<td>82</td>
</tr>
<tr>
<td>Karbhari et al. 1977</td>
<td>178</td>
<td>0</td>
<td>?</td>
<td>21</td>
<td>71</td>
<td>99</td>
<td>93</td>
</tr>
<tr>
<td>Marshall &amp; Newman (1977)</td>
<td>100</td>
<td>0</td>
<td>≥140/90 DBP ≥20 Prevalence %</td>
<td>28</td>
<td>75</td>
<td>94</td>
<td>84</td>
</tr>
<tr>
<td>Gusdon et al. 1977</td>
<td>60</td>
<td>0</td>
<td>≥140/90 DBP ≥20 Prevalence %</td>
<td>22</td>
<td>77</td>
<td>79</td>
<td>50</td>
</tr>
<tr>
<td>Verma et al. 1980</td>
<td>130</td>
<td>?</td>
<td>≥140/90 DBP ≥15 Prevalence %</td>
<td>23</td>
<td>63</td>
<td>62</td>
<td>51</td>
</tr>
<tr>
<td>Campbell, 1978</td>
<td>85</td>
<td>0</td>
<td>≥140/90 DBP ≥15 Prevalence %</td>
<td>53</td>
<td>4</td>
<td>97</td>
<td>66</td>
</tr>
<tr>
<td>Kuntz, 1980</td>
<td>65</td>
<td>0</td>
<td>≥140/90 DBP ≥15 Prevalence %</td>
<td>38</td>
<td>60</td>
<td>68</td>
<td>54</td>
</tr>
<tr>
<td>Thurnau et al. 1983</td>
<td>75</td>
<td>?</td>
<td>≥140/90 DBP ≥15 Prevalence %</td>
<td>37</td>
<td>73</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>Didolkar et al. 1979</td>
<td>39</td>
<td>0</td>
<td>≥140/90 DBP ≥30 Prevalence %</td>
<td>20</td>
<td>41</td>
<td>81</td>
<td>35</td>
</tr>
<tr>
<td>Poland et al. 1980</td>
<td>139</td>
<td>0</td>
<td>≥140/90 DBP ≥20 Prevalence %</td>
<td>32</td>
<td>42</td>
<td>62</td>
<td>34</td>
</tr>
<tr>
<td>Kassar et al. 1980</td>
<td>74</td>
<td>0</td>
<td>≥140/90 DBP ≥20 Prevalence %</td>
<td>27</td>
<td>60</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>Öney &amp; Kaulhausen (1983)</td>
<td>188</td>
<td>0</td>
<td>≥140/90 DBP ≥20 Prevalence %</td>
<td>14</td>
<td>63</td>
<td>75</td>
<td>30</td>
</tr>
<tr>
<td>Degani et al. 1985</td>
<td>100</td>
<td>0</td>
<td>≥140/90 DBP ≥20 Prevalence %</td>
<td>9</td>
<td>67</td>
<td>57</td>
<td>13</td>
</tr>
<tr>
<td>Marx et al. 1980</td>
<td>78</td>
<td>0</td>
<td>≥140/90 DBP ≥20 Prevalence %</td>
<td>22</td>
<td>88</td>
<td>51</td>
<td>33</td>
</tr>
<tr>
<td>Tunbridge 1983</td>
<td>100</td>
<td>0</td>
<td>≥140/90 DBP ≥95 Prevalence %</td>
<td>19</td>
<td>67</td>
<td>57</td>
<td>13</td>
</tr>
</tbody>
</table>

1Main blood pressure criteria for diagnosis of pregnancy-induced hypertensive disorders. DBP ≥20 = increase in diastolic blood pressure of 20 mm Hg or more.
2Predictive value of a positive/negative test.
and abnormal groups. Also the pressor response to noradrenaline appears to be enhanced in hypertensive pregnant patients as compared with normotensive pregnant women (Talledo 1968; Nisell et al, 1985). In the same study in which they investigated the vascular reactivity to adrenaline Raab et al. (1956) also assessed the pressor response to noradrenaline, and found it elevated in normotensive women who later became hypertensive. As with adrenaline, the response showed considerable variability and overlap. No further attempts have been made to use the pressor effects of catecholamines as a test to predict the development of pregnancy-induced hypertensive disorders.

3.2.6 Infusion of vasopressin

Half a century ago two independent groups reported an increased vascular sensitivity to extracts of the posterior pituitary gland (Dieckmann and Michel, 1937; Schockaert and Lambillon, 1937) in preeclamptic patients. Marked rises in blood pressure were noted, sometimes accompanied by oliguria and even precipitation of convulsions. Later investigations confirmed these observations, but found the responses to vasopressin to be extremely variable, both in normotensive and in hypertensive pregnant patients. Infusion of vasopressin in pregnant women is a hazardous procedure and cannot be used for prediction or early diagnosis of hypertensive disorders in pregnancy.

3.2.7 Infusion of angiotensin II

In 1961 Abdul-Karim and Assali reported that intravenous infusion of angiotensin II ("angiotonin") caused a smaller rise in blood pressure in pregnant women than in nonpregnant individuals. The relative refractoriness to the pressor effects of angiotensin II in normal pregnancy was later confirmed in several studies (Chesley et al, 1965; Talledo 1966; Schwarz and Retzke, 1971) and, in addition, it was found that this refractoriness is lost to a marked extent in preeclamptic women (Chesley 1966; Talledo 1968). Gant et al. (1973) used the determination of the sensitivity to the pressor effects of infused angiotensin II to predict the development of pregnancy-induced hypertensive disorders.

In a prospective study in 192 randomly selected extremely young (13-17 years old) and apparently healthy primigravidae, 21% caucasian and 79% black, the effect of infused angiotensin II on diastolic blood pressure was determined sequentially throughout pregnancy. In addition, 10 nonpregnant gynecologic patients (age not reported) were studied as nonpregnant controls. The method used was to determine the amount of angiotensin II infused per kg of bodyweight per minute needed to raise the diastolic blood pressure (Korotkoff 5) by 20 mm Hg (Kaplan and Silah, 1964). The average so-called effective pressor dose (EPD) was found to be about 8 ng/kg/min in nonpregnant individuals and in early (7-10 weeks) pregnancy; it increased approximately two-fold to 15 ng/kg/min by 28 weeks, and fell gradually thereafter to a level of 11-12 ng/kg/min at
term. Up to about 20 weeks' gestation all pregnant women showed this refractoriness to the pressor effects of angiotensin II that appears to be a physiologic feature of pregnancy. However, in women who ultimately developed pregnancy-induced hypertension (blood pressure of 140/90 or higher and/or a rise in diastolic pressure of 20 mm Hg or more) the refractoriness gradually decreased and between 28 and 32 weeks the EPD had returned to nonpregnant values. Of the total group of 192 pregnant women 153 underwent at least one angiotensin II infusion test between the 28th and 32nd week. Of these, 103 remained normotensive, 50 subsequently developed pregnancy-induced hypertension in the third trimester. Of the 103 women who remained normotensive, 13 had an EPD of less than 8 ng/kg/min (positive test), of the 50 women who became hypertensive 45 had at least one positive test. Since this is only part of the prospectively studied group of patients calculation of the sensitivity and specificity of the test is meaningless.

The angiotensin sensitivity test is a rather complicated and time-consuming procedure, which may be the reason that its validity in predicting pregnancy-induced hypertensive disorders has been assessed in very few clinical investigations: there is one other study from the U.S. (Morris et al, 1978), one from Mexico (Orozco et al, 1979), one from Europe (Öney and Kaulhausen, 1982), and one from Japan (Nakamura et al, 1986). The results of these studies are summarized in Table 3.3. The numbers are small, the definitions of a positive test and of pregnancy-induced hypertension are different.

Much of the criticism that has been leveled at reported studies on the use of blood pressure readings and of the roll-over test to predict the development of pregnancy-induced hypertension can also be applied to the few studies on the angiotensin sensitivity test. In particular, it is not mentioned in any of these reports whether or not a double-blind design was used, which raises doubts as to the validity of the calculated test statistics.

As stated already, the angiotensin sensitivity test is too complicated and time consuming to be convenient as a routine screening procedure. However, it may be used for research purposes to define a group of women who, at 28-32 weeks of pregnancy, are at high or low risk of developing pregnancy-induced hypertension (Wallenburg et al, 1986).

3.3 Diagnosis and prediction of pregnancy-induced hypertensive disorders by biochemical markers

Concentrations of a large number of constituents of blood and urine, including hormones, have been shown to change in pregnancy-induced hypertensive disorders. Some of these changes have been proposed to be of value in the prediction or early diagnosis of the disorder and some of them will be discussed in the following paragraphs.
Table 3.3. Validity of angiotensin sensitivity test in reported studies.

<table>
<thead>
<tr>
<th>Authors</th>
<th>EPD (1)</th>
<th>Number of patients</th>
<th>Duration of gestation (wks)</th>
<th>Parity O/M</th>
<th>Pregnancy-induced hypertension definition²</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>p.v.+3</th>
<th>p.v.-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morris et al. (1978)</td>
<td>&lt; 8 (4)</td>
<td>26</td>
<td>29-32</td>
<td>0</td>
<td>DBP ≥90</td>
<td>33</td>
<td>39</td>
<td>7</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>&lt;10 (4)</td>
<td></td>
<td></td>
<td></td>
<td>DBP/ ≥15</td>
<td>67</td>
<td>30</td>
<td>11</td>
<td>87</td>
</tr>
<tr>
<td>Nakamura et al. (1986)</td>
<td>&lt; 8</td>
<td>48</td>
<td>30</td>
<td>O:30</td>
<td>SBP/ ≥30</td>
<td>21</td>
<td>97</td>
<td>75</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>&lt;10</td>
<td></td>
<td></td>
<td>M:18</td>
<td>DBP/ ≥15</td>
<td>80</td>
<td>82</td>
<td>53</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>&lt;12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>74</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Öney &amp; Kaulhausen,</td>
<td>&lt;10</td>
<td>231</td>
<td>28-32</td>
<td>0</td>
<td>≥140/90</td>
<td>76</td>
<td>83</td>
<td>45</td>
<td>95</td>
</tr>
<tr>
<td>(1982)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orozco et al. (1979)</td>
<td>&lt; 8(4)</td>
<td>33</td>
<td>28-32</td>
<td>O:15</td>
<td>≥120/80</td>
<td>89</td>
<td>79</td>
<td>62</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M:18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Effective pressor dose below which test was considered positive
²Main blood pressure criteria for diagnosis of pregnancy-induced hypertensive disorders. DBP/ ≥15 = increase in diastolic blood pressure of 15 mm Hg or more
³Predictive value of a positive/negative test
⁴At least one positive test in serial testing
3.3.1 Plasma volume, hemoglobin concentration, hematocrit

As discussed in chapter 2.2.2 it has been reported by many authors that the average plasma volume is less in patients with pregnancy-induced hypertensive disorders, in particular preeclampsia (MacGillivray 1983; Chesley 1972). There is evidence that plasma volume contraction may precede the blood pressure rise in preeclampsia, at least in some of the cases (Blekta et al, 1970; Gallery et al, 1979; Hays 1985); however, the numbers of patients studied are small, and there is considerable overlap between values of plasma volume in normal and abnormal groups. In addition, dye-dilution techniques for estimating plasma volume are subject to serious criticism (Campbell and MacGillivray, 1980) and they are too complicated to be used as a screening procedure in standard antenatal care.

The contraction of plasma volume that is often observed in preeclampsia may be revealed by an elevation of or a rise in hemoglobin concentration or hematocrit (Sagen et al, 1982). Many obstetricians use serial measurements of the hematocrit to monitor the clinical course of hypertensive disorders in pregnancy. In a large retrospective study comprising over 54,000 singleton pregnancies Murphy et al. (1986) showed a highly significant correlation in nulliparous as well as in parous women between the frequency of occurrence of hypertensive disorders and the hemoglobin concentration at first booking before 24 weeks.

Repeat determinations of hemoglobin concentration or hematocrit are standard procedure in antenatal care. It may be worthwhile investigating in more detail in a double-blind trial the relationship between midtrimester hemoglobin or hematocrit values as indicators of plasma volume and the subsequent course of pregnancy.

3.3.2 Uric acid

In 1925 Stander et al. (Stander et al, 1925) reported raised serum uric acid levels in women with preeclampsia. Many later investigators confirmed this finding and demonstrated a positive correlation between the height of serum uric acid levels, clinical severity of pregnancy-induced hypertensive disorders (Lancet and Fisher, 1956; Thurnau et al, 1983) and perinatal outcome (Redman et al, 1976; Schuster and Wepelmann, 1981; Liedholm et al, 1984). On the basis of the results of a prospective but not blinded study in 332 hypertensive pregnant patients with mixed parity Redman et al. (1976) concluded that plasma uric acid levels constitute a better indicator than blood pressure of prognosis for the fetus. This view is not shared by some other investigators; in a recent book on hypertension in pregnancy uric acid is not even mentioned (Gant and Worley, 1980).

In a study in which the nature of the hypertensive disorder in pregnancy was established by renal biopsy, women with preeclampsia were found to have marked hyperuricemia, whereas uric acid levels were normal in pregnant women with chronic hypertension without preeclampsia (Pollak and Nettles, 1960). Later
studies, however, have shown an association between hyperuricemia, chronic hypertension, and early nephrosclerosis in nonpregnant individuals (Messerli et al., 1980). For that reason it remains to be proven whether or not uric acid levels can be used to distinguish pregnancy-induced hypertensive disorders from chronic hypertensive disease.

Hyperuricemia is nonspecific, it may result from various metabolic disturbances, erythrocyte disorders, impaired renal functions and drugs (diuretics) (Messerli et al., 1980). The cause of the hyperuricemia in pregnancy-induced hypertensive disorders is not well understood; it cannot be attributed solely to a reduced glomerular filtration rate, and various other mechanisms, including increased production by the fetal-placental unit, have been proposed (Wallenburg and Van Kreej, 1978). Some investigators found a correlation between elevated serum uric acid levels and reduced plasma volume in preeclamptic women (Beaufil et al., 1981), a finding that has not been confirmed by others (Gallery et al., 1980).

The results of many studies (Redman et al., 1977; Gallery et al., 1980; Riedel et al., 1981), but not of all (Fay et al., 1985), in normotensive pregnant women suggest that serum uric acid levels may begin to rise before the appearance of hypertension and proteinuria. However, patients studied include nulliparous as well as parous women, some of them with proven renal disease or treated chronic hypertension (Redman et al., 1977), and the presentation of the data does not allow a reliable evaluation of the results. Moreover, the predictive value of uric acid determinations was not assessed in a double-blind fashion in any of these studies.

In conclusion, as all other signs of pregnancy-induced hypertensive disorders hyperuricemia is nonspecific. As yet there is no evidence that determination of uric acid levels can be used as a screening test to predict the later development of pregnancy-induced hypertension, but there certainly is an urgent need for further well-designed, prospective double-blind studies. In patients with established preeclampsia serum uric acid levels appear to reflect fetal prognosis. For that reason uric acid levels can be used in the clinical management of women with a pregnancy-induced hypertensive disorder to monitor the course of the disease.

3.3.3 Calcium excretion

Recently, Taufield et al. (1987)) reported that established preeclampsia is associated with hypocalciuria. The authors investigated a group of 40 pregnant women – 20 nulliparae and 20 multiparae – in the third trimester of pregnancy; 10 were normotensive, 5 had transient hypertension, 6 had chronic hypertension, 7 had chronic hypertension with superimposed preeclampsia, and 12 had preeclampsia. However, the authors used a very broad definition of preeclampsia: hypertension defined as a blood pressure of 140/90 mm Hg, or a rise of 30 mm Hg in systolic pressure, or a rise of 15 mm Hg in diastolic pressure, associated with
a proteinuria of more than 300 mg per 24 hours, or a rise in the serum uric acid level of more than 1 mg per deciliter, a decrease in the platelet count of more than $50 \times 10^9 / l$, or both. None of the subjects received diuretics during the study; but it is not stated whether or not other kinds of therapy were used.

Urinary calcium excretion was not different between normotensive women and patients with transient or chronic hypertension, but it was significantly lower (13-25% of normal pregnant values) in preeclamptic patients. In these patients also the fractional excretion of calcium was reduced, suggesting increased tubular reabsorption. The mechanism of hypocalciuria in preeclampsia is not known. Further prospective studies are needed to support or refute these findings, and to determine whether a decrease in urinary calcium excretion could be used as an early predictor of the disease.

3.3.4 Excretion of prostacyclin metabolites

Evidence is accumulating that a relative deficiency of vascular prostacyclin plays an important part in the development of pregnancy-induced hypertensive disease (Makila et al, 1984). In a recent study FitzGerald et al. (1987) reported that a reduction in the urinary excretion of 2,3-dinor-6-keto-prostaglandin F1 (PGFM), a major metabolite of prostacyclin, precedes the development of clinical disease. Urine was collected at 11-20 weeks, 20-28 weeks, and between 28 weeks and term in 67 pregnant women considered to be at risk of developing pregnancy-induced hypertension on the basis of young age, first pregnancy, black race and/or a history of pregnancy-induced hypertension. Patients were retrospectively allocated to one of four predefined groups (pregnancy-induced hypertension, chronic hypertension, hypertension in labor, or normotension) by observers who were blinded as to the results of the biochemical analysis. Excretion of PGFM was increased throughout pregnancy in all pregnant women as compared with that in normotensive nonpregnant subjects. However, PGFM excretion was significantly reduced in 12 women (18%) who developed pregnancy-induced hypertension later in pregnancy as compared with that in pregnant women who remained normotensive, or who became hypertensive during labor. The authors state that excretion of less than 400 pg PGFM per mg creatinine at 20-28 weeks' gestation was associated with a 65% risk of developing pregnancy-induced hypertension, but they do not provide data to calculate sensitivity and specificity. A study of a larger population is needed to assess the predictive power of determination of urinary PGFM excretion.

3.3.5 Enzymes and hormones

Concentrations of various enzymes in serum or plasma have been studied in patients with a hypertensive disorder in pregnancy, mainly to assess placental function (MacGillivray 1983). Following a brief period of clinical enthusiasm
none of these enzymes has been shown to be of use as an early indicator of hypertensive disease in pregnancy, or as a biochemical measure of its severity. As an extension of earlier studies (Redman et al, 1977; Williams and Jones, 1975) recent investigators have adduced evidence that serum levels of deoxycytidylate deaminase (dCMP) are increased if preeclampsia develops, but not in chronic hypertension (Williams and Jones, 1982). The latter investigators performed at least two determinations of dCMP, one at booking and one in the third trimester, in serum of 2460 pregnant women, the parity of whom is not reported. An abnormally high value was defined as a value equal to or higher than 2 SD above the mean of control values obtained in apparently normal pregnancies. A total of 133 women (5.4%) developed pregnancy-induced hypertension or preeclampsia. All of 73 patients with moderate/severe proteinuric preeclampsia, and 52 of 60 women with mild nonproteinuric pregnancy-induced hypertension had abnormally high levels of dCMP. High levels were also observed in 9 patients with a hepatic disorder. Only 53 other patients had high dCMP levels ("false positives"). It should be emphasized that the majority of the patients already had clinical signs of hypertensive disease at the time of determination of dCMP values. No abnormally elevated levels were found before 24 weeks’ amenorrhea, and very few (4) before 28 weeks, which makes it unlikely that this test can be used to predict the occurrence of hypertensive disorders in pregnancy. This study can be criticized because of the fact that the results were revealed to the physicians and the patients, and that patients were admitted to the hospital antenatally and treated for the sole reason of having abnormally high dCMP levels. Further properly designed studies are required to determine the value of estimation of dCMP - and of the closely related enzyme cytidine deaminase (Jones et al, 1982) - in normotensive and hypertensive pregnancies.

It has been claimed quite recently (Hughes et al, 1980; Toop and Klopper, 1981) that the levels of the placental protein hormone termed pregnancy-associated plasma protein A (PAPP-A) are raised towards the end of the second trimester in women who are likely to develop preeclampsia. The use of PAPP-A determination as a predictive test of preeclampsia has not yet been confirmed in other studies.

The rate at which the placenta clears maternal blood of isotope-labeled dehydroisoandrosterone sulfate (DS) for estradiol synthesis has been postulated to reflect uteroplacental perfusion (Gant et al, 1971). A longitudinal study of the metabolic clearance rate (MCR) of DS in women who developed pregnancy-induced hypertension later in pregnancy showed that during the first half to two-thirds of pregnancy placental perfusion was similar to or even above that in women who remained normotensive. The MCR began to fall 2-4 weeks before hypertension was detected, by which time it was only 35-50% of normal (Worley et al, 1978). These results have not been confirmed in studies by others. In addition, the late occurrence of a fall in MCR-DS and the important interindividual variation makes it unlikely that this test may be used for prediction or early diagnosis of hypertensive disease in pregnancy.
3.3.6 Coagulation factors and platelets

Evidence for the occurrence of abnormal coagulation processes and platelet activation was originally based on the finding in as early as 1893 of fibrin deposits and thrombi in vessels of various organs of women who died of eclampsia (Wallenburg 1987). In later years all factors of the extrinsic and intrinsic coagulation system have been extensively studied in women with hypertensive disorders in pregnancy, either directly or indirectly by means of various clotting tests. A complicated coagulation index has been proposed to predict the clinical progress of preeclampsia (Howie et al, 1976). These studies have recently been reviewed and analyzed in detail (Wallenburg 1987). The results are generally equivocal and do certainly not suggest marked consumption of coagulation factors in most patients with a pregnancy-induced hypertensive disorder. The most frequently occurring hemostatic abnormalities in patients with pregnancy-induced hypertension or preeclampsia are a rise in the factor VIII related antigen (VIII R : AG) level, and a reduced antithrombin III concentration.

Thrombin-induced coagulation, but also vascular endothelial damage and platelet aggregation, increases the circulating levels of factor VIII R : Ag (von Willebrand factor) and the ratio of factor VIII R : Ag to coagulant factor VIII, factor VIII : C (Wallenburg 1987). Several authors have demonstrated an early rise of factor VIII R : Ag and/or of the factor VIII R : Ag /VIII : C ratio in pregnancy-induced hypertension and preeclampsia (Redman et al, 1977) and a positive correlation between plasma factor VIII R : Ag activity and/or the height of the factor VIII R : Ag /VIII : C ratio, and the clinical severity of the disorder (Thornton and Bonnar, 1977; Scholtes et al, 1983), and the degree of hyperuricemia (Redman et al, 1977; Boneu et al, 1980; Whigham et al, 1980), growth retardation and perinatal mortality (Fournie et al, 1981; Whigham et al, 1980; Scholtes et al, 1983). There appears to be a particularly good correlation between plasma factor VIII R : Ag activity and the occurrence of fetal growth retardation, with or without maternal hypertension (Fournie et al, 1981; Whigham et al, 1980; Scholtes et al, 1983; Boneu et al, 1977). It can be concluded from these studies that the finding of an elevated factor VIII R : Ag activity and/or an elevated factor VIII R : Ag /VIII : C ratio constitutes a useful and quite early indicator of the severity of a hypertensive disorder in pregnancy, and in particular of the degree of placental insufficiency. Unfortunately, the suggestive evidence from Redman’s (1977) study that determination of factor VIII R : Ag activity or factor VIII R : Ag /VIII : C ratio early in pregnancy could be used as a test to predict the development of pregnancy-induced hypertensive disease later in pregnancy has not led to further properly designed, prospective and controlled studies.

Decreased levels of antithrombin III (AT III) have been demonstrated in a majority of patients with preeclampsia, but not in pregnant women with chronic hypertension (Weiner and Brandt, 1982; Weenink et al, 1984). Exacerbations and remissions of the disease were reflected in fluctuations of AT III levels, and low AT III concentrations appear to be associated with placental infarction.
and poor fetal outcome (Weenink et al, 1984). No data are available with respect to the possible value of early AT III determinations to predict the development of pregnancy-induced hypertensive disorders later in pregnancy.

Recently attention has been focussed on plasma levels of fibronectin, a glycoprotein involved in coagulation, platelet function, tissue repair, and the vascular endothelial basement membrane. Fibronectin levels were found to be markedly elevated in preeclamptic patients (Stubb et al, 1984; Graninger et al, 1985; Saleh et al, 1987) and correlated with low AT III levels and with the degree of proteinuria (Graninger et al, 1985; Saleh et al, 1987). Lazarchick et al. (1986) published a prospective blinded study in a group of apparently normotensive outpatients. The number of women involved in the study and their parities are not reported. Blood samples for determination of fibronectin levels were obtained at unspecified intervals in the second or third trimester. Seventeen women developed preeclampsia as defined by "widely used criteria" (Hughes 1972). In 16 of these 17 women fibronectin levels were higher than two standard deviations above the mean determined in 23 randomly selected normotensive women. Thirteen of these patients had elevated fibronectin levels detectable 4 weeks or more before the appearance of hypertension. Since the data shows that some women developed preeclampsia already at 16-25 weeks' gestation, it seems likely that at least some patients had chronic hypertension or renal disease. High fibronectin levels have earlier been reported in nonpregnant patients with a nephrotic syndrome and cholestasis (Stathakis et al, 1981). The data presented in the report by Lazarchick et al. (1986) do not allow an assessment of the predictive value of this new biochemical marker.

Many reports in the literature indicate that in patients with a pregnancy-induced hypertensive disorder as a group platelet counts are lower than in uncomplicated pregnancies (Gibson et al, 1982; Giles and Inglis, 1981), and that thrombocytopenia may occur as an early (Redman et al, 1978) but also as a relatively late (Fay et al, 1985) feature of the disorder. Thrombocytopenia is not a feature in pregnant patients with chronic hypertension without superimposed preeclampsia (Gibson et al, 1982; Giles and Inglis, 1981). However, platelet behavior in women with a pregnancy-induced hypertensive disorder is extremely variable (Wallenburg 1987), and reduced platelet counts are not found in all cases, not even of eclampsia (Pritchard et al, 1976; Sibai et al, 1981; Sibai et al, 1982). Results of various large studies suggest that approximately 20% of patients with a pregnancy-induced hypertensive disorder develop thrombocytopenia, varying between 7% in mild pregnancy-induced hypertension to 50% in severe preecclampsia/eclampsia (Giles and Inglis, 1981). Thrombocytopenia is usually mild; in some cases it may be accompanied by hemolysis and elevated liver enzymes, a typical constellation of abnormalities that has been named the "HELLP" syndrome (Weinstein and Brandt, 1982; Aarnoudse et al, 1986). Episodes of platelet activation and consumption are also reflected in increased plasma levels of beta-thromboglobulin (Douglas et al, 1982; De Vries et al, 1983; Socol et al, 1985) and platelet factor 4 (Maki 1983; Borok et al, 1984; Socol et al, 1985)
in some patients with a pregnancy-induced hypertensive disorder, but not with chronic hypertension. The results of these studies indicate that platelet behavior in pregnancy-induced hypertensive disorders is extremely variable, between patients and within one patient. Platelet counts can easily be obtained and repeat counts are an important aid in the clinical management of established hypertensive disease in pregnancy. As yet there are no studies on the predictive value of platelet counts in pregnancy.

In conclusion, determination of factor VIII R : Ag activity and/or of the factor VIII R : Ag/VIII : C ratio, of plasma AT III concentrations, and of platelet counts may be of value in the management of hypertensive disorders in pregnancy. However, there is no convincing evidence that these determinations or that of fibronectin are of any value for prediction or very early diagnosis of the disease.

3.4 Diagnosis and prediction of pregnancy-induced hypertensive disorders by means of ultrasound-Doppler methods

As referred to earlier, it is most likely that the underlying cause of the maternal circulatory maladaptation syndrome, of which pregnancy-induced hypertensive disorders are but one expression, must be sought in a misalliance of fetal trophoblast with maternal tissue in the uteroplacental bed (see chapter 2). This misalliance is thought to block the second wave of cytotrophoblast invasion into the walls of the myometrial portions of the spiral arteries, which normally occurs between 16 and 20 weeks’ gestation. The spiral arteries by-passed by migratory trophoblast and left with an undisturbed nonpregnant architecture fail to dilate (for review see Robertson and Khong, 1987).

The development of continuous wave and pulsed range-gated doppler ultrasound systems has made it possible to study the perfusion of deep-lying vessels such as the arcuate arteries and other branches of the uterine vasculature (Campbell et al, 1983; Griffin et al, 1983; Cohen-Overbeek et al, 1985; Trudinger et al, 1985; Campbell et al, 1986; Schulman 1987). Because of the as yet inevitable errors in the measurement of volume flow (Griffin et al, 1983) most emphasis has been put on the quantitative examination of flow velocity waveforms. The velocity waveforms in the branches of the uterine arteries in normal pregnancy show an increase in end-diastolic flow velocity between 14 and 20 weeks; there appears to be no further change in this pattern of low pulsatility and high diastolic velocity until term (Campbell et al, 1983; Cohen-Overbeek et al, 1985; Trudinger et al, 1985). Since the velocities in end diastole are thought to reflect vascular resistance, the change in flow velocity profile observed in the arcuate arteries in the first half of normal pregnancy is in agreement with the demonstrated occurrence of secondary trophoblast invasion in the wall of the spiral arteries followed by vasodilatation.

In their first study Campbell et al. (1983) observed arcuate artery waveform patterns with reduced end-diastolic velocities suggestive of an elevated uteroplacental vascular resistance in 14 of 31 patients with complicated pregnancies.
Of these 14 patients 10 had proteinuric hypertension and 8 were eventually delivered of a small-for-gestational age infant. Of the 17 patients with normal arcuate artery flow velocity waveforms, only 4 had proteinuric hypertension, and 6 of the fetuses were growth-retarded. These investigations were done in patients with an established disorder of pregnancy. A later article dealing with the same study states that the clinicians who managed the patients had no knowledge of the results (Cohen-Overbeek et al, 1985) but it cannot be excluded that the investigators were aware of the clinical severity of the pregnancy complication, which may have biased the results. The results of this study are supported by those of a larger non-blinded study by Trudinger et al. (1985) who assessed uterine arterial waveforms in 91 patients with complicated pregnancies.

To date the only prospective study in which the predictive value was determined of the occurrence of abnormal arcuate artery waveforms in the second trimester between 16 and 18 weeks' gestation has been reported by Campbell et al. (1986). A group of 149 unselected pregnant women was investigated. Of these 149 women, 17 were delivered elsewhere and there were incomplete records for 6 cases; of the remaining 126 women 41 were nulliparous. Arcuate artery waveforms were determined between 16 and 18 weeks' gestation, the clinicians who took care of the patients were not informed about the results. Of the 76 women with normal waveforms 5 developed pregnancy-induced hypertension, one with fetal growth retardation, and 5 pregnancies were complicated by isolated fetal growth retardation. Of the 50 women with abnormal waveforms 10 developed pregnancy-induced hypertension, 2 of which were complicated by fetal growth retardation, and 10 pregnancies were complicated by fetal growth retardation alone. There was one fetal death in a hypertensive patient. The sensitivity of determination of arcuate artery waveforms at 16–18 weeks' gestation as a test to predict pregnancy-induced hypertension and/or fetal growth retardation is 68%; specificity is 69%. The predictive value of a positive test is 42%, of a negative test 87%. The authors do not present an explanation of the remarkable finding of 40% abnormal waveforms in an unselected sample of pregnant patients, or of the high incidence (24%) of complicated pregnancies.

Measurement of arcuate artery blood flow velocities between 16 and 18 weeks' gestation may diagnose impaired trophoblast invasion of the spiral arteries and may therefore have great potential in predicting pregnancies destined to become complicated by a hypertensive disorder and/or fetal growth retardation. In addition, this method may be useful to monitor the course of the hypertensive disorder and the effects of treatment. However, the errors of measurement (Griffin et al, 1983) and the many possible sampling errors (Wallenburg 1987) need further analysis and assessment. Judgement on the validity of these methods await the results of further well designed and carefully executed prospective clinical studies.
3.5 Conclusion

None of the tests advocated for prediction of pregnancy-induced hypertensive disease has been shown to be clinically useful. Determination of hematocrit values, of serum uric acid concentrations, of platelet counts and, to a lesser extent, of plasma AT III concentration and factor VIII R : Ag /VIII : C ratio are of value in monitoring the progress of established hypertensive disorders in pregnancy. However, both the rather complicated angiotensin sensitivity test and the simple roll-over test could be subjected to further scientific scrutiny to determine their usefulness for research purposes to define a group of women at high or low risk of developing a pregnancy-induced hypertensive disorder.
From the review presented in chapter 3 of methods reported to predict the development of pregnancy-induced hypertensive disorders (PIH) in the course of pregnancy, a preference has emerged for two tests to define women at risk at 28-32 weeks’ gestation. One is the angiotensin-II sensitivity test (AST), which has a good pathophysiologic basis but is rather time-consuming and complicated. The other one is the roll-over test (ROT); it is easy to perform, but its pathophysiologic basis is not understood. However, as judged on the basis of the available literature the validity of both predictive tests remains questionable.

For that reason the present study was designed to contribute to the assessment of the validity of the ROT and the AST in predicting the development of PIH in the third trimester of pregnancy.

4.1 Patients and methods

Ninety healthy normotensive primigravidae attending the Antenatal Clinic of the Erasmus University Hospital Rotterdam were recruited for this study. Informed written and oral consent was obtained in all cases. The women used no medication except oral iron supplements, and their diets were unrestricted. None of the women had a history of hypertension, cardiovascular or renal disease. The duration of pregnancy at the time of recruitment was 24-27 weeks; pregnancies had been uneventful with maximum diastolic blood pressures of 80 mm Hg and adequate fetal growth.

4.1.1 Angiotensin-II Sensitivity Test (AST)

An AST was performed at 28 weeks’ gestation. The women were placed in a quiet room in left lateral position. An intravenous catheter was introduced into the right cubital vein and connected to an infusion pump containing 50 μg of angiotensin-II-amide (Hypertensin, Ciba Geigy B.V., Arnhem, The Netherlands) in 50 ml of dextrose 5% solution. Blood pressure was taken on the left (inferior) upper arm using a standard sphygmomanometer with the cuff at the level of the heart. The point of muffling of the Korotkoff sounds (phase IV) was recorded.
as the diastolic blood pressure. Blood pressure was measured every 5 minutes until a stable diastolic blood pressure was recorded for 15 minutes. The AST was then performed as described by Gant et al. (1973). Infusion was started at a rate of 3 ng per kg of bodyweight per minute, and increased stepwise by 1 ng/kg/min at 5-minute intervals. The minimum amount of angiotensin-II (ng/kg/min) that caused a rise in diastolic blood pressure of 20 mm Hg was defined as the Effective Pressor Dose (EPD). The test was stopped after the EPD had been determined in duplicate or when infusion at a rate of 12 ng/kg/min had not yet caused a rise in diastolic blood pressure of 20 mm Hg. A test with an EPD of 10 ng/kg/min or less was considered positive. The majority of the tests were done by one investigator.

4.1.2 Roll-Over Test (ROT)

In all women an ROT was performed following the AST. In 10 women we also did an ROT before the AST to assess whether the preceding AST would influence the results of the ROT. The women remained in left lateral recumbent position until diastolic blood pressure had returned to its baseline level and had remained constant for 15 minutes. She was then turned on her back and blood pressure was measured immediately and again after 5 minutes; the highest blood pressure after turning supine was recorded. A rise in diastolic blood pressure of at least 20 mm Hg was defined as a positive pressor response. The execution of the ROT in this study is similar to that described by Gant et al. (1974), with the difference that Gant et al. (1974) recorded blood pressure from the right (superior) arm whereas we used the left (inferior) arm. Turning from the left side on the back with blood pressure taken on the superior arm will result in a predictable increase in diastolic blood pressure of approximately 10-12 mm Hg due to increase in hydrostatic pressure relative to the level of the heart (Sobel et al., 1980; Kassar et al., 1980).

For that reason we corrected the level of increase in diastolic pressure of at least 20 mm Hg, as used by Gant et al. (1974) to define a positive test, by 11 mm Hg, and also assessed the significance of a diastolic pressure response of 9 mm Hg or more. The validity of this correction was evaluated in 20 non-pregnant women.

4.1.3 Follow-up

Following the AST and ROT the women received standard antenatal care, in which the investigators were not involved. The results of the tests were not revealed to the women herself or to the attending obstetricians.
4.1.4 Definitions

Pregnancy-Induced Hypertensive Disease (PIH) was defined as the occurrence before labor of a diastolic blood pressure of 95 mm Hg or more on at least 2 occasions 6 or more hours apart in a previously normotensive woman. Pregnancy-induced hypertensive disease with proteinuria (≥0.5 g/l) in the absence of a urinary tract infection was defined as preeclampsia.

The birthweight index is the ratio of observed to ideal birthweight according to the 50th percentile of the distribution of fetal weight corrected for gestational age and fetal sex (Kloosterman 1970). A baby with a birthweight index of 1 has an actual birthweight that is exactly the same as the ideal birthweight according to the 50th percentile. Small-for-gestational age (SGA) babies have a low birthweight index.

4.2 Results

In the 10 women in whom the ROT was performed before as well as after the AST, the change in sequence had no appreciable influence on the results of the ROT.

None of the 90 pregnant women showed an increase in diastolic blood pressure of 20 mm Hg after turning supine, which means that according to Gant's (1974) definition all tests were negative. Using the corrected diastolic blood pressure response of 9 mm Hg or more, 9 women had a positive test.

A positive AST was found in 22 (24.5%) of the women studied. No correlation could be demonstrated between the change in diastolic blood pressure after rolling over and the effective pressor dose of angiotensin-II.

The relationship between the development of pregnancy-induced hypertensive disease and the results of the ROT and AST is presented in Table 4.1.

Table 4.1. The development of pregnancy-induced hypertensive disease as related to the results of the ROT and AST performed in 90 pregnant women at 28 weeks' gestation.

<table>
<thead>
<tr>
<th>Roll-over Test</th>
<th>Angiotensin-II Sensitivity Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in DBP*</td>
<td>EPD (ng/kg/min)</td>
</tr>
<tr>
<td>&lt;9 mm</td>
<td>≥9 mm</td>
</tr>
<tr>
<td>Number of women remaining normotensive</td>
<td>72</td>
</tr>
<tr>
<td>with PIH</td>
<td>9</td>
</tr>
</tbody>
</table>

*Since using the 20 mm Hg level all roll-over tests were negative, only the corrected 9 mm Hg level is presented.
Three women developed PIH late in the third trimester, 9 patients developed preeclampsia. Predictive variables calculated for both tests are presented in Table 4.2.

Table 4.2. Sensitivity, specificity, and predictive values of the ROT and AST performed in 90 pregnant women at 28 weeks' gestation.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>+ test</td>
</tr>
<tr>
<td>Roll-over test*</td>
<td>25</td>
<td>92.3</td>
<td>33.3</td>
</tr>
<tr>
<td>Angiotensin sensitivity test**</td>
<td>91.6</td>
<td>85.8</td>
<td>50</td>
</tr>
</tbody>
</table>

*Positive ROT: Increase in diastolic blood pressure $\geq 9$ mm Hg  
**Positive AST: Effective pressor dose $\leq 10$ ng /kg /min.

There is a positive and significant ($p < 0.05$, Spearman test) correlation between birthweight index and effective pressor dose of angiotensin-II in the range of 5 to 12 ng /kg /min (fig. 4.1).

In the 20 non-pregnant women no change in diastolic blood pressure, recorded from the left (inferior) arm, was observed when they were turned from their left side on their back.

Fig. 4.1. Relationship between EPD at 28 weeks' gestation and birthweight index in 34 women with an EPD below 12 ng /kg /min.
4.3 Discussion

The results of the ROT are disappointing, none of the women showed an increase in diastolic blood pressure of 20 mm Hg or more. Defining a pressor response of 9 mm Hg or more as a positive ROT, 9 women showed a positive ROT, but only 3 of them developed PIH. Using this corrected criterion of a positive test, the sensitivity of the ROT as determined in this study is one of the lowest reported (see Table 3.2, Chapter 3). It is concluded that the ROT is useless as a method to predict the development of pregnancy-induced hypertensive disorders later in pregnancy.

Our results with the AST are in agreement with the results of Öney and Kaulhausen (1982). Based on an unselected European population, with a prevalence of PIH of 15% (13.3% in our study group) these authors determined a predictive value of a positive test of 45%, and of a negative test of 95% (see Table 3, Chapter 3).

We found a significant correlation between angiotensin-II sensitivity at 28 weeks’ gestation and birthweight; no patient with an EPD $\geq 11$ ng/kg/min was delivered of a small-for-gestational age infant (birthweight below 10th percentile). This finding, that has not been reported by other investigators, suggests an association between uteroplacental circulatory insufficiency and vascular sensitivity to angiotensin-II.

In conclusion, the angiotensin-II sensitivity test appears to be a valid method to distinguish at 28 weeks’ gestation between women with a high risk (about 50%) and a low risk (about 2%) of developing a pregnancy-induced hypertensive disorder. The angiotensin-II sensitivity test is rather complicated and too time consuming to be applied as a screening procedure in clinical practice, but it may be considered useful for research purposes.
Chapter 5

ACETYLSALICYLIC ACID (ASPIRIN) AS AN ANTIPLATELET AGENT

Because the next chapters concern the effects of low-dose Aspirin in pregnant women with a pathologic vascular sensitivity to angiotensin-II, this review covers some aspects of Aspirin with regard to its effects on platelet function, with particular attention to its possible adverse effects on the mother, the fetus, and the neonate.

5.1 History

Willow bark (Salix alba), the antipyretic property of which was known in ancient times, yields a bitter glycoside called salicin, discovered by Leroux in 1827. Piria, in 1838, made salicylic acid from salicin. Phenylsalicylate was introduced into medicine in 1886 by Nencki, and Aspirin (acetylsalicylic acid) in 1899 by Dreser and Hoffmann (Smith and Smith, 1966). Aspirin is the most commonly used medicinal agent in the western world, and over 20 to 30 billion tablets are consumed annually in the United States alone (Kelton 1983). The therapeutic efficiency of Aspirin as an analgesic, antipyretic, and anti-inflammatory drug had been universally established for many decades before its mode of action was discovered in 1971 (Vane 1971). In 1971 Vane discovered that Aspirin acts by prevention, or reduction of prostaglandin biosynthesis through inhibition of a key enzyme, cyclo-oxygenase.

5.2 Effects of Aspirin on platelet function and hemostasis

Aspirin inhibits platelet adhesion to collagen under conditions of stasis or low flow. Aspirin does not affect adhesion at physiologic rates of shear and hematocrit levels (Tschopp 1977).

Aspirin also inhibits "irreversible" or second wave aggregation and the associated release reaction induced by agents such as collagen, adenosine diphosphate, and epinephrine (FitzGerald and Sherry, 1982). Platelets obtained from subjects taking single doses of Aspirin demonstrate impaired aggregation response to epinephrine, adenosine diphosphate, and collagen. The platelet aggregation response to adenosine diphosphate and epinephrine after Aspirin intake is limited to a single wave of "reversible" aggregation and both the "oxygen burst" and the release of serotonin, adenosine diphosphate, adenosine triphosphate, platelet fII, and TXA2 is abolished (FitzGerald and Sherry, 1982; Kelton 1983;
Hawiger *et al*., 1987). The effects of Aspirin on thrombin-induced aggregation and secretion are dose-related; inhibition occurs at a low, but not at a high thrombin concentration (Weiss 1983).

Aspirin prolongs the bleeding time through its inhibition of platelet cyclooxygenase activity and the resultant platelet secretory reaction (Hoak 1983). Aspirin acetylates the alanine residue at the active site of platelet cyclooxygenase (Roth and Siok, 1978; FitzGerald and Sherry, 1982; Hawiger *et al*., 1987). The acetyl group of Aspirin is covalently bound to the active site of cyclooxygenase (Verstraete and Kienast, 1986). Consequently, the inhibition of the enzyme caused by Aspirin is irreversible. Platelets lack nuclei and are unable to resynthesize cyclooxygenase. Therefore, following Aspirin administration, TXA2 synthesis in platelets remains impaired for the duration of their life span.

Salicylate alone is a very weak inhibitor of cyclooxygenase and has no measurable effect on platelet aggregation at concentrations achieved in vivo (Kelton 1983). Salicylate partially prevents the inhibitory action of Aspirin, especially on vascular cyclooxygenase activity. After pretreatment with salicylate platelet cyclooxygenase is significantly more sensitive to the inhibitory action of Aspirin than vessel wall cyclooxygenase (Dejana *et al*., 1981; Merino *et al*., 1980). Recently, Buchanan *et al*., (1986) showed that salicylate inhibits 12-HETE production in the platelet, by inhibiting the cytosol associated peroxidase that normally converts 12-HPETE to 12-HETE. Probably impairment of 12-HETE production decreases platelet adhesion. Aspirin may also indirectly suppress the synthesis of lipoxygenase products by enhancing the effects of endogenous lipoxygenase inhibitors such as 15-HETE (Fletcher-Cieutat *et al*., 1985). The antiplatelet effect of Aspirin may therefore, in addition to its inhibition of cyclooxygenase, also be contributed to inhibition of the lipoxygenase pathway.

High doses of Aspirin (1-2 g) prolong the prothrombin time after 2-3 days (Coldwell 1968). Daily administration of 100 and 300 mg of Aspirin has no effect, but doses of 1 to 2 g decrease the level of coagulation factors II, VII, IX and X. The mechanism is unclear, but vitamin K corrects the defect (Goldsweig *et al*., 1976). Salicylate increases the fibrinolytic activity of blood through increased cellular fibrinolysis (Kelton 1983; Moroz 1977).

### 5.3 Importance of the dosage of Aspirin

The inhibitory effects of Aspirin on platelet activity and hemostasis have led to its use as an antithrombotic agent (Kelton 1983). It has been shown to be favorable in the prevention of thrombosis of prosthetic valves and coronary bypasses, and in the prevention of myocardial infarction and other arterial or venous thrombotic lesions (Marcus 1983; Harker and Gent, 1987).

The optimal antithrombotic dose of Aspirin remains disputed. Doses as high as 3.5 g/day and as low as 20-40 mg/day have been reported to be effective in preventing thrombotic events (Kelton 1983). Obviously, the lowest effective dose of any drug is to be preferred, but in the case of Aspirin this may be
a particular important issue because of its concomitant effect on vessel wall cyclo-oxygenase (Shaikl et al, 1980; de Gaetano et al, 1982). Aspirin inhibits endothelial cyclo-oxygenase, but the vessel wall is probably less sensitive than the platelet and has the capacity to generate new cyclo-oxygenase activity when Aspirin is removed from the system (Higgs and Vane, 1983; FitzGerald et al, 1983). TXA2 formation by the platelet can be inhibited by a small dose of Aspirin. A daily dose of 0.45 mg/kg given for seven days produced a cumulative and virtually complete inhibition of platelet TXA2 production without significantly reducing the urinary secretion of prostacyclin metabolites in healthy men and women (Patrignani et al, 1982). Although most investigators found platelet cyclo-oxygenase to be more sensitive to Aspirin than endothelial cyclo-oxygenase, this has not been confirmed in other studies (Masotti et al, 1979; Hoak 1983; Jaffe and Weksler, 1979). Variations in these findings may reflect differences between test conditions and their influence upon the capability of the endothelium to produce PGI2. The endothelial recovery process is dependent upon protein synthesis and reflects the production of new cyclo-oxygenase (fig. 5.1). It was recently shown that Aspirin does not affect the release of endothelial derived relaxing factor (EDRF) (Radomski et al, 1987). EDRF, recently identified as nitric oxide, is a non-prostaglandin inhibitor of platelet function and a powerful vasodilator (Palmer et al, 1987).

![Fig. 5.1. Differential inhibition of platelet TXA2 synthesis by low-dose Aspirin.](image-url)

In the current dispute on the effects of "low" versus "high" doses of Aspirin the pharmacokinetics of the drug should be taken into consideration (Verstraete and Kienast, 1986). Aspirin is a weak acid (pKa 3.5) and therefore exists primarily in the ionized form in blood or body tissues. It spontaneously hydrolyzes to sodium salicylate and the rate of hydrolysis is proportional to the pH (Kelton 1983). The pharmacokinetics of both Aspirin and salicylate are best described by a two-phase model. The half-lives of the alpha-phase of Aspirin and salicylate are 2.7 and 3.8 minutes respectively, and the half-lives of the beta-phase are 15 and 238 minutes, respectively (Rowland et al, 1967). The slow beta-elimination
of salicylate reflects the high degree of plasma protein binding. The excretion and metabolism of salicylate are rate-limited by the unbound plasma concentration.

Aspirin in conventional tablets is rapidly absorbed in the stomach and upper intestine. Peak plasma levels occur 15-20 minutes after oral consumption of Aspirin. For uncertain reasons, women absorb more Aspirin and salicylate (or faster) than men. The sex difference may be of interest given the sex-related difference in efficacy of Aspirin as an antiplatelet agent in certain clinical disorders (Kelton 1983).

Recent studies, using a deuterated Aspirin analogue, have shown that platelets passing through the gut capillaries while an oral dose of Aspirin is undergoing presystemic hydrolysis, are exposed to significantly higher concentrations of Aspirin than platelets in the peripheral circulation (Pedersen and Fitzgerald, 1984). Absorption of a low oral dose of Aspirin causes relatively high concentrations in the pre-systemic (portal) circulation leading to a cumulative inhibition of cyclo-oxygenase in platelets passing through the gut capillaries, whereas the concentration in the peripheral circulation remains too low to affect the enzyme in vascular endothelium (Pedersen and Fitzgerald, 1984; Rosenkranz and Frohlich, 1985; Moncada and Higgs, 1986). Once platelets have been inactivated in the portal circulation, they remain inactivated and therefore inhibition of total body platelet cyclo-oxygenase continues for several days after the last oral intake of Aspirin; restoration of total body platelet cyclo-oxygenase depends on the inflow of new platelets.

5.4 Safety aspects of the use of Aspirin in pregnancy

Aspirin is the most frequently ingested drug in pregnancy, either as a single agent or in combination with other drugs (Corby 1978). In eight surveys totaling over 54,000 patients, Aspirin was consumed at some time during gestation by slightly over 33,000 (61%) (Palmisano and Cassidy, 1969; Forfar and Nelson, 1973; Finningan et al, 1974; Collins and Turner, 1975; Slone et al, 1976; Hill et al, 1977; Harrison et al, 1978; Bodendorfer et al, 1979). The true incidence is probably much higher than this because many patients do not remember having taken Aspirin or consume drug products without realizing that they contain large amounts of salicylates (Palmisano and Cassidy, 1969; Finningan et al, 1974; Harrison et al, 1978). Evaluation of the maternal and fetal effects of Aspirin is thus difficult due to this common, and often hidden exposure. However, some toxic effects on the mother and fetus from large doses of salicylates have been known since 1893 (Jackson 1948).

Aspirin consumption during pregnancy may produce adverse effects in the mother: anemia, antepartum and/or postpartum hemorrhage, and a slight prolongation of gestation and labor (Collins and Turner, 1975; Lewis and Schulman, 1973; Rudolph 1981; Stuart et al, 1982; Stuart 1983). In a prospective but non-blinded Australian study, regular ingestion of high doses of Aspirin
- usually combined with caffeine, phenacetin, or salicylamide – was found to increase the number of complicated deliveries (cesarean sections, breech and forceps (Collins and Turner, 1975). Maternal side effects in one study included non-dose related prolonged bleeding times and dose related vertigo, tinnitus, headache, and hyperventilation (Wolff et al, 1981).

Fetal and newborn effects, other than congenital defects, from Aspirin exposure in utero are said to include increased perinatal mortality, reduced birth weight, congenital salicylate intoxication, and depressed albumin-binding capacity (Palmisano and Cassidy, 1969; Collins and Turner, 1975; Rudolph 1981). No increase in the incidence of jaundice was observed (Palmisano and Cassidy, 1969), and in the Australian study perinatal mortality was usually a result of stillbirths rather than neonatal deaths; premature closure of the ductus arteriosus was not observed (Collins and Turner, 1975; Turner and Collins, 1975). In animals high doses of Aspirin can close the fetal ductus arteriosus due to inhibition of PGE2 and PG12 synthesis (Coceani et al, 1980; Rudolph 1981). However, a large prospective American study involving 41,337 patients, 64% of whom used Aspirin at some time during gestation, failed to show that Aspirin was a cause of stillbirths, closure of the ductus arteriosus in utero, neonatal deaths, or reduced birthweight (Shapiro et al, 1976). The difference between these findings may be due to the chronic or intermittent use of higher doses by the patients in the Australian study (Collins 1981). A recent study by Ylikorkala and his group (1986) demonstrates the importance of the dose of Aspirin ingested by the mother, with regard to its effects on fetal vascular synthesis of vasodilator prostaglandins. They determined prostacyclin and thromboxane generation by umbilical arteries, neonatal urinary excretion of 6-keto-PGF1alpha, and fetal platelet thromboxane synthesis following administration of a single dose of 100 mg or 500 mg of Aspirin to healthy parturients. Fetal and neonatal prostacyclin was significantly reduced following 500 mg of Aspirin, but it was unchanged in infants of mothers receiving 100 mg of Aspirin. Fetal thromboxane synthesis was reduced after 100 mg as well as after 500 mg of Aspirin ingested by the mother.

There are clinical reports suggesting an association between prostaglandin synthesis inhibitors and the persistent pulmonary hypertension syndrome (Levin 1980), but all reports concern indomethacin treatment. From a recent analysis of the literature, it appears that there is no apparent correlation between the occurrence of the persistent pulmonary hypertension syndrome and the use of prostaglandin synthesis inhibitors (Thiery and Amy, 1986). Zuckerman et al, (1984) did not encounter a single case of persistent pulmonary hypertension among the infants born to 315 women treated with indomethacin for tocolysis at 24-35 weeks gestation.

Aspirin given during the week prior to delivery may effect the hemostatic mechanisms of the newborn (Bleyer and Breckenridge, 1970; Corby and Schulman, 1971; Casteels-Van Daele et al, 1972; Haslam et al, 1974; Ekert and Haslam, 1974; Pearson 1978; Haslam 1975). In the initial studies by Bleyer and Breckenridge (1970), 3 of 14 newborns exposed to Aspirin within 1 week
of delivery had minor hemorrhagic phenomena versus only 1 of 17 non-exposed controls. Collagen-induced platelet aggregation was absent in the Aspirin group, and although of less clinical significance, Factor XII activity was markedly depressed. A direct correlation was found between Factor XII activity and the interval between the last dose of Aspirin and birth. In a later study, 10 mothers consumed about 1 g of Aspirin within 5 days of delivery and had increased intrapartum or postpartum blood loss, resulting in markedly lower hemoglobin levels than controls (Stuart et al, 1982; Stuart 1983). Bleeding complications seen in 9 of the 10 infants included numerous petechiae over the presenting part, hematuria, a cephalohematoma, subconjunctival hemorrhage, and bleeding from a circumcision. No life-threatening hemorrhage, effect on Apgar score, or increased hospital stay was found, nor was bleeding observed in 7 mother-infant pairs when Aspirin consumption occurred 6-10 days before delivery (Stuart et al, 1982; Stuart 1983).

An increased incidence of intracranial hemorrhage in premature or low-birthweight infants may occur after maternal Aspirin use near birth (Rumack et al, 1981). Computed tomographic screening for intracranial hemorrhage was conducted on 108 infants 3-7 days after delivery; 17 of these infants were born to mothers who took 1 or more Aspirin tablets (500 mg) within 1 week of delivery, 91 infants had not been exposed to Aspirin. All of the infants were either 34 weeks or less in gestation or 1,500 g or less in birthweight. Intracranial hemorrhage was demonstrated in 53 infants (49%); 12 (71%) were Aspirin-exposed and 41 (45%) were not Aspirin-exposed infants. The conclusions of this study have been challenged and defended (Soller and Stander, 1981; Corby 1981). In view of the potentially serious outcome, however, Aspirin should not be used in doses necessary for pain relief by patients at risk of premature delivery. There are no reports on an association between low-dose (<100 mg) Aspirin and intracranial hemorrhage in newborn infants.

Several studies have examined the possible association between maternal use of Aspirin and congenital defects with findings either supporting or denying such a relationship. In two large retrospective studies, mothers of 1,291 malformed infants were found to have consumed Aspirin during pregnancy more frequently than mothers of normal infants (Richards 1969; Nelson and Forfar, 1971). In a retrospective survey of 599 children with oral clefts, use of salicylates in the first trimester was almost three times more frequent in mothers of children with this defect (Saxen 1975). Reviewing these studies, Collins (1981) notes several biases, including the fact that they were retrospective, which could account for the results. Three other reports of Aspirin teratogenicity involving a total of 10 infants were found (Benawra et al, 1980; McNiel 1973; Sayli et al, 1966). In each of these cases other drugs and factors were present. A recent retrospective study of 300 children with congenital heart disease suggests that there may be a slightly increased risk of certain types of congenital heart disease – in particular aortic stenosis, coarctation, and hypoplastic left heart syndrome (Zierler and Rothman, 1985). However, the Collaborative Perinatal Project monitored 50,282 mother-child pairs, 14,864 of which used Aspirin during the first trimester
Slone et al, 1976). This prospective study did not find evidence of a teratogenic effect with Aspirin.

In summary, the chronic or intermittent consumption of high doses of Aspirin by pregnant women may affect maternal and newborn hemostatic mechanisms, and may be associated with increased perinatal mortality and low birthweight; teratogenic effects are unlikely. It appears prudent not to encourage the usage of analgetic doses of Aspirin in pregnancy for trivial reasons. However, the reported adverse effects are relatively rare and dose-related; there is no evidence that a low dose of Aspirin carries any significant maternal or fetal risks. Nevertheless, if treatment of pregnant women with a low dose of Aspirin is considered it should be started after 12-14 weeks’ gestation, when the risks of inducing congenital heart disease are minimal.
Chapter 6

LOW-DOSE ASPIRIN RESTORES VASCULAR REFRACTORINESS IN ANGIOTENSIN-SENSITIVE PRIMIGRAVIDAE

As discussed in Chapter 2 there is increasing evidence that the physiologic pregnancy-associated vasodilatation and refractoriness to the effects of vasoconstrictor substances depend on a critical balance between the opposing effects of two eicosanoids: prostacyclin (PGI2) and thromboxane (TXA2). In physiologic pregnancy this balance appears to be tilted in favor of the vasodilator effects of vascular PGI2, whereas in pregnancies complicated by pregnancy-induced hypertensive disease the vasoconstrictor effects of TXA2 appear to dominate. A reduced refractoriness to the pressor effects of angiotensin II, known to precede the clinical development of pregnancy-induced hypertension (Gant et al, 1973), could be an early sign of such an imbalance.

In Chapter 5 it was discussed that a low oral dose of Aspirin, in the order of magnitude of 1-2 mg/kg, inhibits production of TXA2 by platelets, with little suppression of vascular PGI2 synthesis. A low dose of Aspirin could thus restore a disturbed PGI2 - TXA2 balance to the dominance of PGI2. If there is indeed a causal relationship between a relative PGI2 deficiency and an elevated sensitivity to the pressor effects of angiotensin II, a low dose of Aspirin could then also restore physiologic angiotensin II refractoriness in angiotensin-sensitive pregnant women.

To adduce evidence to support or refute this hypothesis we investigated the effect of low-dose Aspirin on vascular angiotensin II sensitivity in normotensive pregnant women with a pathologically reduced angiotensin II refractoriness.

6.1 Patients and methods

Healthy primigravidae attending the Antenatal Clinic of the Erasmus University Hospital Rotterdam with an uncomplicated pregnancy of about 26 weeks' duration were given written and oral information about the purpose and design of the study. Sensitivity to intravenously infused angiotensin II was determined at 28 weeks' gestation in 120 women who gave their informed consent. None of the women had a history of hypertension, cardiovascular or renal disease. The course of pregnancy had been uncomplicated in all cases, with maximum diastolic blood pressures of 80 mm Hg. None of the women used any medication except oral iron supplements, and diets were unrestricted.
6.1.1 Angiotensin II Sensitivity Test (AST)

The AST was performed at 28 weeks' gestation by one investigator, as described in 4.1.1. The minimum amount of angiotensin II that caused a rise in diastolic blood pressure of 20 mm Hg was defined as the effective pressor dose (EPD). A positive test was defined as a test with an EPD of 10 ng/kg/min or less.

6.1.2 Study protocol

Of the 120 women 34 had a positive AST and were enrolled in the Aspirin study. According to a randomization list each patient received a coded package with tablets containing 60 mg of plain Aspirin, or matching placebo. The women were instructed to take one tablet daily at breakfast during at least 6 weeks, and at each antenatal visit they were asked how many tablets they had left. Patients were told to report the use of any other drugs, and were explicitly instructed not to use Aspirin.

The AST was repeated at 34 weeks' gestation in women who were still normotensive, and a venous blood sample was drawn for determination of the thrombin-induced production of malondialdehyde by platelets to assess patient compliance (Wallenburg and Van Kessel, 1978). The investigators remained unaware of the results until the code of the study was broken.

The women involved in the study received standard antenatal care, in which the investigators were not involved.

6.2 Results

After the code of the study had been broken there appeared to be 16 women in the Aspirin group, and 18 in the placebo group. The two groups were comparable with regard to age, bodyweight and effective pressor dose at entry into the study (Table 6.1). In the Aspirin group 4 women smoked 10-20 cigarettes per day, as compared with 3 women in the placebo group.

Table 6.1. Characteristics (median, range) of 34 women with a positive AST at 28 weeks' gestation.

<table>
<thead>
<tr>
<th></th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Effective pressor dose (ng/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin group</td>
<td>23 (18-36)</td>
<td>73 (55-96)</td>
<td>7 (6-10)</td>
</tr>
<tr>
<td>Placebo group</td>
<td>24 (19-34)</td>
<td>72 (46-88)</td>
<td>7 (5-10)</td>
</tr>
</tbody>
</table>

The AST was not repeated in 3 women in the placebo group, because they had become hypertensive (diastolic blood pressure of 95 mm Hg or more) before 34 weeks' gestation; in the Aspirin group all women were normotensive (diastolic blood pressure 85 mm Hg or less) at 34 weeks. Thrombin-induced platelet
malondialdehyde production at 34 weeks' gestation was 4.5 ± 1.0 nmol/10^9 platelets in the placebo group, vs 0.48 ± 0.2 in the Aspirin group (p < 0.001, Wilcoxon test); the highest value determined in the Aspirin group was 1.0 nmol / 10^9 platelets.

The results of the second AST at 34 weeks' gestation are presented in Table 6.2. In the Aspirin group significantly more women had returned to a physiologic angiotensin II refractoriness (EPD > 10 ng/kg/min) than in the placebo group (p < 0.02, Fisher test). The difference between the EPD at 34 weeks and that at 28 weeks (ΔEPD) for each women is presented in Figure 6.1. In 6 women of the placebo group the EPD at 34 weeks was even lower than that at 28 weeks, an occurrence not observed in the Aspirin group.

<table>
<thead>
<tr>
<th></th>
<th>28 weeks</th>
<th>34 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤10</td>
<td>&gt;10^1</td>
</tr>
<tr>
<td>Aspirin group (n = 16)</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Placebo group (n = 18)</td>
<td>18^1</td>
<td>10</td>
</tr>
</tbody>
</table>

^1Test not repeated in 3 women
^2p < 0.02 (Fisher test)

Fig. 6.1. The difference between the effective pressor dose at 34 weeks and that at 28 weeks' gestation (Δ EPD) in the Aspirin and the placebo group.
In the placebo group, a significant ($p < 0.01$, Spearman test) negative correlation was found between the EPD and the highest diastolic blood pressure measured before labor (Fig. 6.2); no such correlation was observed in the Aspirin group.

![Fig. 6.2. The negative correlation between Δ EPD and the highest diastolic blood pressure recorded in the placebo group ($p < 0.01$).](image)

6.3 Discussion

The results demonstrate a significant decrease in angiotensin pressor responsiveness in angiotensin sensitive primigravidae in the third trimester, at the end of a 6 weeks' period of ingestion of a daily oral dose of 60 mg of Aspirin. The low cumulative dose of Aspirin caused marked suppression of platelet TXA2 synthesis, as indicated by the reduction of the thrombin-induced formation of malondialdehyde to approximately 10% of placebo values. Malondialdehyde is a stable by-product of platelet TXA2 synthesis; its determination can be used as a sensitive indicator of Aspirin ingestion (Wallenburg and Van Kessel, 1978). The individual malondialdehyde values indicate that women in both groups complied with the prescribed regimen.

Our results were confirmed by Sanchez-Ramos et al. (1987). In an uncontrolled study these investigators obtained a significant enhancement of the physiologic angiotensin II refractoriness in normotensive primigravidae two hours after a
single oral dose of 80 mg of Aspirin. However, our results are in contrast to those of older studies, which reported an increase in angiotensin II sensitivity after ingestion of much higher doses of prostaglandin synthesis inhibitors in normotensive pregnant women (Everett et al., 1978; Jaspers et al., 1981). This discrepancy may be due to the dose of Aspirin ingested. It is known that cumulative doses of Aspirin in excess of 80-100 mg per day result in progressive inhibition of vascular wall prostacyclin biosynthesis (FitzGerald et al., 1983). As discussed in Chapter 2, loss of the local vasodilator effects of PGI2 may result in increased sensitivity to vasopressor substances such as angiotensin II. We did not measure plasma concentrations or urinary excretion of prostacyclin metabolites in our study, so we can only speculate that the low daily dose of 60 mg of Aspirin left vascular prostacyclin synthesis intact. In combination with the demonstrated marked suppression of platelet thromboxane synthesis, this treatment may have restored the physiologic PGI2-TXA2 balance in favor of the effects of PGI2, thus causing an increase in vascular refractoriness to the pressor effects of angiotensin II.

The results of our study support the hypothesis that a balance between the opposing effects of prostacyclin and thromboxane is involved in the pregnancy-induced refractoriness to the pressor effects of angiotensin II. Since elevated vascular angiotensin II sensitivity is a predictor and an early sign of pregnancy-induced hypertensive disorders (Chapter 3), a low dose of Aspirin may not only restore the physiologic pregnancy-induced angiotensin II refractoriness in women with an elevated angiotensin II sensitivity, but it may also prevent the further development of pregnancy-induced hypertensive disorders in these women.
Chapter 7

LOW-DOSE ASPIRIN PREVENTS PREGNANCY-INDUCED HYPERTENSIVE DISEASE IN ANGIOTENSIN-SENSITIVE PRIMIGRAVIDAE

As pointed out in chapter 5, a daily dose of Aspirin as low as 1-2 mg/kg effectively inhibits platelet cyclo-oxygenase and synthesis of TXA2 by platelets, and therefore may correct the putative PGI2 /TXA2 imbalance. This hypothesis was supported by our finding that a low daily dose of Aspirin restores vascular refractoriness in angiotensin-sensitive primigravidae (Chapter 6). Crandon and Isherwood (1979) obtained a history of Aspirin intake during pregnancy from primigravidae, and showed that in women who had taken Aspirin more than once every 2 weeks throughout pregnancy, pregnancy-induced hypertensive disorders were significantly less common than in women who had no history of Aspirin ingestion. Similarly, results of a more recent, randomized but not double-blind study (Beaufils et al, 1985) suggest that Aspirin might prevent recurrent PIH in multigravidae at risk. However, the possible prevention of PIH in primigravidae by suppression of platelet TXA2 production with low-dose Aspirin has not been investigated.

The clinical trial described in this chapter was undertaken to test the hypothesis that a low-dose of Aspirin may correct the putative functional PGI2 /TXA2 imbalance in angiotensin-II sensitive primigravidae and may thus prevent the development of pregnancy-induced hypertensive disease in these women at risk.

7.1 Patients and methods

The trial was designed to include 46 angiotensin-II sensitive primigravidae, 23 in the treatment group and 23 in the placebo group. This number was chosen to be able to demonstrate statistical significance with an alpha of 0.05 (one-sided) and a beta of 0.05, assuming development of pregnancy-induced hypertensive disease in 60% of the untreated women, with a reduction to 15% in the treatment group.

To collect the required number of angiotensin-II-sensitive women, healthy primigravidae attending the antenatal clinic of the Erasmus University Hospital Rotterdam with an uncomplicated pregnancy of 26 weeks duration were given written and oral information about the purpose and design of the study. Sensitivity to intravenously infused angiotensin-II-amide was determined at 28 weeks’ gestation in 207 women who gave informed written and oral consent. None
of the women had a history of hypertension or cardiovascular or renal disease; the course of pregnancy had been uncomplicated in all cases, with a maximum diastolic blood pressure of 80 mm Hg; none of the women had taken drugs except oral iron supplements, and diets were unrestricted.

7.1.1 Angiotensin-II Sensitivity Test (AST)

The technique of the AST is described in chapter 4. The effective pressor dose (EPD) was defined as the minimum amount of angiotensin-II which caused a rise in diastolic blood pressure of 20 mmHg. A positive test was defined as a test with an EPD of 10 ng/kg/min or less.

After introduction of the intravenous catheter blood samples were taken for routine laboratory tests (hemoglobin, hematocrit, platelet count) and, in addition, antithrombin III activity and serum uric acid level were determined.

7.1.2 Study protocol

All 46 women with an EPD of 10 ng/kg/min or less were enrolled in the study at 28 weeks' gestation. Each patient received a coded package with eighty tablets containing 60 mg of plain Aspirin, or matching placebo, according to a randomization list. Patients were told to take one tablet daily at breakfast until delivery and at each antenatal visit they were asked how many tablets they had left. They were asked to report the use of any other drugs, and were explicitly told not to use any Aspirin containing drugs.

All patients received standard antenatal care, with visits every 2 weeks until 36 weeks' gestation, and once a week thereafter. At 33-35 weeks a venous blood sample was drawn for determination of thrombin-induced production of malondialdehyde by platelets (Wallenburg 1982).

Definitions of pregnancy outcome were decided before the start of the trial. Pregnancy-induced hypertensive disease (PIH) was defined as the presence before labor of a diastolic blood pressure of 95 mm Hg or more on at least two occasions 6 or more hours apart. Pregnancy-induced hypertensive disease and concomitant proteinuria (>0.5 g/l) in the absence of a urinary tract infection was defined as preeclampsia. Birthweights were classified according to the percentiles of distribution of weight for gestational age, corrected for parity and fetal sex (Kloosterman 1970). A birthweight below the 10th percentile was defined as small-for-gestational age (SGA).

Differences in relative frequencies between placebo and treatment groups were analyzed with the F test. The Wilcoxon rank-sum test was used to analyze differences in measured variables between the two groups. A p value <0.05 was regarded as significant.
7.2 Results

At the beginning of the study the groups were comparable with respect to age, bodyweight, diastolic blood pressure, and EPD (Table 7.1). In the Aspirin-group 5 women smoked 10-20 cigarettes/day, as compared with 4 women in the placebo-group. Mean (±SD) dose of Aspirin/day/kg bodyweight at 28 weeks' gestation was 0.8 ± 0.2 mg.

Table 7.1. Clinical characteristics (median, range) of angiotensin-II sensitive (n = 46) and angiotensin-II insensitive (n = 161) patients at 28 weeks' gestation.

<table>
<thead>
<tr>
<th></th>
<th>EPD ≤10</th>
<th>EPD &gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspirin n = 23</td>
<td>Placebo n = 23</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>23 17-38</td>
<td>25 19-36</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73 53-100</td>
<td>73 46-91</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>75 70-80</td>
<td>70 60-80</td>
</tr>
<tr>
<td>EPD (ng/kg/min)</td>
<td>7 5-10</td>
<td>7 5-10</td>
</tr>
</tbody>
</table>

Also with regard to the laboratory results at the beginning of the study the groups were comparable (Table 7.2).

Table 7.2. Laboratory results (mean, SD) in angiotensin-II sensitive (n = 46) and angiotensin-II insensitive (n = 161) patients at 28 weeks' gestation.

<table>
<thead>
<tr>
<th></th>
<th>EPD ≤10</th>
<th>EPD &gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspirin n = 23</td>
<td>Placebo n = 23</td>
</tr>
<tr>
<td>Platelet count (N×10^9/L)</td>
<td>226 59</td>
<td>228 54</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>.33 .028</td>
<td>.33 .027</td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>6.9 .65</td>
<td>7.0 .64</td>
</tr>
<tr>
<td>Uric Acid (mmol/L)</td>
<td>.23 .06</td>
<td>.26 .06</td>
</tr>
<tr>
<td>ATIII act. (U/ml)</td>
<td>1.05 .06</td>
<td>1.07 .05</td>
</tr>
</tbody>
</table>

7.2.1 Women with a physiologic pressor response to angiotensin-II at 28 weeks' gestation

Twenty women had an EPD of 11 ng/kg/min; of these, 3 developed mild PIH. None of the 141 women with an EPD above 11 ng/kg/min developed PIH. None of the 161 women was delivered of a SGA infant.
7.2.2 Women with an elevated pressor response to angiotensin-II at 28 weeks’ gestation

The course and outcome of pregnancy in 44 of 46 women in the study group is summarized in Table 7.3. Two patients in the Aspirin-group were excluded from the analysis after the code had been broken. One woman stopped taking her tablets after one week; she remained normotensive and was delivered of a healthy female infant of 2690 g at 40 weeks. The second woman was found to have a high malondialdehyde value of 10.6 nmol/10^9 platelets at 34 weeks; she later disclosed that she had taken her tablets at first very irregularly and had stopped taking them altogether at 32 weeks’ gestation. She developed severe PIH at 39 weeks and was delivered of a healthy female infant of 3710 g.

Table 7.3. Course and outcome of pregnancy in angiotensin-II sensitive patients.

<table>
<thead>
<tr>
<th></th>
<th>Aspirin (n = 21)</th>
<th>Placebo (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of women with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild PIH*</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Severe PIH**</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Week of delivery:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(median and range)</td>
<td>(37-42)</td>
<td>(30-43)</td>
</tr>
<tr>
<td>No of deliveries &lt;37 weeks</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Mode of delivery:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>CS because of PIH</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Fetal weight (g)</td>
<td>3190</td>
<td>3040</td>
</tr>
<tr>
<td>(median and range)</td>
<td>(2380-4320)</td>
<td>(530-4035)</td>
</tr>
<tr>
<td>Weight in relation to gestational age:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of babies P 2.3-10</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>No of babies P &lt;2.3</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Diastolic blood pressure: *95-100 mm Hg; ** ≥105 mm Hg.

In the Placebo-group the mean (±SD) value of malondialdehyde production by platelets at 33-35 weeks’ gestation was 4.6 ± 1.1 nmol/10^9 platelets, versus 0.51 ± 0.3 nmol/10^9 platelets in the Aspirin group (p < 0.001). Nine women had a normotensive pregnancy with diastolic blood pressures below 95 mm Hg and were delivered of term babies with birthweights above the 10th percentile. Two women were delivered of SGA infants. The histories of these two patients will be discussed in more detail.
Patient No 1: A 24-year-old Caucasian gravida-I, diastolic blood pressure 60 mm Hg at 14 weeks' gestation, EPD 6 ng/kg/min at 28 weeks' gestation, remained normotensive throughout pregnancy. In the third trimester fetal growth seemed to slow down, but fetal cardiotocography showed no abnormalities, until diminished variability and prolonged variable decelerations were observed at 39 weeks. She was delivered by cesarean section of a female infant of 2050 g (<P2.3), Apgarscores 3/7/9. Placental weight was 300 g. The neonatal course was uneventful.

Patient No 2: A 27-year-old black African gravida-I, diastolic blood pressure 75 mm Hg at 13 weeks' gestation, EPD 9 ng/kg/min at 28 weeks' gestation, was admitted to the hospital at 29 weeks' gestation because of premature contractions probably induced by a urinary tract infection. Blood pressure on admission was 120/70 mm Hg. She was discharged after antibiotic treatment. Afterwards diastolic blood pressure gradually increased to a maximum of 90 mm Hg. All laboratory tests remained normal, she did not develop proteinuria. At 38 weeks' gestation she was spontaneously delivered of a healthy female infant of 2415 g (P5-P10), Apgarscores 9/10. Placental weight was 500 g. The neonatal course was uneventful.

The remaining 12 (53% of 23) women all developed PIH. One women developed mild PIH at 38 weeks' gestation, she was delivered of a healthy infant with a birthweight just below the 10th percentile. Three women developed severe PIH. Their obstetric histories are described below.

Patient No 3: A 34-year-old Asian gravida-I was booked at 12 weeks' gestation; her blood pressure was 110/80 mm Hg. The EPD at 28 weeks' gestation was 9 ng/kg/min, blood pressure was 110/70 mm Hg. During placebo treatment her blood pressure gradually increased to 140/110 mm Hg at 36 weeks' gestation. All laboratory results remained normal, no proteinuria developed. She was admitted at 36 weeks' gestation with a diagnosis of severe PIH. At 39 weeks' gestation she had a spontaneous delivery resulting in a healthy male infant of 2685 g (P5-P10). Placental weight was 580 g. Blood pressure returned to a normal level within 2 days after delivery. The neonatal course was uneventful.

Patient No 4: A 25-year-old Caucasian gravida-I was booked at 12 weeks' gestation; her blood pressure was 120/75 mm Hg. At 28 weeks' gestation her blood pressure was 120/75 mm Hg, the EPD was 10 ng/kg/min. During placebo treatment the course of pregnancy was uneventful until 39 weeks when a blood pressure of 150/110 mm Hg was found. She was admitted, treatment consisted of complete bedrest. Laboratory tests were normal except an increase in serum uric acid concentration from 0.20 mmol/l at 28 weeks to 0.49 mmol/l on admission, no proteinuria developed. At 40 weeks' gestation she had a spontaneous delivery resulting in a healthy male infant of 3345 g (P25-P50). Placental weight was 750 g. Blood pressure returned to a normal level within 2 days after delivery. The neonatal course was uneventful.
Patient No 5: A 19-year-old Caucasian gravida-I was booked at 10 weeks’ gestation; her blood pressure was 110/65 mm Hg. At 28 weeks’ gestation blood pressure was 110/70 mm Hg, EPD was 10 ng/kg/min. During placebo treatment pregnancy course was uneventful, until at 37 weeks blood pressure increased to 150/105 mm Hg. All laboratory tests remained normal, no proteinuria was observed. She was spontaneously delivered at 38 weeks of a male SGA infant of 2380 g (P2.3-P5). Placental weight 540 g. The neonatal course was uneventful. Blood pressure returned to a normal level within 24 hours.

Of the remaining 8 women 7 developed preeclampsia, 1 patient became eclamptic. The obstetric histories are as follows.

Patient No 6: A 26-year-old black African gravida-I was booked at the antenatal clinic at 15 weeks’ gestation; her blood pressure was 120/75 mm Hg. At 28 weeks’ gestation blood pressure was 120/75 mm Hg, EPD was 7 ng/kg/min. During placebo treatment diastolic blood pressure remained below 85 mm Hg, until 41 weeks’ gestation when she was admitted because her blood pressure had increased to 140/95 mm Hg. On admission all laboratory tests were normal, there was no proteinuria. During the first days following admission her blood pressure rose to 170/110 mm Hg, and she developed sudden and marked proteinuria (>3 g/l). Labor was induced at 42 weeks’ gestation, during labor blood pressure increased to values of 180/130 mm Hg and antihypertensive treatment with intravenous dihydralazine was begun. She complained of a severe headache and showed marked hyperreflexia. A cesarean section was performed and she was delivered of a male infant of 3350 g (P25-P50), Apgarscores 3/8/10. Placental weight was 700 g. Antihypertensive treatment was gradually decreased, blood pressure returned to a normal level without treatment 6 days after delivery.

Patient No 7: A 38-year-old Caucasian gravida-I was booked for antenatal care at 17 weeks’ gestation, blood pressure was 125/75 mm Hg. At 28 weeks’ gestation EPD was 8 ng/kg/min, blood pressure 135/75 mm Hg. At 31 weeks’ gestation she was admitted because of fetal growth retardation and a blood pressure of 145/110 mm Hg. During bedrest without antihypertensive treatment blood pressure gradually increased to 160/120 mm Hg and she developed hyperreflexia and proteinuria (0.5 g/L). Except for the proteinuria and a slight fall in platelet count all laboratory tests remained normal. At 35 weeks’ gestation a cesarean section was performed because it was felt that eclampsia was imminent. She was delivered of a male infant of 1300 g (P < 2.3), Apgar scores were 4/7/9. Placental weight was 300 g. Post partum blood pressure increased to 170/130 mm Hg, antihypertensive treatment with dihydralazine intravenously was started. Two days after delivery antihypertensive treatment could be stopped, blood pressure returned to a normal level 7 days after delivery. The baby died 10 days after birth of necrotizing enterocolitis.
Patient No 8: A 19-year-old Caucasian gravida-I was booked for antenatal care at 13 weeks’ gestation; her blood pressure was 110/70 mm Hg. At 28 weeks’ gestation blood pressure was 120/80 mm Hg, EPD was 5 ng/kg/min. At 32 weeks’ gestation she was admitted because of a blood pressure of 140/90 mm Hg and fetal growth retardation. She was treated with bed rest and blood pressure decreased to 120/80 mm Hg. Laboratory tests showed hemoconcentration (Hb 9.4 mmol/l; Ht 0.46 l/l), and an ATIII activity of 0.80 U/ml. The serum uric acid level had increased from 0.20 mmol/l at 13 weeks to 0.42 on admission.

At 34 weeks’ gestation blood pressure increased rapidly to 170/115 mm Hg, accompanied by the development of marked proteinuria (>3 g/l). She complained about severe headaches and right upper-quadrant abdominal pain. The fetal CTG showed late decelerations and a cesarean section was performed. She was delivered of a female infant of 1250 g (P< 2.3), with Apgar scores 7/9. Placental weight 280 g. The neonatal course was uneventful. Blood pressure returned to normal 4 days after delivery.

Patient No 9: A 31-year-old Caucasian gravida-I was booked at 13 weeks’ gestation; her blood pressure was 120/70 mm Hg. At 28 weeks’ gestation blood pressure was 140/80 mm Hg, EPD was 5 ng/kg/min. She was admitted at 30 weeks’ gestation because of a blood pressure of 130/100 mm Hg, proteinuria (2 g/l) and fetal growth retardation. Laboratory tests showed that the serum uric acid concentration had increased from 0.23 mmol/l at 13 weeks to 0.47 mmol/l on admission; the platelet count had fallen from 195×10^9/l at 28 weeks to 96×10^9/l. She was treated with bed rest and plasma volume expansion which resulted in a fall of the blood pressure to 130/90 mm Hg and a decrease in the amount of proteinuria to <1 g/l. However repeat ultrasound examinations showed an almost complete arrest in fetal growth. At 32 weeks’ gestation the fetal CTG showed late decelerations and a cesarean section was performed. She was delivered of a male infant of 530 g (P< 2.3), Apgar score 4/0, that died almost immediately. Placental weight was 168 g. Post mortem examination was refused. The maternal post-operative course was uneventful, blood pressure returned to a normal level 3 days after delivery.

Patient No 10: A 32-year-old black African gravida-I was booked for antenatal care at 24 weeks’ gestation; her blood pressure was 130/70 mm Hg. At 28 weeks’ gestation blood pressure was 140/80 mm Hg, EPD was 7 ng/kg/min. During placebo treatment the course of pregnancy was uneventful until at 38 weeks’ gestation blood pressure was found to be 145/95 mm Hg. She was admitted at 40 weeks because of an increase in blood pressure to 180/110 mm Hg. Except the presence of proteinuria (0.5-1 g/l) all laboratory results were normal. At 42 weeks cervical ripening was attempted by means of intracervical prostaglandin E2. However, when blood pressure showed a further rise to 180/120 mm Hg and the fetal CTG showed signs of fetal distress, a cesarean section
was performed. She was delivered of a female infant of 3660 g (P50-P75), Apgarscores were 1/5/10. Placental weight was 700 g. After delivery antihypertensive treatment was begun with dihydralazine intravenously, the dosage was gradually decreased. Without antihypertensive treatment blood pressure was found to be normal 11 days after delivery. The neonatal course was uneventful.

Patient No 11: A 24-year-old Caucasian gravida-1 was booked for antenatal care at 21 weeks' gestation; her blood pressure was 120/80 mm Hg. At 28 weeks' gestation blood pressure was 115/75 mm Hg, EPD was 9 ng/kg/min. At 32 weeks' gestation she was admitted for observation because of a sudden rise in blood pressure to 130/95 mm Hg. All laboratory tests were normal. With bed rest diastolic blood pressure fell to 85-90 mm Hg and she was discharged. Afterwards the course of pregnancy was uneventful until 39 weeks' gestation when blood pressure increased again to 150/110 mm Hg. She was readmitted at 40 weeks because of right upper-quadrant abdominal pain and proteinuria (0.5-1 g/L). Except for the proteinuria and an increase in serum uric acid concentration from 0.21 mmol/l at 21 weeks' to 0.36 mmol/l at 39 weeks' gestation all other laboratory tests remained normal. Labor was induced, and she was delivered of a female infant of 3650 g (P75-P90) with Apgarscores of 6/8. Placental weight 900 g. Blood pressure returned to a normal level within 2 days after delivery.

Patient No 12: A 23-year-old black African gravida-1 was booked for antenatal care at 12 weeks' gestation, blood pressure 120/70 mm Hg. At 28 weeks' gestation blood pressure was 130/80 mm Hg, EPD was 5 ng/kg/min. During placebo treatment the course of pregnancy was uneventful, until 38 weeks' gestation when she was admitted because of a blood pressure of 155/100 mm Hg, without proteinuria. She received bed rest only. At 39 weeks' gestation her blood pressure suddenly rose to 180/140 mm Hg and she developed proteinuria (0.5-1 g/l) and severe headaches. The results of laboratory tests were normal except for a marked rise in serum uric acid concentration, from 0.23 mmol/l at 12 weeks to 0.51 mmol/l at 39 weeks' gestation. Antihypertensive treatment with dihydralazine was started, and labor was induced. She was delivered vaginally of a healthy female infant of 2910 g (P10-P25), Apgarscores 9/10. Placental weight was 700 g. Three days after delivery antihypertensive treatment was stopped and her blood pressure had returned to a normal level 6 days after delivery.

Patient No 13: A 20-year-old black African gravida-1 was booked for antenatal care at 20 weeks' gestation, her blood pressure was 115/70 mm Hg. At 28 weeks' gestation blood pressure was 120/80, EPD was 7 ng/kg/min, the uric acid level was 0.27 mmol/l. Following the start of placebo treatment her next appointment was 3 weeks later, but at 30 weeks' gestation she was admitted with eclampsia. On admission she was in coma, blood pressure 170/120 mm
Further laboratory tests showed hemolysis, a low platelet count, ATIII activity 0.44 U/ml, proteinuria > 20 g/l, elevated liver enzymes and serum uric acid (0.88 mmol/l). The fetal CTG showed no signs of distress. Maternal condition was stabilized with dihydralazine and diazepam intravenously under hemodynamic monitoring. She was delivered by cesarean section of a male infant of 1050 g (P5-P10), Apgarscores 7/9. Placental weight was 180 g. The baby went through a period of severe respiratory distress but recovered. Maternal condition improved rapidly. One week after the cesarean section her blood pressure was 120/80 mm Hg without treatment, proteinuria had disappeared, and all laboratory tests had become normal.

Of the 21 women in the Aspirin-group, 15 had an uneventful and normotensive pregnancy, ending in the vaginal delivery of appropriate-for-gestational age infants. Two patients developed mild PIH, in one case associated with fetal death; their obstetric histories can be summarized as follows.

Patient No 1: A 26-year-old black African gravida-I was booked for antenatal care at 20 weeks' gestation; her blood pressure was 130/85 mm Hg. At 28 weeks' gestation blood pressure was 110/80 mm Hg, EPD was 7 ng/kg/min. After having been enrolled in the study she developed gestational diabetes for which she was treated with a diet and insulin. She was admitted at 35 weeks' gestation because of the development of polyhydramnios. At 37 weeks' gestation her blood pressure increased to 140/95 mm Hg, but she developed no proteinuria and showed no increase in serum uric acid levels (<0.20 mmol/l). At 39 weeks' gestation she went into spontaneous labor and was delivered vaginally of a healthy female infant of 3850 g (P75-P90), Apgarscores 9/10. Placental weight was 990 g. Blood pressure returned to a normal level within 24 hours after delivery.

Patient No 2: A 25-year-old Caucasian gravida-I was booked for antenatal care at 12 weeks' gestation, her blood pressure was 115/80 mm Hg. At 28 weeks' gestation blood pressure was 130/80 mm Hg, EPD was 7 ng/kg/min. During the study pregnancy course was uneventful. She presented at 41.5 weeks' amenorrhea with vaginal bleeding and sudden cessation of fetal movements. On admission a blood pressure of 140/95 mm Hg was found, no proteinuria. Ultrasonography confirmed fetal death and she was delivered of a male infant of 3560 g (P50). Post-mortem examination revealed signs of acute asphyxia, but the suspected diagnosis of abruptio placenta could not be confirmed histologically. There were no hemorrhages and histologic examination of the pulmonary vessels and ductus arteriosus showed no abnormalities.

Four patients delivered an infant with a birthweight <P10 but remained normotensive, three of these were Asian women. The history of the fourth, a Caucasian patient will be briefly discussed.
Patient No 3: A 20-year-old Caucasian gravida-I was booked for antenatal care at 15 weeks’ gestation, her blood pressure was 135/80 mm Hg. At 28 weeks’ gestation blood pressure was 125/75 mm Hg, EPD was 5 ng/kg/min. During Aspirin treatment the course of pregnancy was uneventful. At 41 weeks’ gestation she was admitted in spontaneous labor. Because of breech position and variable decelerations on the fetal CTG she was delivered by cesarean section of a female infant of 2600 g (P2.3-P5), Apgarscores were 1/4/7. Placental weight was 450 g. The neonatal course was uneventful.

The number of cesarean sections was significantly higher in the placebo than in the Aspirin-group (p <0.01). The majority of cesarean sections in the placebo group were done because of severe PIH or preeclampsia. There were more premature deliveries and small-for-gestational age babies in the placebo than in the Aspirin-group, but these differences did not reach statistical significance (Table 7.3).

As shown in Table 7.4 blood loss during delivery was similar in both groups (p >0.05), and excessive bleeding did not occur in either group.

Also in the newborns no hemorrhagic complications were observed, and all surviving infants are thriving.

Table 7.4. Estimated blood loss (ml, mean, SD) during delivery.

<table>
<thead>
<tr>
<th></th>
<th>Aspirin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n =21) mean SD</td>
<td>(n=23) mean SD</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without episiotomy</td>
<td>(n = 13) 185 140</td>
<td>(n = 5) 360 230</td>
</tr>
<tr>
<td>With episiotomy</td>
<td>(n = 7) 345 170</td>
<td>(n = 11) 400 200</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>(n = 1) 475</td>
<td>(n = 7) 450 85</td>
</tr>
</tbody>
</table>

7.3 Discussion

The results of this study show that ingestion of a low-dose of Aspirin from 28 weeks’ gestation until delivery prevents PIH and preeclampsia in primigravidae with an increased vascular responsiveness to angiotensin-II at 28 weeks. The predictive value of about 50% found in several European studies (see chapter 4) accords with the 52% of angiotensin-II-sensitive women in our placebo group in whom pregnancy-induced hypertensive disorders eventually developed. In the
Aspirin-group mild PIH occurred in only 2 women (9.5%), late in pregnancy. Determination of thrombin-induced production of malondialdehyde by platelets in both groups of women indicated that they had complied with the prescribed regimen, at least at the time that the determination was done (33-35 weeks' gestation), except for the two women who were subsequently excluded from the analysis. As already mentioned one of these two women developed PIH, the other one was delivered of a small-for-gestational age infant.

The low dose of Aspirin used in this study did not have any apparent adverse effects in mother or fetus. In particular, the occurrence of hemorrhagic complications depends critically on the dose used (Stuart et al, 1982). Blood loss during delivery in the Aspirin-group was the same as in the untreated group. None of the infants born to an Aspirin-treated mother showed detectable hemorrhage, and premature closure of the ductus arteriosus was not observed. At present no infant has developed pulmonary hypertension. The results of a recent study reported by Ylikorkala et al. (1986), discussed in chapter 5, suggest that it is unlikely that such fetal complications would occur with the low dose of Aspirin used in our study.

As discussed in chapter 2 there is increasing evidence that the physiologic vasodilatation and low vascular resistance to flow in pregnancy depend on production of biologically equivalent amounts of vasodilator PGI2 and vasoconstrictor TXA2 in the fetal and maternal circulations. The results of various biochemical studies suggest that in PIH production of these eicosanoids is tilted heavily in favor of TXA2; enhanced sensitivity to angiotensin-II may be the first sign of such an imbalance (Ylikorkala and Makila, 1985; Walsh 1985).

The low dose of Aspirin used in this study suppressed platelet TXA2 synthesis, as indicated by the reduction of thrombin-induced formation of malondialdehyde to approximately 10% of placebo values. Since it seems unlikely on the basis of studies reported in the literature (FitzGerald et al, 1983) that the low dose of Aspirin would have reduced vascular PGI2 production, it may be assumed that Aspirin treatment restored the physiologic PGI2 /TXA2 balance and thus prevented the clinical development of PIH.

In addition to the results of biochemical studies the results of this clinical trial strongly support the hypothesis of an imbalance between the physiologic effects of vasodilator PGI2 and vasoconstrictor TXA2 as a pivotal pathophysiologic mechanism in the development of PIH. Could these results be clinically applied in the prevention of PIH?

A major problem is that we need a reliable and clinically applicable test to identify patients at risk. As discussed in chapters 3 and 4, the ROT is of no value, and other tests such as the MAP-II have a low sensitivity and specificity. The AST is the most reliable predictive test currently available, but it is too cumbersome and time-consuming to be used as a clinical screening test. A recent development is ultrasound measurement of blood flow velocities in uterine arteries and their branches, which may diagnose impaired trophoblast invasion of the spiral arteries and may therefore have potential in predicting the development
of PIH (Campbell et al, 1986). However, the validity of this method is still disputed. The ideal test for early detection would be a simple laboratory test performed on a single blood or urine sample. At longer notice the presence of a potential genetic marker may provide a possibility of identifying the "high risk" woman (Liston 1987).

The application of low-dose Aspirin in obstetric practice to prevent or perhaps treat PIH should await the results of large randomized clinical trials, that are now being prepared or already executed.
Chapter 8

LOW-DOSE ASPIRIN PREVENTS SUPERIMPOSED PREGNANCY-INDUCED HYPERTENSIVE DISEASE IN ANGIOTENSIN-SENSITIVE PRIMIGRAVIDAE WITH CHRONIC HYPERTENSION

Chronic hypertension accounts for approximately one third of all cases of hypertension during pregnancy (Gallery 1988). For the obstetrician the differential diagnosis between chronic hypertension and pregnancy-induced hypertensive disease depends on the gestational age at which hypertension is first noted. When hypertension is found before 20 weeks’ amenorrhea a correct diagnosis of chronic hypertension is likely (Zuspan 1984; Ferris 1988a).

Generally, women with severe and complicated chronic hypertension will only become pregnant if they have been satisfactorily treated; for that reason hypertensive patients who do become pregnant are usually not at risk from acute hypertensive complications, unless they develop superimposed pregnancy-induced hypertensive disease (Gallery 1988). The incidence of pregnancy-induced hypertensive disorders in women with chronic hypertension has been reported to be 20-30%, i.e. 2-7 times higher than in normotensive pregnant women (Redman 1980; de Alvarez and Welt, 1981; Zuspan 1984; Brinkman 1984; de Swiet 1987; Gallery 1988). Perinatal outcome in patients with chronic hypertension who develop superimposed pregnancy-induced hypertensive disease is rather poor, fetal morbidity and mortality are at least five times greater in patients who develop superimposed pregnancy-induced hypertensive disease (Lopez-Llera 1976; Harvey 1987; Gallery 1988; Ounsted 1988). Without complicating pregnancy-induced hypertensive disease, women with moderate chronic hypertension may, as a group, expect normal perinatal outcome (MacGillivray 1983; Zuspan 1984; Robertson 1985).

Gant et al. (1977) studied pressor responsiveness to angiotensin-II in pregnant women with chronic hypertension. This study showed that in gravidae who did not develop superimposed pregnancy-induced hypertensive disease, vascular responsiveness to infused angiotensin-II was markedly blunted compared to results obtained in women destined to develop superimposed pregnancy-induced hypertensive disease. In women, who ultimately developed superimposed pregnancy-induced hypertensive disease, increased sensitivity to angiotensin-II developed as early as 27 or 28 weeks’ gestation.

The study reported in the previous chapter (Chapter 7) showed that low-dose Aspirin prevents the development of pregnancy-induced hypertensive disorders in normotensive primigravidae with an increased vascular angiotensin-
II responsiveness. In the trial reported here we investigated in an identical randomized placebo-controlled double-blind design the effect of low-dose Aspirin, taken daily from 28 weeks' gestation until delivery, on development of superimposed pregnancy-induced hypertensive disease in primigravid women with chronic hypertension, and an increased blood pressure response to intravenous angiotensin-II at 28 weeks' gestation.

8.1 Patients and methods

The trial was designed to include primigravid women with a diagnosis of chronic hypertension. Except for the chronic hypertension all women were healthy primigravidae attending the Antenatal Clinic of the Erasmus University Hospital Rotterdam with an uncomplicated pregnancy of approximately 26 weeks' amenorrhea.

All patients received alpha-methyldopa in an attempt to keep the blood pressure below 90 mm Hg, but no other drugs were taken except oral iron supplements; diets were unrestricted. Only patients with a diastolic blood pressure of 90 mm Hg or less between 20 and 28 weeks' gestation, no proteinuria, a serum creatinine concentration below 70 μmol/l and an adequately grown fetus were selected for this study.

8.1.1 Angiotensin-II Sensitivity Test

The execution and criteria for interpretation of the angiotensin-II sensitivity test (AST) are described in paragraph 4.1.1.

8.1.2 Study protocol

Sensitivity to intravenously infused angiotensin-II was determined at 28 weeks in 20 women who gave informed written and oral consent. Each patient with a positive AST received a coded package with eighty tablets containing 60 mg of Aspirin, or matching placebo, according to a randomisation list. The further treatment protocol was as described in chapter 7.

Chronic hypertension was defined as the presence of a diastolic blood pressure of 90 mm Hg or more determined on at least two antenatal visits before 20 weeks' gestation. Superimposed pregnancy-induced hypertensive disease (PIH) was defined as a rise in diastolic blood pressure of 20 mm Hg or more, in comparison to diastolic blood pressure before 20 weeks' gestation after initiation of alpha-methyldopa treatment. Superimposed preeclampsia was defined as superimposed pregnancy-induced hypertensive disease plus significant proteinuria (≥0.5 g/l) in the absence of a urinary tract infection.

Birthweights were classified according to the percentiles of distribution of weight for gestational age, corrected for parity and fetal sex (Kloosterman 1970).
8.2 Results

8.2.1 Women with a physiologic pressor response to angiotensin-II at 28 weeks' gestation

In 10 of 20 women tested the EPD was $>10$ ng/kg/min, interpreted as a physiologic refractoriness to angiotensin-II. None of these women developed superimposed PIH or preeclampsia; 8 had an uncomplicated pregnancy that ended in a term delivery of healthy newborns with a birthweight above the 10th percentile. One woman was delivered of a small-for-gestational age (SGA) infant, and another woman was delivered prematurely. Their obstetric histories are briefly summarized below.

Patient no 1: A 32-year-old Caucasian gravida-I was booked at 14 weeks' gestation, her diastolic blood pressure was 95 mm Hg. She was treated with 750 mg alpha-methyldopa per day, and her diastolic blood pressure remained about 90 mm Hg. At 28 weeks' gestation the EPD was $>11$ ng/kg/min. At 31 weeks' gestation she was admitted because of fetal growth retardation; on admission diastolic blood pressure was 100 mm Hg. She was treated with complete bed rest, the dose of alpha-methyldopa was increased to 250 mg q.i.d. The platelet count gradually dropped from 207 to $100 \times 10^9$/l, there was no proteinuria, all other laboratory tests were normal. At 38 weeks' gestation labor was induced. Because of fetal distress she was delivered by cesarean section of a male infant of 1600 g ($<2.3$ percentile). Placental weight 300 g. Blood pressure returned to early-pregnancy levels 3 days after delivery. The neonatal course was uneventful.

Patient no 2: A 26-year-old Caucasian gravida-I was booked for antenatal care at 15 weeks' gestation; her diastolic blood pressure was 100 mm Hg. Treatment was started with 750 mg alpha-methyldopa per day. Until the performance of the AST diastolic blood pressure never reached values above 85 mm Hg. At 28 weeks' gestation the EPD was 11 ng/kg/min. Afterwards, diastolic blood pressure gradually increased to 95 mm Hg. All laboratory tests remained normal, except an increase in serum uric acid level from 0.31 to 0.63 mmol/l, no proteinuria developed. Because of premature rupture of membranes she was delivered of a healthy female infant of 2010 g (P25-P50). Placental weight 575 g. The neonatal course was uneventful.

8.2.2 Women with an elevated pressor response to angiotensin-II at 28 weeks' gestation

Ten women showed an increased vascular responsiveness to angiotensin-II at 28 weeks' gestation; 5 received Aspirin and 5 placebo treatment. All women in the Placebo-group developed superimposed PIH or preeclampsia, 3 patients developed severe superimposed PIH (increase in diastolic blood pressure


greater than 30 mm Hg), and 1 patient developed superimposed preeclampsia. Three women were delivered of infants with birthweights above the 10th percentile, 2 patients were delivered of SGA infants. The obstetric histories will be described below.

Patient no. 3: A 40-year-old Caucasian gravida-I was booked at 12 weeks’ gestation; her diastolic blood pressure was 100 mm Hg. She was treated with 1000 mg alpha-methyldopa per day, diastolic blood pressure dropped to 75 mm Hg and remained so until 28 weeks’ gestation. The EPD at 28 weeks’ gestation was 9 ng/kg/min. During placebo treatment her blood pressure gradually increased, she was admitted at 34 weeks’ gestation because of a blood pressure of 170/110 mm Hg. She was treated with bedrest and alpha-methyldopa, the same dosage as before. Serum uric acid levels increased from 0.19 mmol/l at 12 weeks’ gestation to 0.55 mmol/l at 38 weeks’ gestation, platelet count dropped from $201 \times 10^9/l$ at 28 weeks to $109 \times 10^9/l$ at 37 weeks’ gestation, but she did not develop proteinuria. Because of hypertension and fetal growth retardation labor was induced at 38 weeks. She was delivered of a female infant of 2395 g (P5-P10), placental weight 410 g. During labor blood pressure increased to 230/145 mm Hg, and after delivery she complained of severe headache and right-upper quadrant abdominal pain and she developed marked hyperreflexia. Because of imminent eclampsia she was given 300 mg diazoxide intravenously, also an intravenous infusion of diazepam was started. Blood pressure had returned to early-pregnancy levels without additional treatment 4 days after delivery. The neonatal course was uneventful.

Patient no. 4: A 26-year-old Caucasian gravida-I was booked at 6 weeks’ gestation with a diastolic blood pressure of 95 mm Hg. She was treated with 750 mg alpha-methyldopa per day, her diastolic blood pressure decreased to 80 mm Hg and remained so until 28 weeks. The AST at 28 weeks’ gestation was just positive at an EPD of 10 ng/kg/min. During placebo treatment diastolic blood pressure gradually increased, at 32 weeks’ gestation she was admitted because of superimposed preeclampsia (blood pressure 140/100 mm Hg, proteinuria 500 mg/l). Serum uric acid levels increased from 0.20 mmol in the first trimester to 0.62 mmol/l at 38 weeks’ gestation. Eventually blood pressure rose to 170/120 mm Hg. Labor was induced at 38 weeks’ gestation and she was delivered of a healthy female infant of 3080 g (P50-P75), placental weight 600 g. Blood pressure returned without additional treatment to early-pregnancy levels 4 days after delivery.

Patient no. 5: A 24-year-old Caucasian gravida-I was booked at 15 weeks’ gestation; her diastolic blood pressure was 90 mm Hg. She was treated with 1000 mg alpha-methyldopa per day, her diastolic blood pressure decreased to 75 mm Hg. The EPD at 28 weeks’ gestation was 9 ng/kg/min. During placebo treatment blood pressure remained stable until 37 weeks’ gestation, when diastolic blood pressure increased to 105 mm Hg. All laboratory tests were normal. Because
of hypertension labor was induced at 38.5 weeks’ gestation. She was delivered of a healthy male infant of 2930 g (P25-P50), placental weight 580 g. Blood pressure returned to an early-pregnancy level within 3 days after delivery.

Patient no 6: A 27-year-old Caucasian gravida-I was booked at 12 weeks’ gestation with a diastolic blood pressure of 90 mm Hg. She was treated with 1500 mg alpha-methyldopa per day. With this dose diastolic blood pressure remained about 75 mm Hg until the AST was performed at 28 weeks’ gestation. The AST was positive at an EPD of 7 ng/kg/min. During placebo treatment diastolic blood pressure gradually increased to 95-100 mm Hg. Uric acid levels doubled from 0.22 at 28 weeks’ gestation to 0.46 mmol/l at 37 weeks’ gestation, all other laboratory tests were normal. Because of severe headaches she was admitted at 38 weeks’ gestation, blood pressure 150/105 mm Hg. At 39 weeks’ gestation she was delivered spontaneously of a healthy female infant of 3160 g (P50-P75), placental weight 520 g. Diastolic blood pressure decreased to 90 mm Hg after 4 days.

Patient no 7: A 28-year-old Caucasian gravida-I was booked at 12 weeks; her diastolic blood pressure was 95-100 mm Hg. Treatment with alpha-methyldopa was started and the dose was gradually increased to 1500 mg per day, with this dose diastolic blood pressure remained about 80 mm Hg until 28 weeks’ gestation. The EPD at 28 weeks’ gestation was 7 ng/kg/min, at 35 weeks’ gestation she was admitted because of hypertension (150/110 mm Hg). She was treated with bedrest, and the dose of alpha-methyldopa was increased to 2000 mg per day. All laboratory results were normal. Fetal growth was judged to be normal. At 40 weeks’ gestation labor was induced because of an increase in diastolic blood pressure to 120 mm Hg, headaches and hyperreflexia. She was delivered of a healthy male infant of 2720 g (P<5), placental weight 410 g. Blood pressure returned to early-pregnancy levels after 3 days.

No patients in the Aspirin-group developed superimposed PIH, all birthweights were above the tenth percentile. No excessive bleeding occurred during vaginal delivery in the Aspirin-group, and no adverse neonatal effects of Aspirin were noted.

8.3 Discussion

Chronic hypertension implies a state of vascular disease antedating pregnancy. Chronic hypertension in a women predisposes to the superimposition of pregnancy-induced hypertensive disorders in some 20-30% of the patients. In reported series of preeclamptic and eclamptic patients about 10% are said to have chronic hypertension (de Alvarez and Welt, 1981; Ferris 1988a; Gallery 1988). The arteries in the uterus of pregnant women with chronic hypertension often show medial hypertrophy and hyalin degeneration; also vascular stenosis and mural
thrombi are common. These changes as well as reno-vascular alterations may be held responsible for the elevated risk of developing superimposed pregnancy-induced hypertensive disorders (Zuspan 1984; Robertson 1985; Robertson and Khong, 1987).

In this study low-dose Aspirin prevented the development of superimposed pregnancy-induced hypertensive disorders in all women with chronic hypertension with an increased sensitivity to angiotensin-II at 28 weeks' gestation. In the placebo-group all women developed superimposed pregnancy-induced hypertensive disease or preeclampsia. Because of the small number of patients studied these results are not significant but give reason enough to initiate further studies on the potential use of low-dose Aspirin in preventing superimposed pregnancy-induced hypertensive disorders in women with chronic hypertension.
In this chapter some general conclusions will be presented, based on the studies reported in this thesis, in particular with regard to the objectives as outlined in Chapter 1.

Then the various aspects of the study will be considered in the perspective of a possible preventive approach to pregnancy-induced hypertensive disease.

9.1 General conclusions

1. The etiology of pregnancy-induced hypertensive disease remains unknown, but in recent years evidence has been adduced that a pathophysiologic functional imbalance between vasodilator prostacyclin and vasoconstrictor thromboxane-A2 could be of pivotal importance in the development of this syndrome. However, the eicosanoid system has several particularities that make it hazardous to draw conclusions with regard to its physiologic and pathophysiologic effects from results of biochemical studies. Thus the results of biochemical studies provide no definitive proof for the hypothesis that the pathophysiologic development of pregnancy-induced hypertensive disorders depends on a functional imbalance between prostacyclin and thromboxane-A2.

2. On the basis of a critical search of the available literature the roll-over test and the angiotensin-II sensitivity test were selected as the two most promising tests for early diagnosis of pregnancy-induced hypertensive disorders. These two tests were evaluated in an additional clinical study, to assess their validity. The results show that the roll-over test is of no value in screening for pregnancy-induced hypertensive disorders. For the time being the angiotensin-II sensitivity test appears to be the test with the greatest validity. Unfortunately, this test is time-consuming and therefore only useful for purposes of clinical research.

3. Ingestion of a low daily dose (60 mg) of Aspirin, from 28 weeks’ gestation until delivery, prevents the clinical development of pregnancy-induced hypertensive disorders in primigravidae with an increased sensitivity to the pressor effects of angiotensin-II. Vascular refractoriness to angiotensin-II was restored by low-dose Aspirin treatment in women with an increased vascular sensitivity to angiotensin-II at 28 weeks’ gestation. Low-dose Aspirin as used in this study suppresses platelet thromboxane-A2 synthesis, whereas the effect on vascular prostacyclin production is probably negligible. Our finding that the pharmacologic
manipulation of the eicosanoid system by low-dose Aspirin restores vascular refractoriness to angiotensin-II and results in secondary prevention of pregnancy-induced hypertensive disorders strongly supports the hypothesis that the pathophysiologic development of the various signs and symptoms of pregnancy-induced hypertensive disease, including the loss of vascular refractoriness to angiotensin-II, depends on a functional imbalance between vasodilator prostacyclin and vasoconstrictor thromboxane-A2.

9.2 Perspectives of prevention

Pregnancy-induced hypertensive disorders are among the most common complications of pregnancy, and they constitute a major cause of maternal, fetal and neonatal mortality and morbidity. Prevention of pregnancy-induced hypertensive disorders would mean a significant step forwards in prenatal care.

The term "prevention", as used in preventive medicine, not only refers to averting the occurrence of disease (primary prevention) but also to reversing, halting, or at least retarding the disease process before it has become clinically apparent (secondary prevention).

Primary prevention of a disease depends on two main requirements: first, the etiology of the disease must be known, including causative and predisposing factors; second, it must be feasible to intervene, that is to avoid or manipulate such factors as part of a preventive strategy. With regard to pregnancy-induced hypertensive disorders the causative factor or factors of the failure to respond adequately to fetal-maternal immunologic interaction remains unknown, although a lack of, or a defective, gene product has been postulated (Cooper 1980). However, various predisposing factors have been identified, which may have some relevance to primary prevention.

Apart from pregnancy itself, the major risk factor for pregnancy-induced hypertensive disorders is nulliparity. Previous exposure to a fetal allograft has a protective effect, although early abortions prior to the first viable pregnancy offer no demonstrable protection (Campbell et al, 1985). Another risk factor related to the maternal immune response in pregnancy may be a change of partner (Feeney and Scott, 1980). This can lead to the occurrence of pregnancy-induced hypertensive disorders in a later pregnancy, even in a woman who did not develop hypertensive disease in previous pregnancies by another husband. The putative misalliance of fetal trophoblast (Stirrat 1987) with maternal tissue in the uteroplacental bed as a fundamental factor in the etiology of pregnancy-induced hypertensive disorders is further supported by the finding that the risk of developing pregnancy-induced hypertensive disorders may be reduced by prior blood transfusions (Feeney et al, 1977). Other risk factors that have been suggested and may be related to the maternal immune response are consanguinity, a familial history of pregnancy-induced hypertensive disorders, and fetal factors such as twin pregnancies (Hall and Campbell, 1987). Risk factors that are more difficult to relate to the postulated abnormal immune reaction are ethnic origin, young
age, social class, and deficiencies or excesses in nutrition (MacGillivray 1983). With regard to the latter, no convincing evidence has been presented as yet of successful primary prevention of pregnancy-induced hypertensive disorders by means of early dietary manipulation, neither by changes in composition or caloric value, nor by supplementation of vitamins, minerals or trace elements (Hall and Campbell, 1987). Nevertheless, this remains an important area of research, in particular with regard to secondary prevention by means of modulation of prostaglandin synthesis, which will be discussed later. A final risk group to be mentioned is that of women with chronic hypertension who, as a group, have an approximately five-fold greater risk of developing pregnancy-induced hypertensive disorders than normotensive women (Butler and Bonham, 1963). This may be due to some degree of vascular damage precluding an adequate circulatory adaptational response. There is no data from properly controlled clinical trials to support the possibility of primary prevention in this group by pharmacologic (antihypertensive drugs, diuretics) or other strategies.

Secondary prevention not only requires knowledge of pathophysiologic mechanisms of the disease, but also availability of methods of early detection, and of means of intervention and correction of pathophysiologic changes.

The results of the study presented in Chapter 4 have shown that, at this moment, the angiotensin-II sensitivity test is the most valid predictive test, but it is cumbersome and time-consuming, and as such only suitable for clinical research. With regard to secondary prevention of pregnancy-induced hypertensive disease it is of paramount importance to find a reliable, simple test for detection of the earliest stages of the disease.

A few attempts to secondary prevention of pregnancy-induced hypertensive disorders by manipulation of systems involved in the maladaptation have been reported. Attempts using heparin and dipyridamole (Howie et al, 1975; Bonnar and Sheppard, 1981) to prevent or reduce thrombotic lesions in small vessels (uteroplacental vasculature, kidneys etc.) in an effort to bring down blood pressure and to improve placental function have been unsuccessful. In addition, the side effects of anticoagulants in pregnancy can be dangerous to the mother and the fetus (Wallenburg 1987). Also prophylactic treatment with antihypertensive drugs or diuretics has no demonstrated preventive effect (Chesley 1978).

The recognition of a generalized deficiency of prostacyclin in pregnancy-induced hypertensive disorders with a concomitant increase in thromboxane-A2 action (Ylikorkala and Makila, 1985), has led to attempts to reverse this balance by stimulation of prostacyclin or inhibition of thromboxane-A2 synthesis. Dietary supplementation with essential fatty acid precursors has been reported not to decrease the incidence of pregnancy-induced hypertensive disorders, but it reduced the pressor response to infusion of angiotensin-II in pregnant women (O'Brien and Broughton Pipkin, 1983; O'Brien and Broughton Pipkin, 1985). Prostacyclin is available as a pharmacologic preparation for intravenous administration. The short half-life of prostacyclin makes continuous infusion necessary, which precludes its application in the prevention of pregnancy-induced
hypertensive disease. Various other pharmacologic approaches to alter the prostacyclin/thromboxane-A2 balance have been used. Recently, Dombrowski et al. (1986) reported a reduced incidence of preeclampsia in asthmatic patients using theophylline. Theophylline, a phosphodiesterase inhibitor, increases intracellular cyclic adenosine monophosphate in vascular tissue and in platelets, thus reducing vascular reactivity and inhibiting platelet aggregation (Everett et al., 1978). The finding of this study should be interpreted with caution in view of the limited number of patients with preeclampsia and the retrospective nature of the study. Further studies are required to evaluate the preventive potential of phosphodiesterase inhibitors such as theophylline.

In a randomized placebo-controlled double-blind trial we have shown for the first time that it is possible to prevent the development of pregnancy-induced hypertensive disorders with low-dose Aspirin in normotensive women, judged to be at risk because of an increased blood pressure response to intravenously infused angiotensin-II at 28 weeks' gestation.

From a pathophysiologic point of view other drugs that can modify arachidonic acid metabolism or tissue responsiveness to arachidonic acid metabolites, such as thromboxane synthetase inhibitors, thromboxane-A2 receptor blockers and stable prostacyclin-agonists would have the theoretical advantage of leaving vascular prostacyclin synthesis intact. At this moment such drugs have only been used in animal experiments, and in a few women with severe preeclampsia (Van Assche et al., 1984), and nothing is known about their possible adverse effects. Aspirin has the advantage of having been used for almost a century; there is general consensus that the use of low-dose Aspirin is not associated with congenital defects, and adverse effects in mother or fetus are rare and minor.

Further progress in finding an effective approach to prevent pregnancy-induced hypertensive disorders will probably not depend on the development of new drugs, but on finding a reliable and practical test for early detection of women at risk. Low-dose Aspirin could then be used in women to prevent the clinical development of pregnancy-induced hypertensive disease, a disease second only to preterm labor in causing perinatal mortality and morbidity, and cited as the most important cause of maternal mortality in developed and developing countries.
SUMMARY

CHAPTER 1

In chapter 1 a general introduction is presented and the objectives of the thesis are summarized as follows:

1. to review the literature concerning pregnancy-induced hypertensive disease (PIH).
2. to review the literature on methods used for prediction of PIH.
3. to assess the validity of the roll-over test and the angiotensin-II sensitivity test.
4. to investigate if low-dose Aspirin prevents or reverts the clinical signs of PIH in primigravidae with an elevated vascular sensitivity to the pressor effects of angiotensin-II.
5. to evaluate the possibilities of prevention of PIH.

CHAPTER 2

In chapter 2 a survey is given of the pertinent literature on the cardiovascular pathophysiology of pregnancy-induced hypertensive disorders.

In normotensive pregnancy the physiologic vasodilatation in the fetal and maternal circulations seems to depend on a functional balance between vasodilator prostaglandins and vasoconstrictor thromboxane-A2, angiotensin-II, and norepinephrine. The physiologic importance of other vasodilator systems, such as the Kallikrein-kinin system, atrial natriuretic peptides and calcitonin gene related peptide, is controversial.

In pregnancy-induced hypertensive disease production of thromboxane-A2 and prostacyclin is said to be tipped heavily in favor of thromboxane-A2, resulting in vasoconstriction. Vasoconstriction initially causes a reduction in circulating plasma volume, the increase in peripheral vascular resistance can be clinically recognized as an increase in blood pressure.

Although the concept of a functional prostacyclin-thromboxane imbalance allows an explanation for many of the hemodynamic and clinical manifestations of PIH, the hypothesis remains unproven. In addition, the concept explains the pathogenesis, not the etiology of PIH.
CHAPTER 3

In chapter 3 methods used to predict the development of PIH later in pregnancy are reviewed.

The predictive value of blood pressure measurements in the second trimester of pregnancy appears to be low; tests like the cold pressor and the flicker fusion test are only of historical value.

The roll-over test and the angiotensin-II sensitivity test are discussed in some detail. The roll-over test is simple to apply, the angiotensin-II sensitivity test has a good pathophysiologic basis. Predictive values of both tests as reported in the literature make it impossible to reach a conclusion about their validity.

Some biochemical markers, in particular plasma urate levels, antithrombin III activity, and factor VIII-R:Ag, may be of value in monitoring disease progress, but are of no use in predicting PIH. In this respect, measurement of urinary excretion of prostacyclin metabolites looks promising.

At present the predictive value of ultrasound-doppler methods remains controversial.

CHAPTER 4

The study described in chapter 4 was designed to contribute to the assessment of the validity of the roll-over test and the angiotensin-II sensitivity test, in predicting the development of PIH. These tests were selected on the basis of the literature study presented in chapter 3.

The roll-over test and the angiotensin-II sensitivity test were performed on the same day at 28 weeks’ gestation in 90 normotensive primigravidae. The study showed that the roll-over test has no value for the prediction of PIH. The angiotensin-II sensitivity test was found to have a sensitivity and specificity of 92 and 80%, respectively. The predictive value of a positive test was 50%, that of a negative test 99%. The angiotensin-II sensitivity test is a time-consuming test, which precludes its clinical application on a large scale.

CHAPTER 5

Chapter 5 reviews some aspects of Aspirin as an antiplatelet agent. Aspirin potently and irreversibly acetylates and inactivates cyclo-oxygenase.

A low dose of Aspirin effectively inhibits platelet thromboxane-A2 synthesis, vascular prostacyclin production remains relatively unaltered. The chapter closes with a paragraph on maternal and fetal safety aspects of the use of Aspirin in pregnancy. At present there is general consensus that the use of low-dose Aspirin is not associated with congenital defects, and adverse effects in mother or fetus are rare and minor.
CHAPTER 6

In chapter 6 the hypothesis is tested that a low dose of Aspirin corrects a prostacyclin-thromboxane-A2 imbalance, and thus restores the physiologic vascular angiotensin-II insensitivity of pregnancy. Vascular sensitivity to angiotensin-II was determined in 120 primigravidae at 28 weeks’ gestation; an elevated vascular sensitivity to angiotensin-II was demonstrated in 34 women. Each of the positive women received 60 mg of Aspirin or matching placebo in a randomized, double-blind trial design. The angiotensin-II sensitivity test was repeated at 34 weeks. In the Aspirin group significantly more women had returned to a physiologic vascular angiotensin-II insensitivity than in the placebo group. The restoration of vascular refractoriness to angiotensin-II by low-dose Aspirin is most likely due to inhibition of platelet thromboxane-A2 synthesis and correction of a disturbed prostacyclin-thromboxane-A2 balance.

CHAPTER 7

In chapter 7 a randomized, double-blind, placebo-controlled trial is described, in which the effects were investigated of Aspirin taken in a low daily dose of 60 mg, from 28 weeks’ gestation until delivery, on development of PIH in 46 normotensive primigravidae, judged to be at risk of developing PIH because of a positive angiotensin-II sensitivity test. Low-dose Aspirin prevented the development of PIH in 19 out of 21 Aspirin-treated women; two patients developed mild PIH. In the Placebo group, 11 out of 23 women developed PIH, one patient developed eclampsia. No adverse neonatal or maternal side-effects of Aspirin were encountered.

CHAPTER 8

Chapter 8 describes a randomized, double-blind, placebo-controlled trial in which the effects were investigated of low-dose Aspirin, taken daily from 28 weeks’ gestation until delivery, on development of superimposed pregnancy-induced hypertensive disease in 10 primigravidae with chronic hypertension and an elevated vascular sensitivity to angiotensin-II at 28 weeks’ gestation. Low-dose Aspirin prevented the development of superimposed pregnancy-induced hypertensive disease in all women receiving Aspirin; the 5 patients receiving placebo treatment all developed superimposed pregnancy-induced hypertensive disease.
CHAPTER 9

Chapter 9 presents the conclusions of the thesis with regard to the objectives summarized in chapter 1:

1. the etiology of pregnancy-induced hypertensive disease remains unknown, but in recent years evidence has been adduced from biochemical studies that pregnancy-induced hypertensive disease is characterized by a state of prostacyclin deficiency and thromboxane-A2 dominance.
2. the roll-over test is of no value in predicting PIH, but the angiotensin-II sensitivity test allows a valid prediction. Unfortunately, the test is too time-consuming to be suitable for routine screening.
3. low-dose Aspirin restores physiologic vascular refractoriness to angiotensin-II and prevents the clinical development of PIH in angiotensin-II sensitive women at 28 weeks’ gestation.

The possibilities of secondary prevention of PIH as part of routine prenatal care are discussed. Progress in this area will probably not come from the development of new drugs, but will depend on the development of a simple, valid test to predict the development of PIH. With such a test and the use of low-dose Aspirin secondary prevention of PIH may become feasible.
SAMENVATTING

HOOFDSTUK 1

In hoofdstuk 1 wordt een algemene inleiding tot het proefschrift gegeven en de doelstellingen worden als volgt samengevat:

1. een overzicht geven van de literatuur met betrekking tot zwangerschaps-hypertensie en pre-eclampsie.
2. de methoden te analyseren die in de literatuur naar voren zijn gebracht om zwangerschapshypertensie en pre-eclampsie te voorspellen.
3. de waarde te evalueren van de roll-over test en de angiotensine-II gevoeligheidstest.
4. te onderzoeken of een lage dosis Aspirine bij eerst-zwangeren met een verhoogde vasculaire gevoeligheid voor angiotensine-II de klinische symptomen van zwangerschapshypertensie kan voorkomen of verminderen.
5. de mogelijkheden na te gaan voor preventie van zwangerschapshypertensie en pre-eclampsie.

HOOFDSTUK 2

In hoofdstuk 2 wordt een overzicht gegeven van de relevante literatuur betreffende de cardiovasculaire fysiologie van de zwangerschap en de cardiovasculaire pathofysiologie van zwangerschapshypertensie en pre-eclampsie. De fysiologische vaatverwijding die in de normotensieve zwangerschap optreedt in de foetale en moederlijke bloedsomloop lijkt afhankelijk te zijn van een biologische balans tussen vaatverwijdende prostaglandines en vaatvernauwende stoffen zoals thromboxaan A2, angiotensine-II en noradrenaline. Het fysiologische belang van andere systemen met vaatverwijdende effecten, zoals het Kallikreine-Kinine systeem, atrium-natriuretisch peptide en het "calcitonin-gene-related peptide" is controversieel. Er zijn aanwijzingen dat in het geval van zwangerschapshypertensie en pre-eclampsie het evenwicht tussen de synthese van thromboxaan A2 en prostacycline is verschoven in de richting van thromboxaan A2, wat een functionele vasoconstrictie veroorzaakt. De vasoconstrictie veroorzaakt een vermindering van het circulerende plasmavolume, terwijl de toename van de perifere vaatweerstand later tot uiting komt in een stijgen van de bloeddruk. Hoewel het concept van een functionele verstoring van het prostacycline-thromboxaan evenwicht een verklaring geeft voor veel van de hemodynamische en klinische uitingen van zwangerschapshypertensie en pre-eclampsie, is hij nog onbewezen. Er wordt op gewezen dat het genoemde concept een verklaring kan geven voor de pathogenese maar niet voor de etiologie van zwangerschapshypertensie en pre-eclampsie.
HOOFDSTUK 3

In hoofdstuk 3 wordt een overzicht gegeven van methoden, gebruikt om de ontwikkeling van zwangerschapshypertensie en pre-eclampsie te voorspellen. Het blijkt dat de voorspellende waarde van bloeddrukmetingen in het tweede trimester van de zwangerschap laag is; methoden zoals de ”cold pressor test” en de ”flicker fusion test” zijn uitsluitend van historische waarde. De ”roll-over” test en de angiotensine-II gevoeligheidstest worden meer gedetailleerd besproken. De ”roll-over” test is eenvoudig uit te voeren, de angiotensine-II gevoeligheidstest heeft een goede pathofysiologische basis. De in de literatuur gemelde predictieve waarde van beide tests maken conclusies betreffende hun validiteit niet mogelijk. Een aantal biochemische variabelen, vooral de urinezuurconcentratie in plasma, antithrombine-III activiteit en factor VIII-R:Ag hebben hun waarde bewezen bij de bewaking van het ziekteproces, maar kunnen niet worden gebruikt om zwangerschapshypertensie en pre-eclampsie te voorspellen. Wat dit betreft lijkt de bepaling van prostacycline-metabolieten in de urine wel van waarde te kunnen worden. De voorspellende waarde van een aantal ultrageluid-Doppler methoden is op het ogenblik nog controversieel.

HOOFDSTUK 4

Het in hoofdstuk 4 beschreven onderzoek werd uitgevoerd om de waarde na te gaan van de ”roll-over” test en de angiotensine-II gevoeligheidstest voor de voorspelling van zwangerschapshypertensie en pre-eclampsie. Deze twee tests werden uitgekozen voor verder onderzoek op grond van het resultaat van het in hoofdstuk 3 gepresenteerde literatuuronderzoek. Bij 90 normotensieve eerstzwangeren werd op dezelfde dag bij een zwangerschapsduur van 28 weken een ”roll-over” en een angiotensine-II gevoeligheidstest uitgevoerd. De resultaten van het onderzoek tonen duidelijk aan dat de ”roll-over” test geen enkele waarde heeft voor de voorspelling van zwangerschapshypertensie en pre-eclampsie. De angiotensine-II gevoeligheidstest bleek in dit onderzoek een sensitiviteit te hebben van 92% en een specificiteit van 80%. Er werd een voorspellende waarde gevonden van een positieve test van 50% en van een negatieve test van 99%. De angiotensine-II gevoeligheidstest blijkt nogal veel tijd te kosten, waardoor klinische toepassing op wat grotere schaal niet mogelijk is.

HOOFDSTUK 5

In hoofdstuk 5 wordt een aantal aspecten besproken van Aspirine als een tegen thrombocyten-activiteit gericht middel. Aspirine acetyleert en inactiveert het enzym cyclo-oxygenase; de remming is onomkeerbaar. Een lage dosis Aspirine remt op deze wijze de synthese van thromboxaan A2 in bloedplaatjes, maar de synthese van prostacycline door vaatwanden blijft vrijwel intact. Het hoofdstuk
wordt besloten met een paragraaf over de veiligheidsaspecten van het gebruik van Aspirine in de zwangerschap, voor moeder en voor foetus. De algemene conclusie is dat het gebruik van een lage dosis Aspirine in de zwangerschap niet is verbonden met aangeboren afwijkingen en dat andere mogelijke nadelige effecten voor foetus of moeder zeldzaam zijn en van gering klinisch belang.

HOOFDSTUK 6

In hoofdstuk 6 wordt de hypothese getoetst, dat een lage dosis Aspirine een verstoring van het prostacycline-thromboxaan evenwicht zou kunnen corrigeren en daardoor de fysiologische ongevoeligheid voor de bloeddruk verhoogende effecten van angiotensine-II in de zwangerschap zou kunnen herstellen. De gevoeligheid voor angiotensine-II werd bepaald bij 120 eerst-zwangeren met een zwangerschapsduur van 28 weken. Hiervan werd bij 34 vrouwen een verhoogde gevoeligheid voor angiotensine-II vastgesteld. Ieder van deze vrouwen kreeg 60 mg Aspirine of een niet van Aspirine te onderscheiden placebo voorgeschreven in een gerandomiseerde, dubbel-blinde proefopzet. De angiotensine-II gevoeligheidstest werd bij een amenorroe van 34 weken herhaald. In de Aspirine-groep bleken significant meer vrouwen een fysiologische ongevoeligheid voor angiotensine-II te hebben gekregen dan in de placebo-groep. Het herstel van de vasculaire ongevoeligheid voor angiotensine-II na toediening van een lage dosis Aspirine is waarschijnlijk een gevolg van de daardoor veroorzaakte remming van de thromboxaan-synthese in bloedplaatjes en de daardoor veroorzaakte correctie van een verstoord prostacycline-thromboxaan evenwicht.

HOOFDSTUK 7

In hoofdstuk 7 wordt een gerandomiseerd, dubbel-blind, placebo-gecontroleerd onderzoek beschreven, waarin werd nagegaan of een lage dosis Aspirine de ontwikkeling van zwangerschapshypertensie en pre-eclampsie kan voorkomen. Het onderzoek werd uitgevoerd bij 46 normotensieve eerst-zwangeren met een verhoogd risico voor het ontwikkelen van zwangerschapshypertensie of pre-eclampsie wegens een positieve angiotensine-II gevoeligheidstest in de 28e zwangerschapsweek. De zwangeren kregen 60 mg Aspirine of daarvan niet te onderscheiden placebo per dag voorgeschreven van de 28e zwangerschapsweek tot de bevalling. In de Aspirine-groep kwamen slechts 2 gevallen voor van lichte zwangerschapshypertensie. In de placebo-groep ontwikkelden 11 van 23 zwangeren zwangerschapshypertensie of pre-eclampsie, 1 zwangere kreeg eclampsie. In de Aspirine-groep werden geen ongewenste bijwerkingen bij moeder of pasgeborene waargenomen.
HOOFDSTUK 8

In hoofdstuk 8 wordt een gerandomiseerd, dubbel-blind en placebo-gecontroleerd onderzoek beschreven, waarin werd nagegaan of een lage dosis Aspirine ook de ontwikkeling van gesuperponeerde zwangerschapshypertensie en pre-eclampsie kan voorkomen bij zwangeren met chronische hypertensie. Het onderzoek werd uitgevoerd bij 10 eerst-zwangeren met chronische hypertensie en een verhoogde vasculaire gevoeligheid voor angiotensine-II bij een zwangerschapsduur van 28 weken. Evenals de in hoofdstuk 7 beschreven zwangeren kregen zij 60 mg Aspirine of placebo voorgeschreven van de 28e zwangerschapsweek tot aan de bevalling. In de Aspirine-groep trad geen gesuperponeerde zwangerschapshypertensie op, terwijl in de placebo-groep alle 5 patiënten gesuperponeerde zwangerschapshypertensie of pre-eclampsie ontwikkelden.

HOOFDSTUK 9

In hoofdstuk 9 worden de conclusies geformuleerd aan de hand van de doelstellingen van het proefschrift beschreven in hoofdstuk 1.

1. De etiologie van zwangerschapshypertensie en pre-eclampsie is nog steeds niet bekend, maar uit de laatste jaren verricht biochemisch onderzoek komt naar voren dat deze zwangerschapsziekte wordt gekenmerkt door een toestand van prostacycline-deficiëntie en thromboxaan A2-overwicht.

2. De "roll-over" test is voor het voorspellen van zwangerschapshypertensie en pre-eclampsie van geen enkele waarde. Daarentegen is de angiotensine-II gevoeligheidstest waardevol voor de voorspelling van zwangerschapshypertensie en pre-eclampsie, maar deze test kan niet routinematig worden toegepast omdat hij te veel tijd vraagt.

3. Een lage dosis Aspirine herstelt de fysiologische vasculaire ongevoeligheid voor angiotensine-II en voorkomt de klinische ontwikkeling van zwangerschapshypertensie en pre-eclampsie bij zwangeren met een verhoogde angiotensine-II gevoeligheid bij een zwangerschapsduur van 28 weken.

De mogelijkheden voor secundiare preventie van zwangerschapshypertensie en pre-eclampsie als onderdeel van de gebruikelijke prenatale zorg worden besproken. Vooruitgang op dit gebied moet niet worden verwacht van de ontwikkeling van nieuwe geneesmiddelen, maar veel meer van de ontwikkeling van een eenvoudige, betrouwbare test voor het voorspellen van de ontwikkeling van zwangerschapshypertensie. Als een dergelijke test beschikbaar komt kan secundiare preventie van zwangerschapshypertensie en pre-eclampsie met behulp van een lage dosis Aspirine mogelijk worden.
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