PREGNANCY-INDUCED HYPERTENSION IN A RAT HETEROGENEITY MODEL

ZWANGERSCHAPSHYPERTENSIE IN EEN MODEL VAN HETEROGENE RATTEN

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Voor Ted

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CONTENTS

CHAPTER 1
INTRODUCTION

Hypertensive disorders are common in pregnancy and they cause significant morbidity and mortality in the fetus, newborn infant and mother (Chesley 1978). The hypertensive disorders can be divided in pregnancy-induced hypertension (PIH), a disease caused by pregnancy, and pre-existing chronic hypertension, coinciding with pregnancy. PIH may be accompanied by proteinuria, and the clinical entity is then called preeclampsia (PE). Preeclampsia may also be superimposed on pre-existent hypertension.
In addition to the classic signs of hypertension and proteinuria, various other signs and symptoms may occur, indicating involvement of many other systems (MacGillivray 1983).

The etiology of PIH is not known although many hypotheses have been developed (Chesley 1976, 1978). There is evidence that immunologic factors may be involved in the development of PIH (Beer 1978, Redman 1981, Dodson 1982). Immunologic maladaptation to the fetal allograft may be responsible for inhibition of migration of trophoblastic cells into the walls of the spiral arteries, resulting in an inadequate physiologic vascular adaptation in women developing PIH and/or intrauterine growth retardation (Robertson 1986). In man as well as in animals feto-maternal incompatibility of major histocompatibility complex (MHC) antigens seems to be favorable for the course and outcome of pregnancy (Billington 1964, Palm 1974, Beer 1975, Jenkins 1978, Thomas 1985). Incompatibility of non-MHC antigens, however, seems unfavorable in this respect (Palm 1974, Milgrom 1977, Croy 1982,

Dawson 1982) and may be associated with PIH in man (Need 1983). The question as to what kind of pathophysiologic mechanisms are involved in the evolution of a condition of primary fetomaternal vascular maladaptation to a disorder characterized by hypertension or fetal growth retardation, or both, remains unanswered (Robertson 1986). However, there is increasing evidence that a disturbance of the physiologic balance between vasodilator prostacyclin and vasoconstrictor substances like thromboxane may be involved in this maladaptation syndrome, as first suggested by Wallenburg (1979). A shift of the balance towards thromboxane may lead to increased vascular resistance in various organs, including the uteroplacental bed. This may result in hypertension with or without normal organ flow, or in fetal growth retardation due to decreased uteroplacental blood flow, or both (Ylikorkala 1985, Wallenburg 1987).

To investigate the pathophysiologic mechanisms which play a role in the development of PIH an animal model could be of use. Spontaneous PIH does not seem to exist in animal pregnancy, although various pregnancy-related diseases in animals have been described (Douglas 1971). Therefore, several methods were developed in the past to induce hypertension in pregnant animals.

Hypertension has been established in pregnant animals by reducing uterine or uteroplacental blood flow before or during pregnancy (Hodari 1967, Cavanagh 1974, 1985, Abitbol 1976, 1981). A reduced uteroplacental blood flow has indeed been found in PIH (Dixon 1963, Trudinger 1985). However, it seems likely that placental ischemia is a result of the disease, rather than its cause (MacGillivray 1983). Further studies designed to develop animal "toxemia" were performed by influencing nutritional or hormonal status during pregnancy. These studies have yielded no models with pathophysiologic changes comparable to those in human PIH. Only a few studies have been published concerning animal models in which PIH is induced on an immunologic basis

(Seegal 1946, Okuda 1966, Langford 1967). In these experiments animals were sensitized against placental tissue before pregnancy, or they received anti-placenta serum during pregnancy. Hypertension, proteinuria, an increased fetal death rate and reduced fetal weight were found in these animals, perhaps caused by renal and placental damage. However, these models cannot be compared with human PIH, because sensitization against placental antigens as a cause of PIH would imply an increased rate in multiparous women, which is not the case. A detailed review of various hypotheses of the pathophysiology of PIH and of different animal models is presented in chapter 2.

In view of the possible immunologic mechanism in the pathophysiology of PIH we have chosen to study an animal model with different kinds of fetal allograft. In order to increase fetomaternal incompatibility female Wistar rats were mated with Brown Norway males. Pregnancy results were compared with those obtained in Wistar x Wistar couples. Because the Wistar strain is a randomly bred strain with good reproductive capability, incompatibility of MHC antigens within this strain can be expected to be sufficiently strong to warrant a normal outcome of pregnancy. The effect of fetomaternal MHC-antigen incompatibility on the course of pregnancy is not expected to change markedly when Wistar females are mated with Brown Norway inbred males, but, non-MHC antigen incompatibility may be expected to increase. This animal experimental design has been used in an attempt to answer the following questions:

- is it possible to develop PIH or a PIH-like syndrome in animals by means of an immunologic model ?
- if so, are the pregnancy results in such a model comparable with those in human PIH ?
- is it possible to modulate the disease by pharmacologic intervention and could the model thus be of use in testing medication for treatment of PIH ?

The results of maternal variables and of pregnancy outcome are reported in chapter 3. The results of morphometric studies of placentas and fetal livers, to assess possible placental or fetal ischemia, are reported in chapter 4. In order to influence the prostacyclin-thromboxane balance platelet aggregation was inhibited during pregnancy by ticlopidine medication in another group of crossbred rats. The results of these experiments are also reported in chapter 3 and 4.

In chapter 5 results are discussed against the background of the main objectives of this thesis.

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CHAPTER 2
PREGNANCY-INDUCED HYPERTENSIVE DISORDERS: A review of pathophysiologic mechanisms, experimental approaches in animal pregnancy, and spontaneous animal syndromes.

Pregnancy-Induced Hypertension (PIH) is a worldwide disease, mainly but not exclusively occurring in nulliparous women. In most reports the incidence varies between 1-10% (Davies 1971). This variation in reported incidences may be attributed at least in part to problems concerning definition and classification of the disease. These problems are due to the variation in clinical presentation of the disease and to its still unknown etiology. There is no generally accepted nomenclature, but because of the central role of the hypertension we support the term "Pregnancy-Induced Hypertension" (PIH). In this review this term will be used for hypertensive disorders in pregnancy with or without proteinuria.

According to a recent classification a diastolic blood pressure of 90 mmHg or more in the second half of pregnancy or within 48 hours postpartum in a formerly normotensive woman is categorized as PIH. The blood pressure should be measured twice with an interval of at least 4 hours at phase 4 of the Korotkoff sounds. When PIH is accompanied by proteinuria of 300 mg/l or more it is often called preeclampsia (PE) (Davey 1986). The symptom of edema is now generally regarded as too variable and too generally present in uncomplicated pregnancy to be useful as part of the definition.

Apart from the circulation many other systems, such as the coagulation-platelet system, kallikrein-kinin and renin-angiotensin-aldosterone systems, eicosanoid (prostaglandin) and complement systems are involved in the pathophysiology of PIH and we may indeed call it a "disease of cascades" (MacGillivray 1983). It is unclear whether these mechanisms are primarily or

secondarily disturbed, and what the prime mover could be. During normal pregnancy impressive physiologic changes take place in the maternal organism, possibly induced by immunologic adaptation to the fetal allograft. A disturbance of this physiologic adaptation may be responsible for the inhibition of migration of trophoblast cells and the inadequate vascular adaptation of the spiral arteries, which is found early in pregnancy in women destined to develop PIH and/or fetal growth retardation (Robertson 1986). Abnormal maternal biochemical adaptation, in particular an imbalance between prostacyclin and thromboxane action with dominance of thromboxane, is also suspected to be an early feature in this maladaptation syndrome. As a consequence, platelet aggregation is stimulated and maternal vessels become more sensitive to various vasopressor substances, leading to systemic circulatory maladaptation with hypertension and/or local uteroplacental circulatory maladaptation which may lead to fetal growth retardation (fig. 1) (Wallenburg 1987c).

A better understanding of the mechanisms of adaptation and maladaptation to the fetal allograft and their interrelationships could lead to improvement in diagnosis and treatment. In addition to careful clinical observation, experimental animal models may be helpful to unravel the pathophysiologic mechanisms of PIH. There is considerable doubt as to whether PIH occurs spontaneously in animals, it may be a characteristic human disease (Douglas 1971). On the other hand, it is possible that some pathophysiologic mechanisms of PIH occur or can be induced in pregnant animals without leading to all the clinical signs and symptoms of the disease observed in man. Furthermore, animal models could be useful for the further development of an adequate approach to treatment.

In this review we will first consider some of the etiologic and pathophysiologic mechanisms which are thought to be involved in the

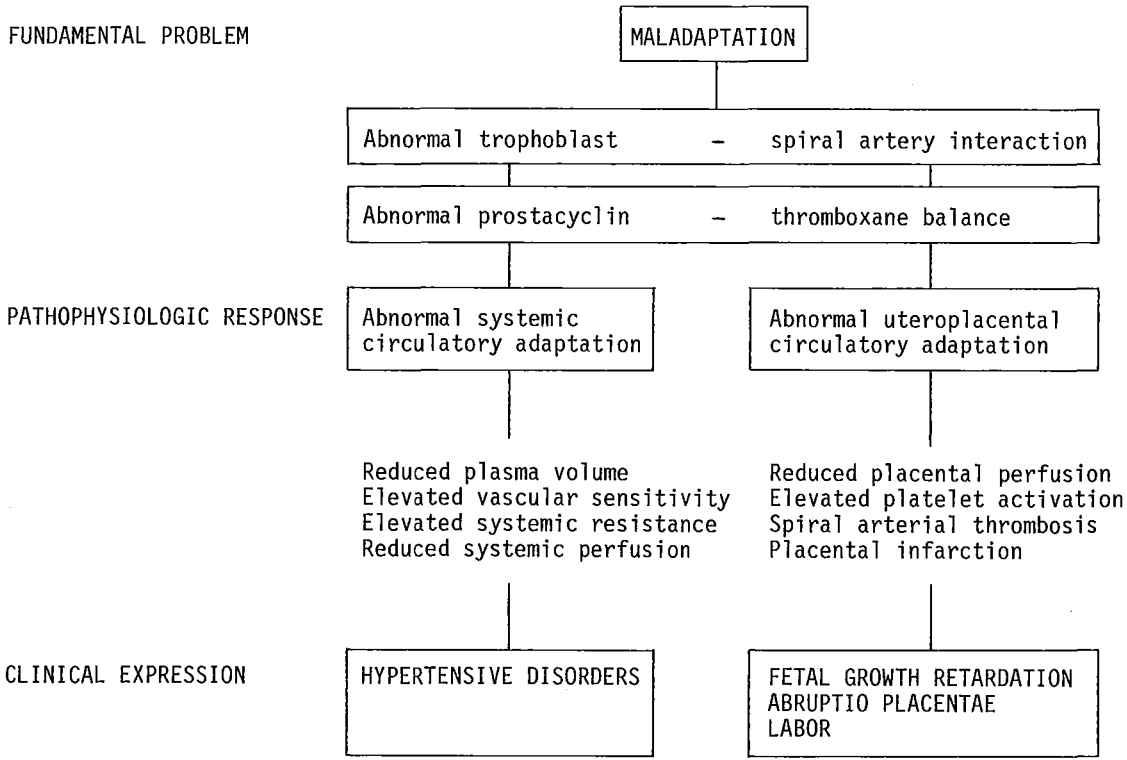

FIG.1 Concept of circulatory maladaptation in pregnancy, leading to maladaptation ("MAD") disease, of which PIH is one of the clinical expressions (Wallenburg 1987c)

development of PIH in man. We will then discuss spontaneous signs of PIH as they occur in animal pregnancy, resembling the disease in man. Finally, various reported attempts to induce the disease in animals will be reviewed.

I HYPOTHESES CONCERNING ETIOLOGIC AND PATHOPHYSIOLOGIC MECHANISMS IN HUMAN PIH.

IMMUNOLOGIC FACTORS

The mechanisms involved in the physiologic protection of allogeneic pregnancies by adaptation of the maternal immune system are complex and not fully understood. They have been extensively reviewed by several authors (Billingham 1981, Beer 1982, Billington 1983, Lala 1983). Because fetomaternal maladaptation may be primarily responsible for the development of several pregnancy related diseases like PIH (Faulk 1981, Anderson 1982, Gille 1982, Wallenburg 1987c) mainstreams of thought concerning the immune mechanisms which may be of importance in normal pregnancy will be discussed first, followed by an assessment of the possible relationship between fetomaternal immune maladaptation and PIH.

Several authors have reported the presence of specific surface antigens on trophoblastic cells in various animal species and in man (Sunderland 1981, Wegmann 1981, Billington 1983, Lala 1983). These antigens are in direct contact with the maternal immune system and can be detected early in pregnancy. Fetomaternal disparity may lead to formation of immune complexes, which theoretically may lead to adaptation but also to rejection of the fetal allograft, depending on the type of immune complex (Gleicher 1979, 1980). In placentae from mice, rats and humans Major Histocompatibility Complex (MHC) antigens of class I, but not of class II, were detected on trophoblastic cells (Wegmann 1981, Sunderland 1981, Billington 1983, Lala 1983, Billington 1986). Incompatibility of MHC class I antigens in the absence of class II MHC

antigens may be responsible for the capacity of trophoblastic cells to induce tolerance instead of rejection in the maternal immune system (Wegmann 1981, Head 1982).

Also non-MHC antigens were found on trophoblastic cells in the rat (Ferguson 1977), the mouse and the human placenta (Billington 1983). Although the role of these non-MHC antigens is far from clear, there is some evidence that incompatibility may exert a negative effect on the outcome of pregnancy. In animal studies an increased occurrence of fetal runting and fetal death has been reported following MHC-compatible but non-MHC incompatible crossbreeding (Palm 1974, Milgrom 1977). After artificial donor insemination in man, an increased rate of PIH was found in women with oligospermic husbands as compared to that in women with aspermic husbands. This has been attributed to sensitization of the women in the oligospermic group against non-MHC sperm specific antigens (Need 1983).

Immunosuppression in pregnancy is modulated by hormones such as estrogens, gonadotrophins, alpha-fetoprotein, placental lactogen and progesterone, of which the latter is probably most important (Beer 1982, Siiteri 1982). Decidualization is another factor which seems to be involved in immunosuppression during pregnancy. It is stimulated by progesterone as well as by fetomaternal antigenic differences (Bell 1983, Lala 1983). Supernatants from decidua-associated suppressor cells have been found to inhibit maternal interleukin-2 dependent cytotoxity (Daya 1987). Immunoprotection may also be achieved by the absorbing capacity of the placenta for immune complexes (Beer 1982).

In summary, fetomaternal MHC incompatibility, progesterone production and decidualization seem to be important factors with regard to adaptation of the mother to the conceptus. On the other hand, non-MHC incompatibility could be unfavorable in this process of adaptation.

The possibility of an immunologic basis underlying the etiology of PIH has

been discussed by several authors (J.S.Scott 1976, J.R.Scott 1976, Kitzmiller 1977, Beer 1978, Redman 1981, Dodson 1982). PIH is predominantly, although not exclusively, a disease of nulliparae. Although PIH is a rare occurrence after a previous normotensive pregnancy, an increased incidence has been observed in pregnancies following a change of partner (Feeney 1978). A decreased incidence of PIH was reported by Stevenson (1976) in consanguineous marriages, and an increased incidence was reported in marriages of dissimilar race (Alderman 1986). These observations suggest that genetic dissimilarity may contribute to the development of PIH. Blood transfusion early in pregnancy has been reported to provide some degree of protection against the development of PIH (Feeney 1977). The same protection may be obtained through prolonged exposure to paternal semen (Marti 1977). Both may be the result of sensitization of protective maternal immune responses by foreign (paternal) antigens. Increased compatibility of Human Leucocyte Antigen (HLA = MHC antigens) between husbands and wives has been found in severe cases of PIH by some investigators (Jenkins 1978, Redman 1978a), but could not be confirmed by others (Persitz 1983). A reduced production of maternal anti-HLA was observed in pregnancies complicated by PIH (Jenkins 1977), but no specific HLA antigen was found to be associated with PIH (Redman 1978a, Jenkins 1978, Persitz 1983). The role of non-HLA antigens is uncertain, but, as mentioned before, exposure to incompatible non-HLA antigen may be involved in the development of PIH (Need 1983). Changes in cellular response have also been noted in PIH; an impaired maternal lymphocyte response to the fetus was described by Jenkins (1978) and Birkeland (1979). However, this hyporesponsiveness may be a consequence rather than the cause of the disease (Griffin 1979). In patients with PIH natural killer cell activity, which could play a role in rejection of the fetus, was reported to be increased (Toder 1983) or decreased (Alanen 1982).

Circulating immune complexes (CIC) may account for the enhanced platelet

activation which is found in PIH (Gibson 1982). Platelet-associated IgG and IgM and complement were found to be increased in preeclamptic patients as compared with normotensive controls (Massobrio 1985, Rote 1987, Samuels 1987, Burrows 1987). An inverse correlation between platelet count and the amount of platelet associated immunoglobulins was demonstrated in two studies (Rote 1987, Burrows 1987); the increased levels of platelet associated immunoglobulins may be due to increased CIC. Increased levels of CIC in patients with PIH have been reported by some authors (Schena 1979, Vasquez Escobosa 1983, Medcalf 1983, Massobrio 1985), but were not confirmed by others (Gleicher 1980, Balasch 1981). Elevated levels of immune complexes have also been demonstrated in tissues of patients with PIH such as the skin (Houwert-de Jong 1982), the kidney (Petrucco 1974) and the placental vessel wall (Kitzmiller 1973).

Elevated levels of complement have been found in placentas of women with PIH (Sinha 1984), suggesting increased maternal-fetal interaction. Using immunofluorescent techniques, complement (C3) deposition was found in the spiral arterial walls in the first trimester of pregnancy. High levels of C3 were found more frequently in primigravidae than in multigravidae, and the spiral arteries of primigravidae with high C3 levels often showed vascular lesions as described in PIH (Lichtig 1985). The demonstrated absence of the physiologic changes in the walls of the spiral arteries of women with PIH (Brosens 1972, Gerretsen 1981) could be a result of immunologic reactions (Robertson 1986). It is noteworthy that this phenomenon is not only observed in patients with PIH, but also in normotensive pregnancies complicated by fetal growth retardation (Sheppard 1981). The physiologic changes in the spiral arteries during pregnancy are caused by early trophoblast migration, a feature common to several animal species and man. The invading trophoblast is surrounded by leucocyte infiltration, which suggests an immune response (Pijnenborg 1981).

PLACENTAL FACTORS

Some investigators consider uteroplacental ischemia to be a precipitating factor in the development of PIH (Page 1972, Speroff 1973, Willems 1977). Ischemic villous lesions, placental infarcts, are a feature of PIH; their frequency of occurrence and their extent are related to the severity of the hypertensive disorder (Wallenburg 1969). Old observations of reduced placental blood flow in PIH as measured by injection of radioactive sodium in the choriodecidual space (Mc Clure Brown 1953, Dixon 1963) are supported by recent studies using ultrasound Doppler (Trudinger 1985, Campbell 1986). The placental changes in PIH are known to be secondary to ischemia (Wallenburg 1973), but it is not clear if the reduction of blood flow precedes or follows the onset of PIH (Rushton 1984).

Placental ischemia could explain some of the pregnancy complications frequently associated with PIH, such as fetal growth retardation and fetal death. However, there is no explanation for the onset of the rise in blood pressure and the general vasoconstriction as the result of chronic placental ischemia itself, especially not in cases without fetal growth retardation.

THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Uteroplacental blood flow is modulated by several vaso-active substances. In particular prostaglandins and the renin-angiotensin system are thought to be involved in the pathophysiology of PIH.

Other vaso-active substances may as well play a role in the modulation of uteroplacental blood flow and can also have general vasomotor effects.

Estrogens have weak vasodilator properties (Wallenburg 1981b), and it is not likely that they are directly involved in the pathophysiology of PIH (MacGillivray 1983). Catecholamines were found to be increased, decreased or unchanged in PIH (Poland 1978, Davey 1981, Tunbridge 1981, Pedersen 1983, Øian 1986) and the involvement of catecholamines in PIH is uncertain.

Because of its strong vasoconstrictive properties attention has been focussed on angiotensin. In normotensive pregnancy all components of the renin-angiotensin-aldosterone system are elevated (Skinner 1972, Weir 1975, Wilson 1980, Hsueh 1982, Pedersen 1982, Symonds 1983). In women with PIH plasma renin concentration (PRC) and plasma renin activity (PRA) are both lower than in normotensive pregnancy (Brown 1966, Weir 1973, Pedersen 1982). Plasma aldosterone concentrations in PIH were also found to be decreased (Weir 1975, Pedersen 1982, 1983). Angiotensin converting enzyme concentration appears to be elevated in PIH (Goldkrand 1986). Reports on angiotensin II (AII) levels in PIH are controversial; levels were found to be decreased (Weir 1973) or increased (Symonds 1975, Broughton Pipkin 1981). These controversial results may be due to inappropriate measurements because of immunologic cross-reactions with AII degradation products (Derkx 1987).

There is general agreement that the physiologic unresponsiveness to exogenous AII in pregnancy is reduced or even absent in patients with PIH (Abdul-Karim 1961). The increase in vascular sensitivity to AII begins before the onset of clinical signs of PIH (Gant 1973, Oeney 1982). In the nonpregnant state the vascular response to endogenous AII is mainly modulated by changes in plasma volume and renin activity, but in pregnant women prostaglandins may be of more importance in this regard (Worley 1979).In pregnancy the vascular refractoriness to AII can be abolished by administration of prostaglandin inhibitors like indomethacin or a high dose of aspirin (Everett 1978, Jaspers 1981). In the isolated placental cotyledon AII response is attenuated by the prostaglandins PGE_1, PGD_2 and 6-βPGI_1, a stable analogue of prostacyclin (PGI_2) (Glance 1986).

In pregnancy the vasopressor renin-angiotensin system seems to be counterbalanced by the vasodilator effects of prostaglandins (Wallenburg 1981b), and perhaps an imbalance between these two systems could induce signs of PIH. In patients with PIH Broughton Pipkin (1981) found higher

venous levels of AII in uterine and umbilical blood than in peripheral venous blood, which suggest production of AII in the placenta. A role of the placenta in conversion of AI to AII is supported by in vitro studies of the placental cotyledon (Glance 1984).

There is a close relationship between prostaglandin, AII and renin production. PGI_2 stimulates renin release (Whorton 1977), which may be expected to increase AII, in the presence of a sufficient amount of renin substrate. Angiotensin II itself stimulates PGI_2 in lungs and kidneys as shown by Mullane (1980) in vivo in dogs. The in vitro production of PGE and PGI_2 was enhanced when AII was injected into the fetal circulation of the human placental cotyledon (Glance 1985).

PROSTAGLANDINS

Prostaglandins are vasoactive substances, which are thought to play an important role in the modulation and regulation of the utero-placental circulation (Wallenburg 1981a). During pregnancy there is a physiologic increase in the production of prostaglandins E_2 and I_2, which have vasodilator action in the utero-placental area (Wallenburg 1981b).

It has been suggested that, in physiologic pregnancy, vasodilator prostaglandin E could counteract the vasoactive effect of AII (Novy 1980). In hypertensive pregnancies prostaglandin E_1 and E_2 levels in placental tissue and urine were found to be reduced as compared with those in normotensive pregnancies (Alam 1973, Demers 1976, Robinson 1979, Hillier 1981, Pedersen 1983). However, it is now known that prostaglandin I_2, prostacyclin, is a much more potent vasodilator and the most potent physiologic inhibitor of platelet aggregation (Moncada 1979). In normotensive pregnancy plasma levels and urinary excretion of PGI_2 metabolites are elevated (Lewis 1980, Goodman 1982, Brash 1983), possibly with a peak during the first (Greer 1985c) or second trimester (Bolton 1981).

In PIH PGI_2 activity in vitro was found to be reduced in maternal, placental and umbilical vessels (Bussolino 1980, Remuzzi 1980 a+b). Also in vitro production of 6-keto-PGF1α, the stable metabolite of PGI_2, was shown to be reduced in umbilical vessels (Downing 1980, Carreras 1981, Stuart 1981, Dadak 1982, Mäkilä 1983) and in placental tissue (Walsh 1985 a+b). PGI_2 activity was also found to be decreased in amniotic fluid from patients with PIH (Bodzenta 1980). The concentration of PGI_2 metabolites was decreased in amniotic fluid (Ylikorkala 1981b), plasma (Yamaguchi 1985, Greer 1985c) and urine (Goodman 1982) obtained from patients with PIH, as compared with concentrations in normotensive pregnant women. In addition, a decreased prostacyclin biosynthesis was observed preceding the clinical signs of PIH (Fitzgerald 1987a). However, these data were not confirmed by all investigators. Moodley (1984) found no decrease in 6-keto-PGF1α concentration in amniotic fluid from patients with PIH; plasma levels were found to be unchanged (Ylikorkala 1981a) or even elevated in PIH (Koullapis 1982). Mäkilä (1984) found the same in vitro placental 6-keto-PGF1α production in normotensive pregnancy as in PIH. The high plasma levels of 6-keto-PGF1α , which were found by Yamaguchi (1985), were attributed to methodologic errors by some authors (Greer 1985b). In general, however, most investigators seem to agree that the vasoconstrictive status in patients with PIH is most likely a result of deficient PGI_2 action (Briel 1981, Lewis 1983, Sitsen 1983, Spitz 1984, Ylikorkala 1985, Lippert 1986).

Prostacyclin not only counterbalances the vasoconstrictive effects of the renin-angiotensin system, but also those of another eicosanoid: thromboxane A_2 (TXA_2). TXA_2 has strong vasoconstrictive and platelet aggregating properties and is released mainly by activated platelets in nonpregnant as well as in pregnant women (Moncada 1979, Fitzgerald 1987b). Data about TXA_2 concentrations, as measured by its stable hydrolysis product

TXB_2 , in normal pregnancy are conflicting and have been found increased (Ylikorkala 1980) or decreased (Greer 1985a). Excretion of major urine metabolites of thromboxane increases in pregnancy (Fitzgerald 1987b). In PIH in vitro production of TXB_2 was found to be increased in the placenta (Mäkilä 1984, Walsh 1985b) or unchanged in umbilical arteries (Mäkilä 1983). Plasma levels of TXB_2 were found to be elevated (Koullapis 1982) or unchanged (Yamaguchi 1985) and amniotic fluid levels unchanged (Ylikorkala 1981b, Moodley 1984). Increased malondialdehyde formation by platelets, which also reflects TXA_2 production, was found in pregnancies complicated by fetal growth retardation with or without hypertension (Wallenburg 1982).

It may be concluded that published data on PGI_2 and TXA_2 production or concentrations are disparate. Perhaps the functional balance between PGI_2 and TXA_2 may be more important than their actual concentrations; when the balance shifts in favor of TXA_2, hypertension may be the result. Disturbance of the placental PGI_2/TXA_2 balance could lead to local vasoconstriction, enhanced platelet aggregation and thrombosis, thus leading to placental ischemia and infarction (Wallenburg 1981a). Such a shift between PGI_2 and TXA_2 was demonstrated by measurements of the in vitro production of PGI_2 and TXA_2 metabolites in placental tissue (Mäkilä 1984, Walsh 1985b). A reduced ratio of in vitro PGI_2 over TXA_2 production appeared to be associated with decreased placental intervillous blood flow (Mäkilä 1986). Indirect evidence that a shift in the balance of PGI_2/TXA_2 may be involved in the development of PIH, with or without fetal growth retardation, is based on the prevention of PIH in women with an increased vascular sensitivity to exogenous AII (Wallenburg 1986) and of fetal growth retardation in women at risk (Wallenburg 1987d) by treatment with low doses of aspirin during pregnancy. A pathologically increased sensitivity of the vasculature to AII is reversed by low dose aspirin medication (Wallenburg 1987a). These effects are explained by the fact that a low dose of aspirin suppresses TXA_2

synthesis with minimal effects on PGI_2 synthesis, and thus restores the dominance of PGI_2.

Dietary factors can interfere with prostaglandin metabolism. Although vitamin E deficiency in animals has shown to reduce PGI production (Karpen 1981, Spitz 1983), there is as yet no indication that vitamin E deficiency is involved in the pathogenesis or pathophysiology of PIH in man (Entman 1984). Prostaglandin metabolism can also be manipulated by the use of essential fatty acids. It has been shown in pregnant patients that vascular sensitivity to AII can be reduced by means of a diet containing primrose oil, which contains a substantial amount of linoleic acid, a precursor of arachidonic acid (O'Brien 1983). In clinically observed patients with PIH a slight, but not significant, improvement of blood pressure was found following administration of linoleic acid (Morrison 1984). Eicosapentanoic acid (EPA) and its precursor linolenic acid inhibit platelet aggregation, because of transformation into TXA_3, which does not interfere with platelet aggregation, and into the potent antiaggregating agent PGI_3 (Dyerberg 1978). In male volunteers using an EPA enriched diet, prolonged bleeding times, decreased TXA_2 and increased PGI_3 production were found. In addition, the systolic blood pressure decreased as well as the blood pressure response to noradrenaline and (not significantly) AII administration (Lorenz 1983). There is, however, no evidence that an absolute or relative lack of essential fatty acids is involved in the pathogenesis of PIH.

THE COAGULATION-PLATELET SYSTEM

In normal pregnancy the coagulation system is in an activated state with raised levels of nearly all coagulation factors (Stirling 1984, Wallenburg 1987b). Some investigators have proposed that thrombin-dependent disseminated intravascular coagulation (DIC) could be involved in the pathophysiology or even pathogenesis of PIH. According to Page (1972) DIC could be attributed in

PIH to an accelerated spill of trophoblastic elements into the maternal circulation, due to placental ischemia. In patients with PIH 20 times more trophoblastic elements were found in the uterine vein at cesarean section than in normotensive women (Jäämeri 1965). After eclampsia McKay (1953) found fibrin thrombi in several organs, which he attributed to DIC. He compared eclampsia to the general Shwartzman phenomenon, perhaps triggered by degradation products from decidua or placenta. Although Sheehan and Lynch (1973) also found fibrin thrombi after eclampsia, this was, in their opinion, not caused by DIC, but the result of ischemia due to vasospasm. In women with PIH an increase in the factor VIII R Ag/VIIIC ratio as compared with that in normotensive pregnancy was reported by several authors (Redman 1977b, Fournie 1981, Scholtes 1983). Although this can be the result of thrombin mediated coagulation, it is more likely that it is caused by increased vascular endothelial damage or release of platelet factor VIII, since the ratio is predominantly increased because of an elevated factor VIII R : Ag (Fournie 1981, Scholtes 1983). Other coagulation factors, as measured directly or indirectly, do not show changes suggestive of marked consumption in most cases of PIH (Howie 1971, Condie 1976, Gibson 1982, Wallenburg 1987b, Burrows 1987).

The turnover of ^{125}I-labelled fibrinogen appeared to be increased in patients with PIH in comparison with normotensive pregnancies (Kuijpers 1982). A thrombin-mediated mechanism was suggested because of the return of the fibrinogen turnover to normal during low-dose heparin medication. An increased ^{125}I-turnover, which returned to normal after low-dose heparin, was also found in normotensive women during bedrest of at least one week (Kuijpers 1982). This finding could, at least in part, explain differences in coagulation patterns between normotensive and hypertensive patients, since most coagulation studies in hypertensive pregnant patients have been performed under conditions of bedrest. Increased thrombin activation in

patients with PIH has also been suggested on the basis of increased levels of fibrinopeptide A (Douglas 1982), but the elevation of fibrinopeptide A was not confirmed in later studies (Wallmo 1984, Burrows 1987). The results of soluble fibrin monomer measurements (cryofibrinogen or protamine sulphate/ethanol gel precipitation assays) are variable, but increased levels were found in some of these studies (Gibson 1982). Soluble fibrinogen/ fibrin monomer complexes as measured by chromatography were found to be elevated in selected patients with severe preeclampsia (McKillop 1976, 1977, Edgar 1977, Beller 1979). Evidence of increased fibrinolysis has been found by several authors with measurement of fibrinogen/fibrin degradation products (FDP) and fragment E in advanced cases of PE (Henderson 1970, Bonnar 1971, Howie 1971, Gordon 1976). Other investigators, however, could not find increased FDP levels, even not in eclampsia (Pritchard 1976, Dunlop 1978).

Direct measurements of plasminogen and indirect measurements of plasminogen activation by the euglobulin lysis time appeared to be variable and did not result in consistent differences between normotensive pregnancies and PIH/PE (Gibson 1982, Wallenburg 1987b). Decreased levels of the physiologic clotting inhibitor antithrombin III were found in patients with PIH and were associated with placental infarction and poor fetal outcome (Weiner 1982, Weenink 1983).

Because the severity of PIH and the presence or absence of fetal growth retardation, which may be associated with coagulation disorders, is often not mentioned, it is difficult to compare the results of these studies and to draw conclusions. Increased thrombin-dependent coagulation may be a feature in some women with severe PIH, but DIC appears to be an exception, even in eclampsia (Gibson 1982, Wallenburg 1987b).

In patients with PIH the average platelet count appears to be reduced as compared with that in normal pregnancy (Bonnar 1971, Howie 1971, Dunlop 1978, Boneu 1980, Giles 1981). A fall in platelet concentration was reported to be

an early feature of the disease (Redman 1978b) and may also be present in mild cases of PIH (Dunlop 1978, Giles 1981, O'Brien 1986). Platelet life span seems to be reduced in PIH (Rakoczi 1979, Boneu 1980), in particular when PIH occurs in combination with fetal growth retardation (Wallenburg 1982). Increased platelet activation as assessed by β-thromboglobulin levels (Redman 1977a, Boneu 1980, Douglas 1982) and a reduced platelet aggregation in vitro in response to various agents (Wigham 1978, Kelton 1985) was found in patients with PIH.

Although thrombocytopenia may be a result of thrombin-dependent coagulation, the majority of patients, most of them with mild PIH, have no signs of intravascular coagulation, as discussed previously. For that reason it is more likely that platelet activation and consumption are thrombin-independent, and caused by an abnormal platelet-vessel wall interaction in the non-endothelialized spiral arteries in the presence of an imbalance of PGI_2 /TXA_2 (Wallenburg 1987b). Increased plasma levels of fibronectin, a suggested marker for endothelial damage, were reported in patients with PIH (Saleh 1987). The increase was even found before the onset of clinical signs of PIH (Lazarchik 1986). The vascular endothelial injury in PIH may be caused by vasospasm or by immune complexes (Saleh 1987). The occurrence of a direct effect of immune complexes on platelets in patients with PIH, leading to increased platelet activation and consumption has also been suggested (Bern 1981, Gibson 1982, Medcalf 1983, Massobrio 1985, Rote 1987, Samuels 1987, Burrows 1987).

It can be concluded that fetomaternal immunologic maladaptation may be responsible for the decreased trophoblast invasion of spiral arteries early in pregnancy in women destined to develop PIH and/or fetal growth retardation. The disturbed balance of PGI_2/TXA_2 action which seems to be present in PIH with/without growth retardation can possibly be attributed to

immunologic fetomaternal maladaptation, perhaps by direct uteroplacental endothelial damage and decreased PGI_2 production or by increased platelet activation and TXA_2 production. The shift in PGI_2/TXA_2 balance could lead to an impaired uteroplacental function and placental ischemia, a process which may aggrevate the effect of the unbalanced PGI_2/TXA_2 ratio. Meanwhile an imbalance between prostaglandin action and the renin-angiotensin system may lead to the increased vascular resistance which is a characteristic feature of PIH.

II SPONTANEOUS PIH-LIKE SYNDROMES IN ANIMALS

Some of the signs and complications of PIH have also been described to occur spontaneously in pregnant animals, although usually without hypertension (Douglas 1971, Seidl 1979). Complications such as neurologic signs and liver and/or kidney lesions, described in the milk-fever syndrome and after starvation in several animal species, are not pathognomonic of PIH in man. A syndrome with an increased frequency of fetal death, proteinuria and hemorrhagic lesions in uterus, placenta, liver and kidneys was described in pregnant guinea pigs with aortic hypoplasia. However, in this syndrome blood pressure appeared to be lower than in healthy pregnant guinea pigs (Seidl 1979).

In non-human primates pregnancy-related convulsions were described in several parous chimpanzees, one primigravid chimpanzee, two multiparous orang-utangs, one Stuhlmann's monkey and one rhesus monkey (Chez 1975). The disease process was lethal in most cases. In the case of the primigravid chimpanzee generalized edema and glomerular capillary endothelial swelling were found (Stout 1969).

A placenta with a large number of infarcts, excessive fibrinoid and atherosis

of decidual vessels was described following an apparently uncomplicated pregnancy in a Patas monkey, which had delivered a normal size stillborn fetus (Gille 1977). In a colony of 98 Patas monkeys edema and proteinuria was observed in 6 animals (Palmer 1979). In only 2 of them blood pressure was recorded and appeared to be elevated when compared with that in 15 normal pregnant animals. However, 64 of 98 pregnant animals (3 of 6 affected animals) received the carcinogenic agents ethylnitrosurea or diethylnitrosamine, and from the data it is not clear whether or not the hypertensive animals were among them. Another observation was made by Baird (1981), who found convulsions and edema in a 9-year-old parous gorilla. Spontaneous delivery resulted in the birth of a live infant 3 weeks after the "eclamptic" seizure. The placenta was smaller than normal and showed infarcts.

Microscopically syncytial knots and hyaline degeneration were seen. From the data presented above we must conclude that there is no convincing evidence that PIH occurs spontaneously in animals, not even in nonhuman primates.

III ANIMAL EXPERIMENTS

Various experiments have been performed in an attempt to induce hypertensive syndromes in pregnant animals. A suitable animal model of PIH must meet several requirements.

a) The model should allow investigation of major signs of PIH such as hypertension and proteinuria.

b) The model should allow investigations of additional signs, such as thrombocytopenia, liver and kidney lesions, or impaired fetoplacental circulation.

c) Results in experimentally manipulated pregnant animals should always be compared with those obtained in nonpregnant, normal pregnant or sham-operated pregnant animals.

Experimental approaches that could be found in the literature will be assessed against the background of these requirements. Many approaches have involved experimental uteroplacental ischemia, but administration of hormones and vasoactive substances, dietary manipulation, and immunologic approaches have also been used. Because the pathophysiologic pathway of PIH in man appears to develop some time before the onset of clinical symptoms, we consider chronic experiments in animals to be most relevant, although acute experiments, involving special effects of the disease, may also provide valuable data. In this review animal models with renal hypertension will not be discussed because the pathophysiologic mechanisms which lead to hypertension are not related to pregnancy.

UTEROPLACENTAL ISCHEMIA

Uteroplacental ischemia has been experimentally induced by reduction of uteroplacental blood flow at several levels: the placental level, the level of the uterine and ovarian arteries, and the level of the abdominal aorta below the renal arteries, in order to prevent direct effects on the renal blood flow. In most experiments the reduction of uteroplacental blood flow was obtained by mechanical means.

The first experiments involving uteroplacental ischemia were of the acute type. Impairment of the uteroplacental circulation was obtained by clamping the aorta below the level of the renal arteries, by clamping the uterine arteries, by dissection of the ovarian arteries, and by distension of the uterine cavity by a balloon. In all of these models a sudden rise in blood pressure was observed, which returned to normal after normalization of the circulation. In nonpregnant controls blood pressure did not change

(Ogden 1940, van Bouwdijk Bastiaanse 1950, Gyönggyössy 1958).

Another method to induce placental ischemia consisted of placement of Z-sutures through the placentae in pregnant rabbits (Berger 1963, 1964). A rise in blood pressure was observed within 25-45 minutes after placing the Z-sutures, followed by a period of time during which blood pressure remained constant or decreased slowly. After 18 hours renal arteriograms showed a constriction of the renal arteries and arterioles. The same results were obtained in nephrectomized rabbits, but not in rabbits with Z-sutures placed through the uterine wall only. Blood from a nephrectomized rabbit drawn after placement of a placental Z-suture evoked an increase in blood pressure in a nonpregnant recipient (Berger 1963).

In a second paper Berger (1964) reported the results of transfer of plasma from Z-sutured hypertensive nephrectomized pregnant rabbits to normal pregnant rabbits. Most of these rabbits, which were observed for 20 to 72 hours, developed hypertension and proteinuria. Vasoconstriction as measured by arteriography was found in all animals, first observed in the renal and later in the uterine and ovarian arteries; the placentae became ischemic. According to this paper (Berger 1964) an irreversible vicious circle could be established experimentally, with prolonged hypertension and placental ischemia. This effect could also be established in nephrectomized pregnant rabbits, but only slightly in nonpregnant rabbits. The humoral factor was also found in plasma of pregnant animals after ligation of one common iliacal artery. Induction of placental ischemia by AII infusions also resulted in a vicious circle of prolonged or increased hypertension, vasoconstriction and increasing placental ischemia (Berger 1964). These results have never been confirmed by other investigators. Smith (1966) and Bregulla (1977) failed to obtain hypertension in pregnant rabbits by placing Z-sutures through the site of placentation. Wardle (1973) found that Z-sutures in the placentae of pregnant rabbits led to severe shock in most animals which

aborted soon because of abruptio placentae. Increased proteinuria, increased fibrinogen turnover and a decrease in the concentrations of fibrinogen and platelets were found, perhaps resulting from an increased inflow of placental thromboplastin, in particular into the lungs. Mild hypertension was found several days after the operation in 3 of 12 animals only.

Douglas (1967) produced chronic uterine and placental ischemia by wrapping the uterine horns of rats and rabbits with cellophane before pregnancy. In treated rats blood pressure rose slightly during pregnancy, but significantly after pregnancy as compared with a control group. The amounts of proteinuria did not differ from those in the control group. Fetal death occurred in 90% of cases with placental ischemia. In rabbits there was an increase in proteinuria after birth, but no change in blood pressure.

Experimental subacute or chronic placental ischemia was induced in pregnant dogs by partly occluding the uterine arteries by means of clips together with complete ligation of the ovarian arteries 10-15 days before term (Kumar 1962). A rise in blood pressure was observed to occur after 2 or 3 days, but it returned to normal after 6 days. Placental blood flow as measured by clearance of radioactive sodium was reduced to 50-75% of its baseline value, but it also returned to normal within 7 days, presumably as a result of the development of a collateral circulation. Eight animals were operated again on the third and seventh day after the first operation, and the uterine arteries were ligated completely, one per operation. Three dogs developed hypertension, which persisted after delivery, and five delivered prematurely. When animals were operated three times, proteinuria occurred which persisted until one week after delivery. No blood pressure rise was observed in nonpregnant or sham-operated dogs. No histologic studies were done (Kumar 1962).

In nonpregnant dogs Hodari and Hodgkinson (1967, 1967) ligated the ovarian arteries completely and the uterine arteries partly by placing teflon bands

around them. Arterial blood pressure was recorded in the femoral artery and arteriograms were made in banded and nonbanded animals, before pregnancy and after they had become pregnant. Arterial blood pressure in nonbanded pregnant and in nonpregnant dogs (banded or nonbanded) was similar, but in banded pregnant dogs in which the normally occurring distension of the uterine arteries was prevented by the teflon bands an increase in blood pressure occurred at as early as 3 weeks' gestation, with a gradual rise throughout the remainder of pregnancy. One month post partum blood pressure had returned to normal. Proteinuria was more often found in banded than in nonbanded pregnant dogs. Arteriograms showed a reduction of the diameter of uterine arteries in banded animals, although this was in part compensated for by extensive collateral circulation. Fetal and placental weights tended to be lower in the banded dogs, although the differences were not significant. Histopathologic investigations were not reported and sham-operations were not done.

A similar procedure was performed in guinea pigs (Golden 1980). Of 6 animals which were bred after one week, two aborted. In pregnancy there was a slight fall in blood pressure in normal animals but an increase in both systolic and diastolic blood pressure in banded animals. Banding the uterine arteries did not alter blood pressure in nonpregnant animals. Proteinuria was only observed in the banded pregnant group. In banded pregnant animals creatinine levels increased, and the kidneys showed deposition of protein-like material in the tubules and in the glomeruli, which appeared to be swollen and hypercellular. Fetuses of banded animals were smaller, but the placentae did not differ from those obtained from normal animals. There were no sham-operated animals.

In an experiment with banding of the uterine arteries in nonpregnant rats and rabbits, with or without ligation of the ovarian arteries, Bregulla (1977) failed to obtain hypertension in these animals after they had become pregnant.

Uterine arterial banding experiments have also been performed in nonhuman primates. In baboons, the uterine arteries were partially occluded and the ovarian arteries transected; the animals were bred afterwards. The results were compared with those obtained in normal pregnant and in sham-operated animals (Cavanagh 1972, 1974, 1977). In banded pregnant animals hypertension, proteinuria, a decrease in renal blood flow and an increase in renal and peripheral resistance were found to occur in the course of pregnancy. Hemoglobin values did not differ, but uric acid levels in the ischemic group were higher than those in normal pregnant baboons. Although coagulation studies showed no abnormalities, immunofluorescent staining for fibrin or fibrinogen in glomeruli of hypertensive animals was positive. The histologic changes in the kidneys varied from slight to lesions indistinguishable from those seen in human preeclampsia. These lesions were also found in a group of animals banded during pregnancy. Birthweights are not given. These important observations have not been confirmed by other investigators. In the rhesus monkey, banding of the uterine arteries before pregnancy resulted in a reduced number of spiral artery entries into the intervillous space and an increase in fetal loss, but no hypertension or proteinuria was observed (Misenhimer 1970).

Abitbol (1976a, 1976c, 1977b, 1981) produced experimental "toxemia" in rabbits, dogs and monkeys by constricting the aorta during pregnancy, just below the level of the renal arteries. The degree of stricture appeared to be critical. When it was too loose, no effects were seen and when it was too tight it resulted in placental separation, fetal death and/or paralysis of the hind legs. In general, the diameter of the aorta had to be reduced to 1/3 or 1/4 of its original size. Sham-operated and nonoperated pregnant and nonpregnant animals served as controls. In treated rabbits the average systolic blood pressure was markedly raised. About 40% of the ischemic group delivered prematurely and in about 50% one or more fetuses were macerated.

The average weight of fetuses of comparable length of gestation was decreased. Kidney lesions similar to those observed in PIH in man, such as mesangial proliferation, focal thickening of the glomerular basement membrane, swelling of endothelial cells and subendothelial depositions probably of fibrinogen were found in several animals. Focal necrosis and subcapsular hemorrhage was observed in the liver of some animals (Abitbol 1976a). In placentae, syncytial degeneration and syncytial knots, acute and older infarcts were seen in cases of "severe toxemia". Mild lesions, such as congestion of capillaries and sometimes small infarcts, were seen in rabbits which did not develop all the clinical signs and in which ischemia had lasted only a few days (Abitbol 1976b).

In female dogs of mixed breed, blood pressure and blood flow in the abdominal aorta were measured 1-3 weeks after placement of an aortic stricture below the renal artery (Abitbol 1976c). Values were compared with those obtained in a group of dogs in which the blood pressure was measured immediately before and after constriction, and with values obtained in sham-operated dogs. Because of hemorrhage all dogs, but one, had to be sacrificed at the end of this measurement. Immediately after placement of the stricture there was a significant drop in blood flow and pressure in the abdominal aorta below the constriction, but no change in the thoracic aorta. When measured 1-3 weeks after the operation, a significant rise in systolic and diastolic blood pressure as compared with normal pregnant and sham-operated pregnant dogs was found in the thoracic aorta in 9 out of 14 ischemic pregnant animals, and blood flow in the abdominal aorta was decreased. Marked proteinuria was demonstrated in 3 of 14 dogs. Birthweights are not given. Kidney, liver and placental lesions, as described in the ischemic pregnant rabbit, were also seen in the ischemic pregnant dogs, and were shown to be related to the duration of ischemia (Abitbol 1976c). Blood flow changes in the abdominal aorta were only observed after a constriction of more than 50%. When the

stricture was removed after 10 days, blood pressure below became equal to blood pressure above the removed stricture, and hypertensive ischemic dogs remained hypertensive, although they were only observed for a couple of hours (Abitbol 1977b). Coagulation studies in ischemic pregnant dogs revealed a fall in the concentration of platelets and fibrinogen as compared with normal pregnant dogs, and in some cases FDP's were detected (Abitbol 1978). In a later study, the same investigators placed a vascular occluder and a flow transducer around the abdominal aorta, which made it easier to produce a continuously monitored constriction. Hypertension and marked proteinuria were observed in about half the number of 21 ischemic pregnant dogs. Fetal outcome is not mentioned. Histologic lesions were as described above, and more severe in proteinuric than in nonproteinuric animals (Abitbol 1981).

Finally, the same investigators produced an aortic constriction in pregnant monkeys (Macaca Mulatta) by means of a silk suture, reducing the aortic diameter 3 or 4 times (Abitbol 1977a). One sham-operated pregnant animal, one nonoperated pregnant animal and one nonpregnant animal served as controls. Blood pressure changes, proteinuria, kidney-, liver-, and placenta changes were similar to those observed in other experimental animals, as described above, and comparable to those observed in human PIH. Again, the effects on the fetus are not presented.

Banding of the aorta below the level of the renal arteries has also been performed in pregnant baboons (Cavanagh 1985). Animals were operated at 100 days' gestation, and aortic blood flow below the constriction was reduced by 55% to 60%. Results were compared with those obtained in sham-operated animals. Hypertension occurred near term, and proteinuria and increased renal vascular resistance were found. Light and electron-microscopic changes in kidney biopsies were similar to those described in human PIH. The number of platelets was slightly decreased and uric acid levels increased, although to a lesser extent near term. Fetal and placental weights, and amniotic fluid

volumes were lower in hypertensive than in normotensive baboons, but maternal plasma volumes were equal.

In a recent study, aortic constriction below the renal artery level in pregnant rabbits resulted in hypertension, proteinuria, thrombocytopenia and hypovolemia when compared with nonoperated nonpregnant, as well as with sham-operated pregnant and nonpregnant animals (Losonczy 1986-87). Increased arterial norepinephrine levels and a positive correlation between the arterial norepinephrine concentration and blood pressure were observed in pregnant animals with aortic constriction, and uterine venous AII concentration appeared to be elevated. An increased vascular response to exogenous norepinephrine or AII was also a feature in treated pregnant rabbits. Uterine venous plasma prostanoids (PGE_2, 6-keto-PGF1α , PGFm, TXB_2) were all elevated in animals with aortic constriction as compared with the levels in sham-operated pregnant and nonpregnant animals, but arterial plasma prostanoid levels were not different between groups. However, when compared with the nonoperated nonpregnant animals, the levels of 6-keto-PGF1α , PGFm and TXB_2 were not significantly different. Histologic examination revealed focal placental necrosis in about half of the fetuses, and fibrin depositions in maternal glomeruli as shown by immunofluorescence. No light microscopic lesions were observed in kidneys or livers of animals with aortic constriction. Increased occurrence of fetal death and decreased birthweights were observed in rabbits with aortic constriction (Losonczy 1986-87).

In conclusion, several experiments in which the investigators attempted to induce chronic placental ischemia resulted in hypertension, proteinuria, lesions in several maternal organs, and sometimes fetal growth retardation or fetal death (table 1). The best documented pathologic changes were reported following experimental chronic placental ischemia induced by banding of the aorta during pregnancy, or by banding of the uterine arteries combined with

dissection of the ovarian arteries before or during pregnancy. Only the studies of Cavanagh (1974, 1985), Abitbol (1976a, 1976b, 1977, 1981) and Losonczy (1986-87) meet most of the criteria for a suitable animal model as stated before. The failure of some investigators to obtain similar results in apparently identical experiments may be due to the kind of animals used in their experiments, or to failure to obtain a critical grade of ischemia necessary to induce the syndrome. The syndrome in animals induced by placental ischemia resembles that observed in human PIH with (severe) fetal growth retardation. However, this does not necessarily mean that uteroplacental ischemia is primarily responsible for the development of PIH. The observation of increased levels of vasodilating prostanoids in placental ischemic rabbits is at variance with findings in human PIH, and may be an indication of another pathophysiologic mechanism.

STEROID HORMONES

Because of the impressive changes in hormonal production in pregnancy, with their possible effects on blood pressure and fluid retention, the role of hormones in the etiology and pathogenesis of PIH has been subject of various investigations (MacGillivray 1983). Animal experiments have been performed in which hormones were administered in an attempt to induce a PIH-like syndrome; the experiments will be briefly discussed.

In pregnant rats 5 mg of progesterone i.m. daily resulted in a rise in systolic blood pressure. The animals also developed proteinuria and liver and kidney lesions when pregnancy went on to near term. The placentas showed degeneration of Langhans' and syncytial cells, and focal hemorrhage. No lesions were found in organs of nonpregnant control animals (Symeonidis 1950). The occurrence of maternal and fetal death with histologic features of DIC was described by Stamler in pregnant rats following daily intramuscular injection of 10 mg progesterone, in particular when parturition was delayed

TABLE 1

Investigator	animal species and number	experiment	acute/chronic	hypertension	proteinuria	fetus	PA	DIC
Ogden et al. 1940	5 dogs 1 cat	clamping Aa.ut. ligation Aa.ov.	acute	yes	not reported			
v.Bouwdijk Bastiaanse et al. 1950	6 dogs	ditto	acute	yes	not reported			
Gyöngyössi et al. 1958	6 dogs, 11 cats	distension ut.cavity	acute	yes	not reported			
Berger et al. 1963	7 rabbits 3 nephrectom.	Z-sutures placenta	(sub)acute	yes	not reported			
Smith et al. 1966	6 rabbits	ditto	acute	no	not reported			
Wardle et al. 1973	16 rabbits	ditto	subacute	3 animals mild hypert.	yes	abortion in most animals		a,b
Bregulla et al. 1977	15 rabbits	ditto	subacute	no				
Berger et al. 1964	7 rabbits	plasma transfer after Z-sutures pl.	subacute	yes	yes	increased f. death		
	4 rabbits	pharmacol. induced plac. ischemia						
Douglas et al. 1967	30 rats	wrapping uterine horns before pregn.	chronic	after pregn. no	after pregn. no	f.death		
	4 rabbits					f.death		
Kumar 1962	10 (3) dogs	dissection Aa.ov. (repeated) partly occlusion Aa.ut. during pregn.	subacute chronic	yes	yes	increased f. death		

Hodari 1967	20 dogs	banding Aa.ut. dissection Aa.ov. before pregn.	chronic	yes	yes	decreased f.weight?	
Golden et al. 1980	6 guinea pigs	ditto	chronic	yes	yes	decreased f. weight	+
Bregulla et al. 1977	20 rats 7 rabbits	banding Aa.ut. with/without dissection Aa.ov. before pregn.	chronic	no			
Misenhimer et al. 1970	6 rhesus monkeys	banding Aa.ut. before pregn.	chronic	no	no	increased f. death	
Cavanagh et al. 1974	10 baboons 3 baboons	banding Aa.ut. dissection Aa.ov. before pregn. ditto during pregn.	chronic	yes	yes		+ b
Abitbol et al. 1976	51 rabbits	banding abd. aorta during pregn.	chronic	yes	yes	decreased f.weight, f.death	+ b
Abitbol et al. 1976	14 dogs	ditto	chronic	yes	yes		+ a,b
Abitbol 1981	21 dogs						
Abitbol et al. 1977	10 monkeys	ditto	chronic	yes	yes		+ b
Cavanagh et al. 1985	9 baboons	ditto	chronic	yes	yes	decreased f. weight	+ b
Losonczy et al. 1986-87	21 rabbits	ditto	chronic	yes	yes	decreased f.weight, f.death	b

PA + = histologic lesions comparable to those found in PIH
DIC a = laboratory findings compatible with DIC
b = histologic findings compatible with coagulation
Aa.ut. = uterine arteries
Aa.ov. = ovarian arteries

for several days (Stamler 1961, 1971, 1977). In these experiments blood pressure was not measured. The lesions described in all these experiments may well have been caused by the toxic effects of the very high doses of progesterone, although the results obtained by Symeonidis and Stamler were not confirmed by Hester (1953), who also treated pregnant rats with 5-10 mg progesterone daily.

Adrenocortical hormones may be involved in the pathogenesis of hypertension and of the development of edema through retention of salt and water. The latter sign, however, cannot be considered characteristic of PIH since it is also found in a high proportion of normotensive pregnant women (Davey 1986). Parker (1980) found no significant differences between the production of desoxycorticosterone (DOCA) in normal pregnancy and in pregnancies complicated by PIH. A syndrome with signs resembling those in severe PIH was induced in uni-nephrectomized nonpregnant rats. The rats were made hypertensive by the administration of desoxycorticosteroneacetate (DOCA) and a high intake of sodium (Masson 1951, Page 1955). After renin administration a further rise in blood pressure, proteinuria, severe edema and sometimes convulsions developed. Demonstrated lesions consisted of diffuse small hemorrhages and thrombi in several organs; in the kidneys swelling of endothelial cells was observed and tubular casts were formed (Masson 1952). A rise in blood pressure following high sodium intake and DOCA has also been described in uni-nephrectomized pregnant rats (Douglas 1971).

Marked proteinuria and considerable weight gain was observed in 4 out of 20 pregnant rats; 2 animals developed convulsions. Hemorrhagic lesions were seen in lungs, adrenals and placentas. The livers showed focal necrosis, and renal glomeruli were often occluded by endothelial swelling and fibrilar or granular deposits. The kidneys were most severely affected in animals who were treated during the whole pregnancy. Compared with a control group of pregnant rats an increased fetal death rate was observed in the DOCA-NaCl

treated animals (Douglas 1971). Vascular sensitivity to exogenous AII appeared to be increased in DOCA-NaCl treated pregnant rats (Douglas 1984). On the other hand, the physiologic fall in blood pressure near term which occurs in normotensive as well as in spontaneously hypertensive pregnant rats (Terragno 1983, Lorenz 1984), was also observed in DOCA-NaCl treated pregnant rats (Parks 1978). Such a fall in blood pressure was not found when the rats were treated with indomethacin, suggesting an intermediary role of the prostaglandin system (Parks 1978). Indeed, in pregnant rats near term there is a rise in the vascular production of prostacyclin in vitro and in the plasma concentration of prostacyclin metabolites (Williams 1978, Wilson 1982, Terragno 1983). In contrast to what usually occurs in the preeclamptic patient, the DOCA-NaCl treated rat still shows a fall in blood pressure near term, perhaps because prostacyclin production has remained within physiologic limits. It may be concluded that the rise in blood pressure and the development of organ lesions induced by DOCA in pregnant as well as in nonpregnant rats is probably due to unphysiologic doses of DOCA (and renin). Although the DOCA-NaCl-renin model may be used for the study of hypertension in pregnancy, it has produced no evidence of involvement of DOCA in the pathogenesis of pregnancy-induced hypertension.

NUTRITIONAL FACTORS

Despite a good number of investigations there is still no evidence that nutritional factors are involved in the etiology and pathophysiology of PIH (Chesley 1978, MacGillivray 1983). However, some nutritional factors may interfere with the regulation of blood pressure, in particular because of their possible influence on the production of prostanoids.

In 5 late-pregnant sheep a rise in mean arterial blood pressure and peripheral resistance, and a fall in uterine artery blood flow and glomerular filtration rate were observed following 72 hours of fasting. In addition, the

development of proteinuria and kidney lesions characteristic of human PIH was observed. The same treatment produced no changes in nonpregnant ewes (Thatcher 1986). In a subsequent report results were presented of platelet counts and function, and of prostacyclin and thromboxane measurements in this animal model (Keith 1987). In the hypertensive animals platelet counts were decreased, and plasma 6-keto-PGF1α and TXB_2 levels showed a slight but not significant decrease as compared to the situation before starvation. Treatment with a selective thromboxane synthetase inhibitor restored mean arterial blood pressure and platelet counts and function to values comparable to those before starvation. After inhibition of thromboxane synthesis TXB_2 levels were not significantly different from values before and during starvation; levels of 6-keto-PGF1α were even higher than before starvation, perhaps due to the shunting of substrate from thromboxane to prostacyclin synthetase. The possible pathophysiologic mechanism in this animal model may be the development of an imbalance of prostacyclin-thromboxane action, perhaps caused by reduced prostacyclin action in ketonemic animals (Keith 1987).

The PGI_2/TXA_2 balance may also be influenced by vitamin E. Already in the early sixties vitamin E deficiency was reported to lead to trophoblast degeneration, placental infarction, and DIC in pregnant rats (Stamler 1959, McKay 1963, 1967). In vitamin E supplemented nonpregnant rats a higher aortic production of PGI_2 in vitro and a lower TXB_2 production by platelets was found, as compared with that in vitamin E deficient rats (Karpen 1981, Valentovic 1982). This effect may be due to protection of platelets and vessel wall from accumulating lipid peroxydes by the free radical scavenging effect of vitamin E (Okuma 1980, Srivastava 1986).

In pregnant rats a vitamin E deficient diet reduced PGI_2 production in mesometrial triangles and aorta, and lipid peroxyde plasma levels increased. Effects were more marked when the diet was more oxidized. Fetal weight was

lower in the vitamin E deficient group (Spitz 1983, 1985). In these experiments no data on blood pressure or proteinuria were reported. Douglas (1966), however, could not find a rise in blood pressure or proteinuria in vitamin E deficient pregnant or nonpregnant rats. In spontaneously hypertensive nonpregnant rats a diet deficient in essential fatty acids resulted in a rise of systolic blood pressure (Church 1977). Supplementation of linoleic acid, the precursor of the prostaglandin-1 and -2 series, prevented the development of hypertension in nonpregnant rats fed on a 1.5% salt solution (Triebe 1976, Ten Hoor 1978); this effect was attenuated by a high dose of aspirin (Ten Hoor 1978). In nonpregnant rats, made hypertensive by renal artery constriction and contralateral nephrectomy, only a small and non-significant fall in systolic blood pressure was observed with a linoleic acid enriched diet as compared with the effects of a standard diet in a control group (Mahoney 1983). The aortic PGI_2 and renal PGI_2 and PGE_2 synthesis in the linoleic acid supplemented group did not differ from that in the standard diet group (Mahoney 1983).

In man Eicosapentanoic acid (EPA) as well as its precursor linolenic acid may have an influence on blood pressure and platelet aggregation because of conversion into PGI_3, the active counterpart of PGI_2, and synthesis of TXA_3, the much less vasoactive and thrombogenic counterpart of TXA_2 (Lorenz 1983). In rats, however, there is some evidence that the effects of EPA and linolenic acid caused by increased synthesis of the prostaglandin-3 series are overwhelmed by their influence on the production of PGI_2 and TXA_2, because of inhibition of cyclo-oxygenase (Scherhag 1982). A decreased PGI_2 production with an EPA and/or linolenic enriched diet has been observed in nonpregnant rats (Hornstra 1979, 1981, Scherhag 1982, Mahoney 1983). In contrast, Hamazaki (1982) reported an increased production of PGI_2 from aortic segments in vitro in male Wistar rats with an EPA enriched diet as compared to control rats. The release of platelet TXA_2 has been reported to

be slightly (Scherhag 1982) or significantly (Hornstra 1979, 1981) decreased in nonpregnant rats fed an EPA or linolenic acid enriched diet, and PGI_3 production was found to be rather small or even absent in these nonpregnant rats (Hornstra 1981, Hamazaki 1982).

The effects of EPA and linolenic acid on systolic blood pressure in nonpregnant rats appear to be variable; it was found to be slightly but not significantly decreased in hypertensive rats after renal artery constriction and contralateral nephrectomy (Mahoney 1983) and increased in normal nonpregnant rats (Scherhag 1982).

The effects of dietary supplementation of essential fatty acids on prostaglandin synthesis and blood pressure in pregnancy are not known, since all the experiments mentioned earlier were performed in nonpregnant animals. So far there are no data that essential fatty acids are involved in the pathophysiology of PIH, but it remains an interesting field for further investigations.

VASOACTIVE SUBSTANCES

Blood pressure in pregnant animals appears to be the same or lower as compared to that in nonpregnant animals (Berssenbrugge 1980, Donker 1983, Paller 1984, Venuto 1984, Losconzy 1986-87, Weiner 1987), which is possibly caused by an increased synthesis of vasodilatory prostaglandins. Inhibition of prostaglandin synthesis by meclofenamate did not change mean arterial blood pressure in either pregnant or nonpregnant rabbits or rats (Donker 1983, Paller 1984, Venuto 1984), but in pregnant sheep an increase in mean arterial blood pressure was found following indomethacine (Rankin 1979a). Following captopril, an inhibitor of angiotensin I converting enzyme, mean arterial blood pressure was slightly decreased in pregnant rats and significantly so in pregnant rabbits, but it remained unchanged in nonpregnant rats and rabbits (Donker 1983, Paller 1984). Blockade of

α-receptors resulted in a slight decrease of blood pressure in nonpregnant and a more pronounced decrease of blood pressure in pregnant rabbits (Berssenbrugge 1980, Venuto 1984).

In analogy to human pregnancy a reduced vascular responsiveness to exogenous vasoactive substances like AII, norepinephrine and vasopressin has been observed in rats, rabbits and guinea pigs during pregnancy (Berssenbrugge 1980, Donker 1983, Paller 1984, Venuto 1984, Weiner 1987). The reduced vascular response in pregnancy to vasoactive substances seems to be caused by circulating vasodilatory agents, since this phenomenon could not be shown in vitro in studies with isolated arterial strips from pregnant rabbits (Moisey 1983). The blunted response to exogenous AII in pregnancy can also not be attributed to reduced AII receptor affinity or receptor number, or increased occupancy of vascular AII receptors by high circulating levels of endogenous AII, as shown in pregnant rats (Paller 1984). The reduced vascular pressor response in pregnancy is more likely to be produced by the increased synthesis of vasodilatory prostaglandins like PGI_2 and PGE_2. Inhibition of prostaglandin synthesis by cyclo-oxygenase inhibitors has been shown to increase the vascular response to exogenous AII, noradrenaline and vasopressin in pregnant animals, in many of which the pressor response became equal to that in nonpregnant animals. In nonpregnant animals inhibition of prostaglandin synthesis had little or no influence on the pressor response of exogenous vasoactive substances (Donker 1983, Paller 1984, Venuto 1984, Weiner 1987).

The reduced blood pressure is possibly, and the decreased pressor response to exogenous vasoactive substances in pregnancy is probably the result of an increased production of vasodilatory prostaglandins like PGE_2 and PGI_2, which has been demonstrated in rats and rabbits (Venuto 1982, 1984, Donker 1983, Terragno 1983). Endogenous AII and norepinephrine both play a role in the maintenance of blood pressure in pregnancy. The concept that elevated levels

of AII and norepinephrine in pregnancy are necessary to counteract the vasodilatory activity of prostaglandin (Venuto 1984) is supported by the decreased plasma renin activity, which was observed after inhibition of prostaglandin production in pregnant rabbits (Donker 1983).

Not only the systemic circulatory effects of prostaglandins and vasoactive substances are important, but in particular also their effects on the uteroplacental circulation. Inhibition of prostaglandin synthesis by indomethacine in pregnant sheep resulted in a fall in placental and nonplacental uterine blood flow (Rankin 1979a). In pregnant rabbits a significant fall in placental blood flow was observed following indomethacine, but nonplacental uterine blood flow was unchanged (Katz 1981). Administration of the vasodilator prostaglandin PGI_2 increased uterine blood flow in nonpregnant sheep (Resnik 1980, Clark 1981). In pregnant sheep prostacyclin has been reported to increase uterine blood flow (Clark 1982). However, Rankin (1979b) found an increase in nonplacental uterine blood flow only, whereas placental flow appeared to be significantly decreased. These changes were associated with a marked drop in blood pressure, which may in part explain the fall in uterine placental blood flow. The decrease in uterine placental blood flow following PGI_2 could also be a result of enhanced responsiveness to AII in uterine placental vessels, as observed in pregnant ewes receiving AII and PGI_2 infusions. In contrast, responsiveness in renal and uterine nonplacental vessels to exogenous AII appeared to be reduced during PGI_2 infusion (Parisi 1985). Vasodilator PGE_2 in pregnant sheep was shown somewhat unexpectedly to reduce total uterine and placental uterine blood flows; this effect may be due to myometrial contraction (Rankin 1976, Clark 1982) because PGE_2 administered to the fetus caused an increase in placental uterine blood flow after being transferred across the placenta (Rankin 1976).

All the observations above have been made in normotensive pregnant animals, which makes it difficult to extrapolate them to human pregnancy complicated by PIH. However, just like in human pregnancy, a decrease in vasodilator prostaglandin action in animal pregnancy may have important effects on vasoconstriction and blood flow, although the effects of vasodilator prostaglandins on the uteroplacental circulation are still unclear.

IMMUNE SYSTEM

Publications concerning animal models to induce PIH on an immunologic basis are limited to descriptions of experiments with sensitization to placental extracts or administration of antiplacenta sera. In nonpregnant rats sensitization against homologous placental extracts has been reported to result in an increase in blood pressure, proteinuria and glomerulonephritis. Pregnancy occurring some months later ended in abortion, intrauterine death or poor fetal growth, but blood pressure was not recorded in these experiments (Okuda 1966).

Administration of rabbit anti-rat placenta serum to nonpregnant and pregnant rats was found to cause chronic glomerulonephritis and an increased abortion rate (Seegal 1946). After administration of rabbit anti-rat placenta serum hypertension developed in pregnant, but not in nonpregnant rats, and kidney lesions were found in pregnant and nonpregnant rats (Langford 1967). The renal lesions caused by rabbit anti-rat placenta serum may be due to antigenic cross reactivity shared by placental and renal antigen. This could explain the increased blood pressure and proteinuria, but Langford (1967) observed hypertension in pregnant rats only. As the rabbit anti-rat placenta serum also effects trophoblast cells, in particular those that are in direct contact with the mother (Franke 1971), it is tempting to speculate that placental damage was involved in the development of the hypertension in these animals.

If sensitization of the maternal immune system against placental and kidney antigens were an important mechanism in the pathogenesis of PIH, one would expect an increase in the frequency of occurrence of PIH in multiparous women. This is not the case, and a simple immunologic model as mentioned above cannot explain the many features of the disease in man.

Like in man an interplay between rejection and adaptation of the fetus by the maternal immune system can be expected in animals. An increase in litter size, and in placental and fetal weight was found after crossbreeding of inbred rat and mouse strains (Billington 1964, Beer 1975, Beer et al 1975). This effect was diminished by removal of paraaortic lymph nodes before mating, which suggests that not only heterosis, but also maternal immune reactions play a part (Beer 1975, Beer et al 1975).

Thus, like in man, histoincompatibility seems to favor pregnancy. This protective effect is perhaps the result of an enhanced maternal antibody response and a weakened or eliminated cell-mediated immune response to the fetal allograft due to exposition to incompatible class I MHC trophoblast antigens, in the absence of class II antigens (Wegmann 1981, Head 1982). Hyporesponsiveness to paternal alloantigens is increased with increasing parity (Head 1982), suggesting an increase in formation of blocking antibodies. The heterogeneic advantage of crossbreeding was not found in all inbred strain combinations and in some crossbreedings of inbred mouse and rat strains incompatibility of non-MHC antigens appeared to have a negative effect on the outcome of pregnancy (Palm 1974, Milgrom 1977, Croy 1982, Dawson 1982). In these experiments the relationship between fetomaternal immunologic interaction and the problem of PIH was not investigated.

IV CONCLUSIONS

Spontaneous "PIH-like" syndromes are rare in pregnant animals and in most cases they have been insufficiently documented. Major signs like hypertension and proteinuria were usually not found or reported. Pre-existent diseases such as hypertension or renal disorders were not ruled out. Many of the signs of spontaneous "PIH" in animals are of metabolic origin, and can also be observed in nonpregnant animals. It remains doubtful whether spontaneous PIH really exists in animal pregnancy.

Several factors seem to be involved in the pathogenesis and pathophysiology of PIH in man, some of which have been studied in animal pregnancy. Prostaglandins are important in the regulation of blood pressure in pregnancy, but their effects on uterine, placental and fetal blood flow are not fully understood. In PIH in man the PGI_2/TXA_2 balance appears to be an important factor in the development of the disease. The role of nutritional factors in human PIH is most likely limited. However, some nutritional factors may influence the prostaglandin metabolism and thus interfere with the regulation of blood pressure. For that reason the demonstrated influence of essential fatty acids and vitamin E on the prostaglandin metabolism is interesting, and may have a bearing on the pathophysiology of PIH and perhaps also on the treatment of the disease. Further investigations of these dietary factors could be useful. An interesting observation has been made in ketonemic pregnant sheep following starvation. These animals developed hypertension, proteinuria, decreased placental blood flow, and changes in platelets, prostacyclin and thromboxane production. Inhibition of thromboxane synthetase in these animals appeared to be beneficial.

The vasoregulatory mechanisms of the renin-angiotensin system seem to play a secondary part in the pathogenesis and pathophysiology of PIH. Although progesterone may influence vascular refractoriness to various vasoactive

metabolites, and DOCA has been shown to increase blood pressure in animals, a role of steroid hormones remains questionable.

In various animals with experimentally induced placental ischemia hypertension, proteinuria, coagulation disorders and histological lesions were observed. However, even in severe human preeclampsia fetal growth retardation is not always present, and in animals with induced placental ischemia the production of vasodilatory prostaglandins appeared to be increased, perhaps as a compensatory mechanism. It cannot be concluded that placental insufficiency is a primary phenomenon in the pathogenesis and pathophysiology of PIH.

The role of immunologic factors in PIH is unclear. In man as well as in animals MHC-incompatibility appears to be favorable with regard to the outcome of pregnancy. In contrast, non-MHC incompatibility may have a negative effect on the progeny. Further immunologic experiments in animals are needed to support or refute the concept that PIH is a disease of immunologic maladaptation of the mother to the fetal allograft. The relationship between immunologic dysfunctioning and the production of vasoactive substances, especially prostacyclin and thromboxane, needs further investigation.

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CHAPTER 3
PREGNANCY-INDUCED HYPERTENSION IN CROSSBRED RATS*
I Maternal and fetal outcome of pregnancy

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ABSTRACT

Because of the possible influence of fetomaternal incompatibility on the occurrence of pregnancy-induced hypertensive disease and the outcome of pregnancy a comparative study was performed in 25 Wistar female rats bred with Wistar males, 33 Wistar females bred with Brown Norway males and 12 Wistar females bred with Brown Norway males treated with the antiplatelet drug ticlopidine.
Thirteen nonpregnant Wistar females were used as controls. Blood pressure near term was reduced in all pregnant animals as compared with nonpregnant controls, but the decrease in bloodpressure was less pronounced in crossbred pregnancies. Maternal weight and uterine arterial 6-keto-PGF1α production were lower in crossbred than in non-crossbred animals and proteinuria was found to be increased. In the crossbred ticlopidine group, 6-keto-PGF1α production was in the same range as that in nonpregnant animals and blood pressure and proteinuria were comparable with those in the non/crossbred group. It is concluded that fetomaternal incompatibility in this model increases the susceptibility of developing a pregnancy-induced hypertensive disorder.

INTRODUCTION

The etiology of pregnancy-induced hypertension (PIH) in man remains unknown. Although symptoms resembling those of PIH have been reported to occur in animal pregnancies, it is unlikely that the disease develops spontaneously in animals (Douglas 1971). Various experimental approaches have been used to induce PIH in pregnant animals, mostly based on the induction of placental ischemia. However, placental ischemia is not always present in human PIH, and there is evidence that immunologic factors may be involved in the development of PIH (Beer 1978, Redman 1981). HLA-dissimilarity between mother and fetus seems to protect against the development of PIH (Jenkins 1978, Redman 1978). In experimental animals fetomaternal compatibility has been shown to result in reduction in litter size, fetal and placental weight, possibly due to immunologic factors (Billington 1964, Beer 1975). However the results of these animal experiments do not elucidate the pathogenesis of PIH since blood pressure was not measured.

To investigate the effects of immunologic factors on circulatory adaptation, course and outcome of pregnancy we developed a heterogeneous rat model, by crossbreeding female Wistar rats with Brown Norway males. Maternal variables such as blood pressure, proteinuria and weight were determined, and litter size and fetal weight were assessed. Because there is increasing evidence that the balance of vasodilator substances such as prostacyclin (PGI_2) and vasoconstrictors such as thromboxane A_2 (TXA_2) is involved in the pathogenesis of PIH (Wallenburg 1981, Ylikorkala 1985), the in vitro production of PGI_2 by uterine arteries was also studied.

MATERIALS AND METHODS

Seventy-three virgin Wistar rats were mated at 8-10 weeks of age and conception was confirmed by inspection of the vaginal plug. After mating animals were kept in standard cages, 6 animals per cage. Food and water were

supplied ad libitum. Forty-eight Wistar females were mated with Brown Norway males, and 25 Wistar females were mated with Wistar males. Thirteen nonpregnant virgin Wistar rats, aged 8-10 weeks, served as controls.

In order to study the influence of prostaglandins on the outcome of pregnancy 14 of the crossbred animals received the anti-platelet drug ticlopidine, administered in a dose of 25 mg on day 6, 8, 11, 13, 15 and 18 of pregnancy by means of a stomach canula. With this dose, effective inhibition of platelet aggregation in rats is achieved for several days (Ashida 1978).

Systolic blood pressure was measured on day 19 of pregnancy in conscious rats using a tail cuff (Palbol 1979). Measurements were repeated with intervals of 2 minutes until 3 stable values were obtained. On day 20 urine was collected after spontaneous voiding and the degree of proteinuria was assessed using Albym-test reagent sticks (Boehringer Ingelheim). Anesthesia was then induced with pentobarbitone sodium, the animal was weighed, the abdomen was opened, and the uterine arteries were removed and stored in 0.1 M Tris buffer, pH 7.4 at 4° C. The whole uterus was removed and the animal was killed with an overdose of pentobarbitone sodium. The numbers of living and dead fetuses were recorded, and all fetuses were weighed. Nonpregnant rats were treated in the same fashion and sacrificed at 8-10 weeks of age.

Equal parts of uterine arteries were used for measurement of 6-keto-PGF1α production. Each part was cut into small pieces (Mc Ilwain tissue chopper) at 4° C. The pieces were incubated in siliconized aggregation tubes (Paytoon) at 37° C under stirring (900 rpm) in 0.5 ml of Tris buffer (50 mM; pH 6.8) for 1 hour, and 20 μl of the supernatant was taken for radioimmunoassay of 6-keto-PGF1α using commercial RIA-kits (NEN, Boston, USA). The sensitivity of the assay is within a range of 10-1000 pg. Amounts were found in ranges of 30-70% B/Bo. Cross reactivity of 6-keto-PGF_2 and PGE_2 was $\leq$ 8%, and of other eicosanoids $\leq$ 0.1 %. Data were expressed as nanograms per sample of uterine artery.

TABLE 1 Systolic Blood Pressures and Production of 6-keto-PGF1α (mean ± SD) by Uterine Arterial Tissue in NonPregnant Rats, and in Crossbred and Non-Crossbred Pregnant Rats.

	Wistar nonpregnant	W x W non-crossbred	W x Br.N. crossbred	W x Br.N. (ticlopidine) crossbred
Experimental animals	(n = 13)	(n = 25)	(n = 33)	(n = 11)
Systolic blood pressure (mmHg)	108 ± 5	89 ± 5 A	99 ± 7 AB	89 ± 8 A
6-KetoPGF1α (ng/sample)	1.93 ± 0.90	3.04 ± 1.17 A	2.42 ± 1.24 C	1.65 ± 0.43 DE

A. $p < 0.005$ compared with Wistar nonpregnant
B. $p < 0.005$ compared with WxW and WxBrN (ticlopidine)
C. $p < 0.05$ compared with WxW
D. $p < 0.005$ compared with WxW
E. $p < 0.025$ compared with WxBrN

TABLE 2 Maternal Weight and Fetal Outcome (means ± S.D.)

	Wistar nonpregnant	W x W	W x Br.N	W x Br.N (ticlopidine)
Animals	(n=13)	(n=25)	(n=33)	(n=11)
Maternal weight (gram)	177.94±21.79	338.13±41.26 A	267.80±38.28 AB	262.32±41.85 AB
Littersize		11.20± 2.62	8.73± 2.91 C	10.27± 3.72
Fetal weight (gram)		3.47± 0.65	3.63± 0.32 D	3.14± 0.56
Fetal death		21 E	7	0

A $p < 0.005$ compared with W nonpregnant
B $p < 0.005$ compared with WxW
C $p < 0.005$ compared with WxW
D $p < 0.005$ compared with WxBrN(ticlopidine)
E $p < 0.005$ compared with WxBrN and with WxBrN(ticlopidine)

Averages and standard deviations of fetal and maternal weight, systolic blood pressure and 6-keto-PGF1α production were calculated. Differences were statistically analyzed using Wilcoxon's test, and $p < 0.05$ was considered to represent significance.

RESULTS

In the crossbred group 4 animals, 3 of which were receiving ticlopidine, appeared to be not pregnant and for this reason they were removed from the study. Systolic blood pressure appeared to be significantly lower in all pregnant animals as compared with that in nonpregnant animals (Table 1). The lower systolic blood pressure was more pronounced in the non-crossbred and in the ticlopidine crossbred animals: the mean values of these two groups were significantly different from those in the untreated crossbred group. In all pregnant animals 6-keto-PGF1α production by uterine arterial tissue was significantly elevated as compared with that in nonpregnant controls, but 6-keto-PGF1α production was lower in crossbred than in non-crossbred animals. Values in ticlopidine treated crossbred rats were even lower than those in nonpregnant animals (Table 1).

The highest maternal weight near term was found in the non-crossbred group, which could only in part be attributed to the total weight of the fetuses. In the untreated crossbred group litter size was significantly lower than that in the non-crossbred group. The highest mean fetal weight was observed in the untreated crossbred group. Little difference in fetal weight between groups was found and although mean fetal weight was highest in the untreated crossbred group the mean fetal weight in the non-crossbred group was higher when corrected for litter size (fig. 1).

Fetal death occurred significantly more often in the Wistar x Wistar group than in the other groups (table 2). In almost all rats at least a trace of proteinuria could be found. In the crossbred group (without ticlopidine)

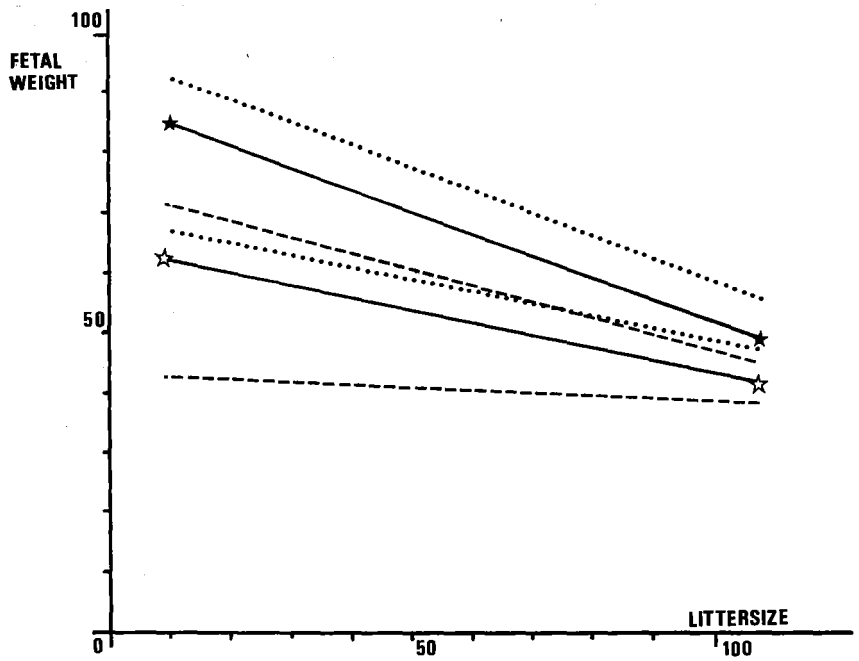

FIG. 1: Correlation between relative fetal weight (%) at day 20 of gestation and relative litter size (%). Fetal weight of 5.20 g and litter size of n=16 were taken as 100%.

★——★ = Wistar x Wistar, r=0.86 (·····90% confidence limits)

☆——☆ = Wistar x Br. Norway, r=0.65 (_____90% confidence limits)

proteinuria ≧ 0.3 g/l occurred more frequently (58%) than in the non-crossbred group (24%) and in the ticlopidine crossbred group (22%).

In the crossbred Wistar - Brown Norway group (without ticlopidine) litter size was significantly smaller than in the other groups. When the Wistar x Brown Norway group (without ticlopidine) was divided into a group with litter size ≦ 8 and a group with litter size 10-12, no differences between groups could be demonstrated (table 3), except for the number of dead fetuses which was 1 in the group with litter size 10-12, and 6 in the group with litter size ≦ 8.

TABLE 3 Wistar x Brown Norway, different litter size

Litter size group	≤ 8	10-12
Animals	(n=12)	(n=17)
Syst. blood pressure (mm Hg)*	102 ± 8	99 ± 8
6-keto-PGF1α (ng/sample)*	2.63± 1.67	2.42± 0.98
Maternal weight (gram)*	245.60±25.54	283.61±36.72
Litter size*	5.50± 2.20	10.71± 0.69
Fetal weight (gram)*	3.74± 0.41	3.63± 0.25
Fetal death	6	1

* means ± S.D

DISCUSSION

Prostacyclin (PGI_2), an important endogenous vasodilator with strong platelet anti-aggregation potency, and thromboxane (TXA_2), with opposite effects, are supposed to be important pathogenetic factors in human PIH. A shift of the physiologic balance between PGI_2/TXA_2 to the dominance of the effects of TXA_2 could explain the vasoconstriction and enhancement of platelet aggregation that occurs in PIH (Ylikorkala 1985). In rat pregnancies near term increased levels of 6-keto-PGF1α have been found in uterine, placental and arterial tissue, amniotic fluid and uterine venous plasma (Zamecnik 1980, Wilson 1982). We also found elevated in vitro production of 6-keto-PGF1α and a significantly lower blood pressure in pregnant animals when compared with nonpregnant animals. However, the blood pressure appeared to be higher and 6-keto-PGF1α production lower in the crossbred group when compared to that in the Wistar x Wistar group. Although the capacity to produce 6-keto-PGF1α is at least influenced in part by the fetoplacental unit (Wilson 1983) we could not find differences in 6-keto-PGF1α production between crossbred animals

with small (≤ 8) and normal sized (10-12) litters. Proteinuria tended to be elevated in crossbred untreated animals.

The reduced 6-keto-PGF1α production by uterine arteries in vitro and the higher blood pressure resemble the symptoms of PIH in man. Fetal growth retardation, a common finding in PIH in man, was not observed in our rat model, and in fact the number of fetal deaths was larger in the Wistar x Wistar group. However, it cannot be excluded that the smaller litter size in the Wistar x Brown Norway group may have been caused by early fetal death followed by complete absorption.

In order to study the influence of prostaglandins on the results obtained in our model some of the crossbred rats were treated with ticlopidine. Ticlopidine is a powerful anti-platelet agent with a permanent effect on the platelet membrane. Ticlopidine does not inhibit platelet cyclooxygenase and has no effect on thromboxane synthetase, but it enhances the inhibitory effect of prostacyclin on platelet aggregation (Panak 1983). In the crossbred rats treated with ticlopidine we found a lower blood pressure and a reduced production of 6-keto-PGF1α as compared with that in untreated crossbred animals. A decrease of 6-keto-PGF1α production would be expected to lead to hypertension, unless TXA_2 production is also reduced with a shift of the PGI_2 /TXA_2 balance towards PGI_2. Platelet aggregation is a common feature in PIH in man, in particular when combined with fetal growth retardation, and could be a major source of TXA_2 production. In most cases thrombocytopenia in PIH is mediated by thrombin independent mechanisms (Gibson 1982, Wallenburg 1987). Ticlopidine has no direct effect on TXA_2 production, but during ticlopidine treatment in man decreased levels of TXB_2 (the major stable metabolite of TXA_2) were found following in vitro supplementation of arachidonic acid in patients with enhanced platelet aggregation. Platelet TXB_2 production was not influenced by thrombin stimulation (Gensini 1983). In our model no TXB_2 measurement or platelet life-span studies were performed,

but the reduced blood pressure in combination with a decreased 6-keto-PGF1α production suggests a further suppression of TXA_2 due to inhibition of platelet aggregation by ticlopidine.

In our experiments fetomaternal incompatibility had no favorable effect on pregnancy outcome in contrast with the improved pregnancy outcome reported in case of increased major histocompatibility complex (MHC) antigen incompatibility by crossbreeding two inbred strains (Billington 1964, Beer 1978). We did not use 2 inbred strains, but a random bred Wistar strain and an inbred Brown Norway strain. MHC incompatibility in the random bred Wistar strain can be expected to be strong enough to warrant normal pregnancy outcome. Incompatibility of non-MHC-antigens may have a negative effect on pregnancy outcome (Palm 1974, Dawson 1982), which may explain the unfavorable outcome in our model. Further investigation in this animal model is required to provide information on the relationship between the immune system and PIH.

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CHAPTER 4
PREGNANCY-INDUCED HYPERTENSION IN CROSSBRED RATS*
II Quantification of histologic expression in placenta and fetal liver

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ABSTRACT

To investigate the role of immunity on the course of pregnancy, with special emphasis on changes found in human pregnancy-induced hypertension, pregnancies of 44 crossbred female Wistar rats x Brown Norway males were compared with those of 25 Wistar x Wistar couples, with particular reference to 6-keto-PGF1α production by uterine arteries, and the histologic features of placenta and liver. All female rats were of the same age and nulliparous. Eleven crossbred animals received the platelet aggregation inhibiting agent ticlopidine during gestation. After crossbreeding, elevated blood pressures, increased levels of proteinuria, and a reduced uterine arterial 6-keto-PGF1α production were found, in analogy with findings in pregnancy-induced hypertension (PIH) in man. These changes were in part prevented by treatment with ticlopidine. Increased labyrinthine giant cells, trophoblast proliferations, and extramedullary hematopoiesis in fetal liver were found after crossbreeding, suggestive of placental and fetal ischemia. These changes were only partly prevented in placentas after ticlopidine medication. It is concluded that fetomaternal incompatibility in this model may lead to a disturbance of the maternal uteroplacental adaptation and fetal and placental hypoxemia.

INTRODUCTION

The immune system is supposed to play a role in the pathogenesis of Pregnancy-Induced Hypertension (PIH) (Beer 1978, Redman 1981). In animal experiments fetomaternal incompatibility seems to be advantageous for the outcome of pregnancy (Palm 1974, Beer 1975), although exceptions exist (Palm 1974, Dawson 1982). However, none of the reported experiments allows conclusions with regard to the pathogenesis or pathophysiology of PIH.

In an attempt to investigate the effects of incompatibility on the course of pregnancy, in particular with regard to the development of PIH, we designed an immunologic heterogeneity model by crossbreeding female Wistar rats with Brown Norway males. In this model maternal expressions of PIH, such as an elevated blood pressure, increased levels of proteinuria, and decreased in vitro 6-keto-PGF1α production by uterine arteries, were found, which closely resembles PIH in man. These signs could in part be prevented by ticlopidine treatment during pregnancy (Hutten, accepted for publication).

Because PIH is often accompanied by an impaired placental circulation, expression of fetoplacental ischemia was investigated in the animal model. Proliferation of trophoblast cells and fetal extramedullary hematopoiesis (EMH) were chosen as indicators of placental and fetal ischemia, respectively. In the labyrinthine placenta of the rat syncytial trophoblast cells and labyrinthine giant (trophoblast) cells can be distinguished. Quantification of placental labyrinthine trophoblast cells and liver extramedullary hematopoietic cells was performed in order to study possible signs of increased ischemia in the crossbred group.

MATERIALS AND METHODS

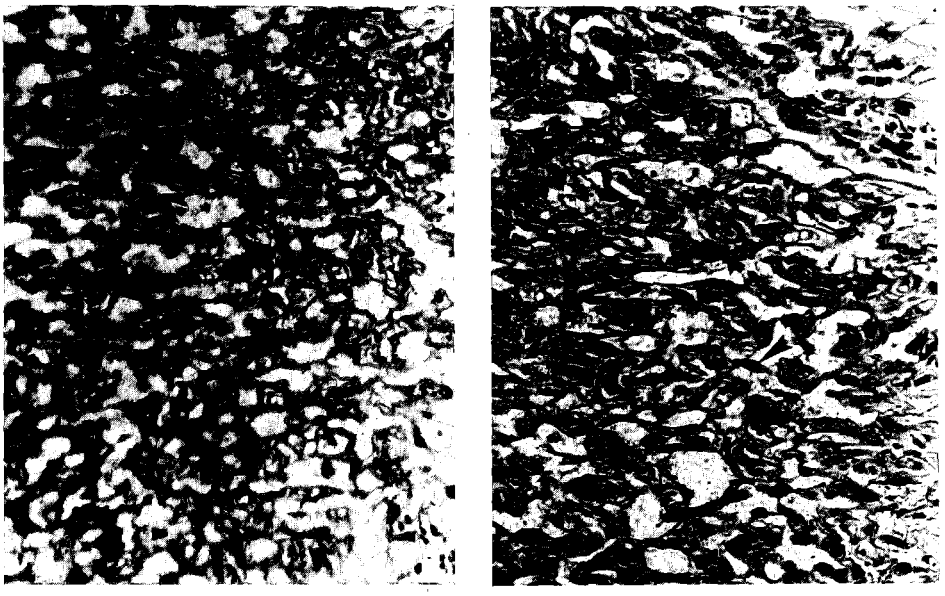
Seventy-three nulliparous Wistar rats were mated at 8-10 weeks of age and conception was confirmed by inspection of the vaginal plug. Animals were kept in standard cages, 6 animals per cage. Food and water were supplied

ad libitum. Forty-eight Wistar females were mated with Brown Norway males, 25 Wistar females were bred with Wistar males. Thirteen nonpregnant nulliparous female Wistar rats aged 8-10 weeks served as controls.

Because platelet aggregation is common in PIH in man and may influence the prostacyclin-thromboxane balance which may interfere with the disease (Wallenburg 1987), platelet aggregation was inhibited in 14 crossbred rats by means of treatment with the antiplatelet agent ticlopidine in a dose of 25 mg on day 6, 8, 11, 13, 15 and 18 of pregnancy (Hutten, accepted for publication).

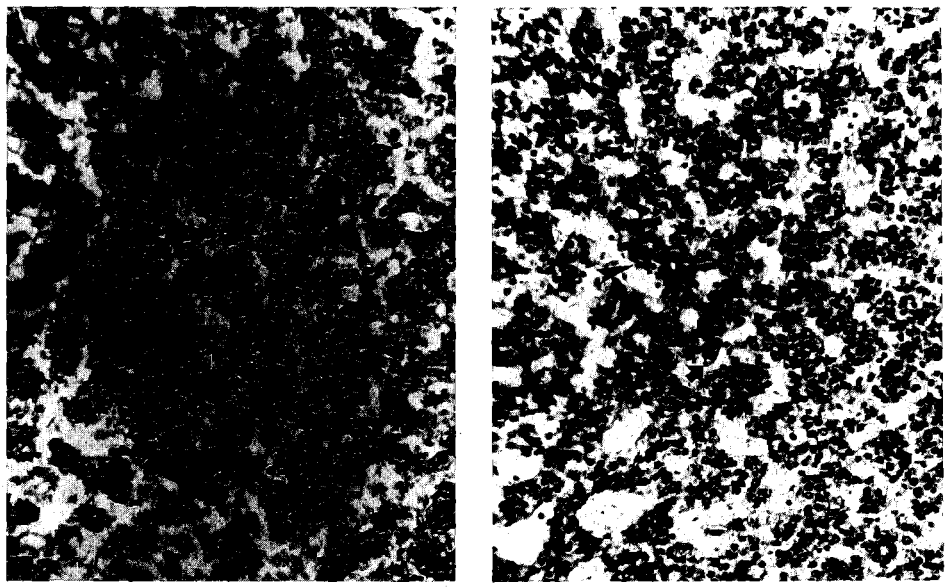
Animals were sacrificed on day 20 of pregnancy. Fetuses and placentas were fixed in 70% ethanol for at least 24 hours. Of each pregnancy one fetus and placenta were embedded in paraplast following routine histologic procedures. Placentas were cut transversally through the center and fetuses sagitally through the midline and vertebral column. Sections (4 μ m) were stained with hematoxylin without counterstain (see figures 1 and 2). The sections were examined, using established methods of morphometric quantitation (Aherne 1982). The adequate sample size for counting placental trophoblast and giant cells and fetal liver EMH cells was determined using the minimal sample size assessment through the method of running means. A sample size was chosen that resulted in average values and standard deviations within 2,5% of final means and standard deviations. Reproducibility studies revealed intra- and inter-observer variation coefficients less than 3% for the cells investigated.

Area selection for extraction of parameters was performed as follows: In the placenta the center was found using an eyepiece grid in the microscope (Nikon UWF and 2 x Plan-apochromat objective) and a line parallel to the placental base was chosen. This line was divided in four equal parts. Because perfusion of blood is lowest in the lateral parts of the placenta, studies on placental ischemia were performed in these parts. Eight photographs of adjoining areas from the lateral parts of this virtual line were taken per placenta using a



A B

FIG. 1. Placentas from Wistar x Wistar (A) and Wistar x Brown Norway (B). ➧ arrow at labyrinthine giant cell, ➤ arrow at trophoblastic cell (hematoxylin staining, original magnification: 200 x).



A B

FIG. 2. Fetal livers from Wistar x Wistar (A) and Wistar x Brown Norway (B). ➧ arrow at hepatocyte, ➤ arrow at island of EMH (hematoxylin staining, original magnification: 400 x).

20 x objective and 10 x photoocular. In the fetuses the center of the upper abdominal transversal diameter was measured. From there a line was drawn at a right angle through the diaphragm. From the transsection of this line and the subdiaphragmatic liver capsula, 2 frames per direction were taken, in ventral and dorsal directions, using a 40 x objective and a 10 x photoocular. Images were projected on a grated screen (80x100 cm) divided into 10x10 cm squares, using a standard projector. Cell populations were analyzed in a random sequence, without the investigator being aware of the clinical background of the animal; nuclei were identified as either trophoblastic, giant cell or "other" in the placenta, and the subpopulations were counted per slide. The number of placental cells were expressed as number of nuclei per square mm of tissue. In the livers nuclei were identified as hepatocytic or non-hepatocytic, assuming that the majority of the non-hepatocytic nuclei are related to EMH.

For details of histology see figures 1 and 2. Results of cell counting were tabulated and means and standard deviations were calculated. Differences were statistically analyzed using Wilcoxon's test, and $p < 0,05$ was considered to represent significance.

RESULTS

One animal in the crossbred group and three in the ticlopidine treated crossbred group were not pregnant. Numbers of labyrinthine trophoblast and giant cells were found to be significantly elevated in the crossbred group, as compared to those in the non-crossbred group (Table 1). Ticlopidine treatment had no demonstrable effect on the number of labyrinthine trophoblast cells. However, ticlopidine significantly reduced the number of labyrinthine giant cells ($p < 0.001$), although it remained higher in the crossbred group than in the non-crossbred animals. Liver cells as analyzed by hepatocyte nuclei counting did not differ between the three groups.

The amount of EMH, as analyzed by absolute numbers of non-hepatocytic nuclei, and the EMH/liver cell index were significantly increased in livers of crossbred fetuses. No significant difference was apparent between the group with and that without ticlopidine treatment.

TABLE 1 Histologic Features (means ± S.D.) of Placenta and Fetal Liver in Experimental Animals and Controls.

	WistarxWistar	WistarxBr.Norway	WistarxBr.Norway ticlopidine
Animals	n = 25	n = 33	n = 11
Labyr.trophobl. cells nuclei (10^3 mm^{-2})	1.5 ±0.35	2.3 ± 0.39 A	2.1 ± 0.45 A
Labyr.giant cells nuclei(10^3 mm^{-2})	0.31±0.06	0.58± 0.07 B	0.44± 0.06 B,C
EMH (nucl/slide)	76.94±27.17	101.12±30.85 A	107.91±38.38 A
Hepatocytes (nuclei/slide)	47.62±10.60	50.57±11.20	54.55±14.03
EMH/hepatocytes ratio	1.64± 0.42	2.01± 0.63 A	2.14± 1.07 A

A = $p < 0.01$ compared with WxW
B = $p < 0.001$ compared with WxW
C = $p < 0.01$ compared with WxBrN

DISCUSSION

The chorioallantoic placenta in man as well as in rats is hemochorial. The human placenta is of the villous type, whereas the rat placenta contains more differentiated areas like the labyrinth, the basal zone and a layer of giant cells (Garbis-Berkvens, accepted for publication). Only the labyrinth

contains fetal vessels, which are surrounded by 3 layers of trophoblast cells: one layer of metabolically active labyrinthine giant cells and two layers of syncytial trophoblast cells. The blood flow in the rat placenta is regulated through a central vessel, and hypoxia may be expected to occur first in the lateral parts (Garbis-Berkvens, accepted for publication).

The results of our study show that crossbreeding Wistar with Brown Norway rats stimulates the development of labyrinthine trophoblast and giant cells in placentas and EMA in fetal livers when compared with non-crossbred Wistar x Wistar rats. Ticlopidine treatment during pregnancy in the crossbred group tends to prevent the placental but not the fetal liver changes.

In human pregnancy cytotrophoblastic activity and formation of syncytial knots is known to be stimulated by placental ischemia (Rushton 1984). In analogy with the changes seen in human hypoxic placentas, proliferation of both types of trophoblastic cells in the rat placenta may also be caused by hypoxemia.

In the fetus we have found increased extramedullary hematopoiesis in the liver after crossbreeding, a feature that is also observed in experimentally growth-retarded guinea pigs (Lafeber 1981). Increased fetal hematopoiesis due to chronic hypoxia can also be found in human fetal and neonatal pathology (Naeye 1977, D'Souza 1981).

The signs of hypoxia found in placentas and fetal livers after crossbreeding are consistent with the earlier described increase in blood pressure which is regarded as a reaction to decreased placental perfusion, proteinuria and reduced in- vitro uterine artery 6-keto-PGF1α production in these animals (Hutten, accepted for publication). The picture closely resembles that of PIH as it develops in man.

Because increased platelet consumption is a common feature in PIH in man and appears to be associated with a disturbed PGI_2 /TXA_2 balance (Wallenburg 1987), platelet aggregation was inhibited in a group of crossbred animals by

means of ticlopidine treatment. Placental changes during ticlopidine treatment are suggestive of a slight but significant improvement in placental oxygenation. Such an effect was not observed in the fetal liver, and in fact after ticlopidine treatment fetal weight also appeared to be decreased (Hutten, accepted for publication). Perhaps the increased average litter size in rats receiving ticlopidine (10,27 vs 8,73) in combination with the slight improvement of the placental function may be responsible for the lower average fetal weight in this group.

In the model described in this report fetomaternal incompatibility appeared to be unfavorable for the outcome of pregnancy. In man an increased incidence of spontaneous abortion (Komlos 1977, Thomas 1985) and PIH (Jenkins 1978, Redman 1978) was found in cases of HLA compatibility. In animals incompatibility of MHC antigens also favors the outcome of pregnancy (Palm 1974, Beer 1975). However, incompatibility of non-MHC antigens can have a negative effect on pregnancy (Palm 1974, Dawson 1982). Perhaps the incompatibility of these non-MHC antigens has been responsible for the negative effects on pregnancy in our crossbred group. Further immunologic studies are required to answer this question.

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CHAPTER 5
GENERAL DISCUSSION

This thesis presents an approach to develop pregnancy-induced hypertension (PIH) in animals by means of an immunologic model. Hypertensive pregnancy disorders in man may be considered a clinical expression of maladaptation in pregnancy. Maladaptation disease develops early in pregnancy, a long time before the onset of clinical signs. There is evidence that maladaptation of the maternal immune system to the fetal allograft is involved in the etiology of PIH. Increased fetomaternal MHC-antigen compatibility and non-MHC-antigen incompatibility may be responsible for such maladaptation, and may therefore lead to the disease. Fetomaternal maladaptation is possibly responsible for the inadequacy, or even absence, early in pregnancy of trophoblast invasion in the walls of the spiral arteries. Later in pregnancy it may be responsible for the increased platelet activation, which is even observed in mild cases of PIH, by formation of immune complexes. This may lead to a disturbed prostacyclin/thromboxane balance, which appears to be an important mechanism in the development of clinical signs of PIH. The elevated thromboxane activity in patients with PIH, resulting from enhanced platelet activity, leads to local vasospasm and increased platelet aggregation. This may impair uteroplacental blood flow, in particular in case of insufficient trophoblast migration, which may lead to endothelial damage and a further decrease in prostacyclin production. There may also be a direct effect of immune complexes on the production of prostacyclin by placental endothelial cells. The pathophysiologic pathway, that leads from maladaptation to a disturbed prostacyclin/thromboxane balance, and finally to the clinical manifestations

of PIH, is far from clear. To elucidate this problem animal experiments may be helpful. In this thesis the influence of parenteral heterogeneity was investigated with regard to signs of PIH, and of fetoplacental ischemia. In all pregnant Wistar rats near term lower systolic blood pressures and higher in vitro uterine artery 6-keto-PGF1α production were found as compared with nonpregnant Wistar rats. After crossbreeding with Brown Norway males, however, the reduction in systolic blood pressure and the increase in 6-keto-PGF1α production in these animals appeared to be less than in non-crossbred Wistar females. A negative correlation was found between the in vitro synthesis of 6-keto-PGF1α and systolic blood pressure in pregnant rats with and without ticlopidine treatment, whereas in nonpregnant animals such a correlation was completely absent (appendix 2). This supports the concept that prostacyclin is involved in the regulation of blood pressure in pregnancy, but not in nonpregnant animals. In addition to the higher systolic blood pressure and lower 6-keto-PGF1α production a tendency to increased proteinuria was observed in crossbred animals, and there were signs of increased fetoplacental ischemia. As compared with non-crossbred animals extramedullary hematopoiesis in the fetal liver and numbers of placental labyrinthine trophoblast and giant cells appeared to be increased in crossbred rats as a result of increased fetoplacental ischemia. In agreement with these histologic findings a lower mean fetal weight (after correction for litter size) was found in crossbred rats as compared with non-crossbred rats. The number of macroscopically observed fetal deaths, however, appeared to be highest in the non-crossbred group. This may perhaps be attributed to early fetal death and complete resorption of the fetus in crossbred animals, which might explain the reduced mean litter size in this group. The higher blood pressure, proteinuria, reduced 6-keto-PGF1α production, and signs of fetoplacental ischemia in crossbred pregnant animals occurred spontaneously without intervention before or during pregnancy.

This picture strongly resembles that of human PIH. The only difference between the two groups was introduced by heterogeneous paternity. As the fetoplacental unit is a major source of prostacyclin, the reduced in vitro 6-keto-PGF1α production observed in crossbred rats might be explained by the decreased mean litter size in this group. This may explain the reduced decrease in blood pressure near term, but it cannot explain the increased proteinuria and fetoplacental ischemia in the crossbred animals. Moreover, the results obtained in crossbred animals with a litter size comparable with that found in non-crossbred animals were similar to those found in the entire crossbred group or in the crossbred animals with small litters. It can be concluded that crossbreeding Wistar females with Brown Norway males increases the susceptibility to developing symptoms comparable with human PIH, most likely because of immunologic fetomaternal maladaptation.

The results of treatment of the crossbred females with the antiplatelet drug ticlopidine during pregnancy suggest that the model is appropriate for the study of medical intervention. In the treated animals systolic blood pressures and the degree of proteinuria were found to be similar to those observed in non-crossbred animals. In vitro synthesis of 6-keto-PGF1α was reduced and comparable with nonpregnant values. It seems likely that ticlopidine decreased blood pressure by reducing thromboxane production, thus shifting the prostacyclin-thromboxane balance in favor of prostacyclin. As ticlopidine has no known direct effect on prostacyclin production, the observed reduction in 6-keto-PGF1α production may be due to an indirect effect caused by other vasoactive systems, such as the renin-angiotensin or kallikrein-kinin systems, which are known to be closely interrelated with prostacyclin production.

Histologic examination of the placentas of ticlopidine-treated crossbred rats showed signs of less severe hypoxia than that observed in placentas of untreated crossbred rats, but still more than observed in non-crossbred rats.

The rate of extramedullary hematopoiesis in the fetal liver of the treated rats was similar or even higher than that in untreated crossbred rats. Mean fetal weight was also lower in the treated group, but no fetal deaths were observed. The lack of improvement in fetal weight by ticlopidine treatment may be a result of the larger litter size and marginal placentation, in combination with a crude correction of blood pressure. In summary, the female Wistar rat mated with a Brown Norway male is susceptible to signs resembling human PIH. The model seems suitable for further studies in the field of the immunologic etiology and of the pathophysiologic pathways leading to the clinical signs of PIH. In addition, this model allows investigations into the effects of drug treatment of PIH on maternal and fetal condition.

SUMMARY

In CHAPTER 1 the main objectives of this thesis are described. There is a need for suitable animal models to investigate the etiology and pathophysiologic mechanisms in pregnancy-induced hypertension (PIH). Because of the possible immunologic basis of PIH an animal model was investigated with different kinds of fetal allograft. There is some evidence that in human and animal pregnancy MHC-antigen incompatibility has a positive influence on the course and outcome of pregnancy, whereas the influence of non-MHC-antigen incompatibility seems to be a negative one. Compatibility for MHC-antigens and incompatibility for non-MHC-antigens may be associated with the development of PIH. In order to increase non-MHC-antigen incompatibility without gross changes of MHC-antigen incompatibility, Wistar female rats were mated with Brown Norway males. Results were compared with those obtained in Wistar x Wistar pregnancies. In some of the crossbred animals platelet aggregation was inhibited by ticlopidine in an attempt to manipulate the prostacyclin/thromboxane balance.

With the use of this animal model a study was undertaken with the following objectives:

- to develop PIH or a PIH-like syndrome by means of an immunologic model;
- to investigate whether or not course and outcome of pregnancy in such a model are comparable with that known to occur in human PIH;
- to modulate the disease by pharmacological treatment and to investigate if the model could be of use in testing medication for treatment of PIH.

In CHAPTER 2 a review is given of the mechanisms that may be involved in the development of PIH in man, in spontaneous PIH-like syndromes in animals, and in experimental animal models of PIH. The concept of maternal immunologic adaptation to the fetal allograft and the possible role of maternal maladaptation in the etiology of PIH are explained.

An important step in the pathophysiology of the disease is attributed to a disturbed balance in prostacyclin and thromboxane activity, perhaps in interaction with other vasoactive systems such as the renin-angiotensin system. The disturbed balance between the vasoactive prostaglandins may be the result of an immunologic process.

Animal models are discussed in which placental ischemia was established by vascular occlusion, placental lesions were obtained on an immunologic basis, by means of steroid hormones, or by dietary manipulation in attempts to induce PIH. Although some signs were obtained comparable to those found in human PIH, none of the reviewed animal models appears to be satisfactory. The possible relationship between early fetomaternal maladaptation and the development of PIH has not been investigated in any of these models.

In CHAPTER 3 the results of maternal and fetal outcome of crossbred pregnancies are presented and compared with those obtained in non-crossbred pregnancies, and in crossbred pregnancies treated with ticlopidine. Blood pressure near term was reduced in all pregnant animals as compared with that in nonpregnant controls, but the decrease in blood pressure was less pronounced in crossbred pregnancies. Maternal weight and uterine arterial 6-keto-PGF1α production were lower in crossbred than in non-crossbred animals, and the severity of proteinuria was found to be increased. In the crossbred group treated with ticlopidine, 6-keto-PGF1α production was in the same range as that in nonpregnant animals, and blood pressures and proteinuria were comparable with those in the non-crossbred group. It is concluded that fetomaternal incompatibility in this model increases the

susceptibility of developing a pregnancy-induced hypertensive disorder.

In CHAPTER 4 the results of morphometric quantitation of fetal livers and placentas with regard to morphologic signs of fetoplacental hypoxic distress are presented. Increased labyrinthine giant cells, trophoblast proliferations, and extramedullary hematopoiesis in the fetal liver, suggestive of placental and fetal ischemia, were found after crossbreeding. These changes were prevented in part in placentas of ticlopidine-treated animals.

It is concluded that fetomaternal incompatibility in this model may lead to a disturbance of the maternal uteroplacental adaptation, and to fetal and placental ischemia.

In CHAPTER 5 the results of the heterogeneity model are discussed with regard to the main objectives of this thesis. It is concluded that immunologic heterogeneity in crossbred pregnancies of Wistar females with Brown Norway male rats increases the susceptibility to developing signs similar to those observed in human PIH. The model can be used for further studies on immunologic factors involved in the development of PIH, but also for investigation of the pathophysiologic pathways which lead to PIH. In addition, the model allows the study of the effects of drug treatment on maternal and fetal condition.

SAMENVATTING

In HOOFDSTUK 1 worden de belangrijkste doelstellingen van dit proefschrift omschreven. Er is een behoefte aan geschikte proefdiermodellen, waarin de etiologie en de pathofysiologische mechanismen van zwangerschapshypertensie onderzocht kunnen worden. Gezien de mogelijkheid van een immunologische basis van zwangerschapshypertensie werd een proefdiermodel met verschillende typen foeten als allo-"transplantaat" bestudeerd. Er zijn aanwijzingen dat in de zwangerschap, zowel bij mens als dier, incompatibiliteit van MHC-antigenen een gunstige invloed op het verloop en het resultaat van de zwangerschap heeft, terwijl non-MHC-antigeen incompatibiliteit een ongunstig effect lijkt te hebben. Compatibiliteit voor MHC-antigenen en incompatibiliteit voor non-MHC-antigenen is mogelijk betrokken bij het ontstaan van zwangerschaps-hypertensie. Om een toename van de non-MHC-antigeen incompatibiliteit te bewerkstelligen met minimale veranderingen van de MHC-antigeen incompatibiliteit, werden vrouwelijke Wistar ratten gedekt met Brown Norway mannetjes. De resultaten werden vergeleken met de resultaten zoals die verkregen waren bij Wistar x Wistar zwangerschappen. In verscheidene van de gekruiste dieren werd de plaatjesaggregatie geremd door ticlopidine in een poging de prostacycline/ thromboxaan balans te beinvloeden.

Met dit proefdiermodel werden de volgende doelstellingen bestudeerd:

- het ontwikkelen van zwangerschapshypertensie of een erop lijkend syndroom met behulp van een immunologisch model;
- het onderzoek of het verloop en resultaat van de zwangerschappen in zo'n model vergelijkbaar zijn met die zoals die bij zwangerschapshypertensie bij

de mens beschreven worden;

- de farmacologische beinvloeding van het ziektebeeld en toetsing van het model op de mogelijke bruikbaarheid voor het uittesten van medicamenteuze behandeling van zwangerschapshypertensie.

In HOOFDSTUK 2 wordt een overzicht gegeven van de mechanismen die een rol spelen in de ontwikkeling van zwangerschapshypertensie bij de mens, bij spontaan voorkomende - op zwangerschaps-hypertensie gelijkende - ziektebeelden bij dieren en bij proefdiermodellen met zwangerschaps-hypertensie. Het concept van maternale immunologische adaptatie ten aanzien van de allogene foetus en de mogelijke rol van een maternale adaptatie-stoornis in de etiologie van zwangerschapshypertensie worden uiteengezet.
Een belangrijke stap in de pathofysiologie van de ziekte wordt toegeschreven aan een verstoorde balans in de activiteit van prostacycline en thromboxaan, mogelijk in interactie met andere vasoactieve systemen zoals het renine-angiotensine systeem. De verstoorde balans tussen deze vasoactieve prostaglandinen wordt mogelijk veroorzaakt door een immunologisch proces.
Er worden proefdiermodellen besproken, waarbij gepoogd werd zwangerschaps-hypertensie te induceren door middel van placentaire ischemie door vasculaire occlusie, dan wel werden placenta laesies verkregen op basis van een immunologisch mechanisme, c.q. door middel van steroid hormoontoediening of dieetmanipulatie. Hoewel enige gelijkenissen gevonden werden met afwijkingen die gezien worden bij vrouwen met zwangerschapshypertensie, bleek geen van de vermelde proefdiermodellen bevredigend. De mogelijke relatie tussen een reeds vroeg intredende foetomaternale adaptatiestoornis en de ontwikkeling van zwangerschapshypertensie werd in geen van deze modellen bestudeerd.
In HOOFDSTUK 3 worden de resultaten getoond van gekruiste zwangerschappen voor wat betreft het maternale en foetale beloop en vergeleken met de resultaten, verkregen in niet gekruiste zwangerschappen en gekruiste

zwangerschappen, behandeld met ticlopidine. De bloeddruk à terme was in alle zwangere dieren lager dan in de niet-zwangere controlegroep, maar deze vermindering in bloeddruk was minder uitgesproken in gekruiste zwangerschappen. Het maternale gewicht en de 6-keto-PGF1α produktie van de arteriae uterinae waren lager in gekruiste dan in niet gekruiste dieren en er was een toegenomen proteinurie. In de gekruiste groep die met ticlopidine werd behandeld, was de 6-keto-PGF1α produktie vergelijkbaar met die zoals in niet- zwangere dieren; de bloeddruk en proteinurie waren vergelijkbaar met die zoals in de niet gekruiste groep. Geconcludeerd wordt dat de foetomaternale incompatibiliteit in dit model de vatbaarheid voor het ontwikkelen van een zwangerschapshypertensie verhoogt.

In HOOFDSTUK 4 worden de resultaten van de kwantitatieve morfologie van foetale levers en de placenta's getoond met betrekking tot morfologische kenmerken van foetoplacentaire hypoxie.
Een toename van labyrintaire reuscellen, trofoblast proliferaties en extramedullaire hematopoiese in de foetale lever, die suggestief is voor placentaire en foetale ischemie, werd gevonden in gekruiste zwangerschappen. Deze veranderingen werden deels voorkomen in placenta's van met ticlopidine behandelde dieren. De conclusie is dat foetomaternale incompatibiliteit in dit model tot een verstoring van de maternale uteroplacentaire vascularisatie leidt en tot foetale en placentaire ischemie.

In HOOFDSTUK 5 worden de resultaten van dit heterogene zwangerschapsmodel bediscussieerd in het licht van de doelstellingen van dit proefschrift. Geconcludeerd wordt dat de immunologische heterogeniteit in gekruiste zwangerschappen van Wistar vrouwtjes met Brown Norway mannetjes de vatbaarheid tot het ontwikkelen van afwijkingen die vergelijkbaar zijn met die, welke in zwangerschapshypertensie bij de mens gevonden worden, verhoogt.

Het model kan gebruikt worden voor verdere studies naar mogelijke immunologische oorzaken van zwangerschapshypertensie, maar ook voor verder onderzoek naar de pathofysiologische mechanismen die leiden tot zwangerschapshypertensie. Bovendien is het model geschikt voor studies naar het effect van medicamenteuze behandeling op de maternale en foetale conditie.

APPENDIX 1

W.nonpr.	6-keto-PGF1α (ng/sample)	syst. BP (mmHg)	proteinuria
1	1.51	105	±
2	2.34	105	±
3	1.95	110	±
4	4.22	110	±
5	2.67	115	±
6	1.90	110	-
7	2.73	107.5	+
8	1.17	120	±
9	1.82	102.5	±/+
10	1.12	105	-
11	1.40	107.5	±/+
12	1.17	100	±
13	1.04	110	+

- = negative

± = trace

+ = 0.3 g/l

++ = 1 g/l

W x W	6-keto-PGF1α (ng/sample)	syst.BP (mmHg)	proteinuria	litter size *	fetal death	fetal weight (gram ± SD)
1	3.77	85	+	12		3.16 ± 0.27
2	3.90	90	±	8		4.48 ± 0.16
3	4.42	87.5	±	10		4.77 ± 0.28
4	2.08	90	±	12		4.00 ± 0.28
5	3.33	90	±	12		4.37 ± 0.25
6	1.82	90	±	12		3.33 ± 0.19
7	4.55	85	+	15		3.04 ± 0.25
8	3.90	90	±	13		3.13 ± 0.23
9	2.03	90	±	16		2.98 ± 0.23
10	1.22	100	±	12		3.11 ± 0.46
11	3.58	85	±/+	11	2	2.73 ± 0.33
12	2.73	92.5	±	12		2.98 ± 0.21
13	1.90	85	±	13	1	4.28 ± 0.30
14	2.86	87.5	±	12		3.20 ± 0.13
15	5.20	92.5	+	13	1	3.12 ± 0.24
16	4.00	85	±	9	3	2.82 ± 0.56
17	2.86	80	±	12	1	3.01 ± 0.49
18	1.61	92.5	±	10		3.41 ± 0.15
19	4.03	95	++	9	2	3.60 ± 0.16
20	1.77	100	+	10	1	3.27 ± 0.31
21	2.29	80	±	12	1	3.30 ± 0.29
22	2.44	95	±	11		3.40 ± 0.34
23	1.87	95	+	4	2	4.21 ± 0.85
24	5.38	85	±	6	7	3.38 ± 0.10
25	2.44	85	-	14		4.59 ± 0.24

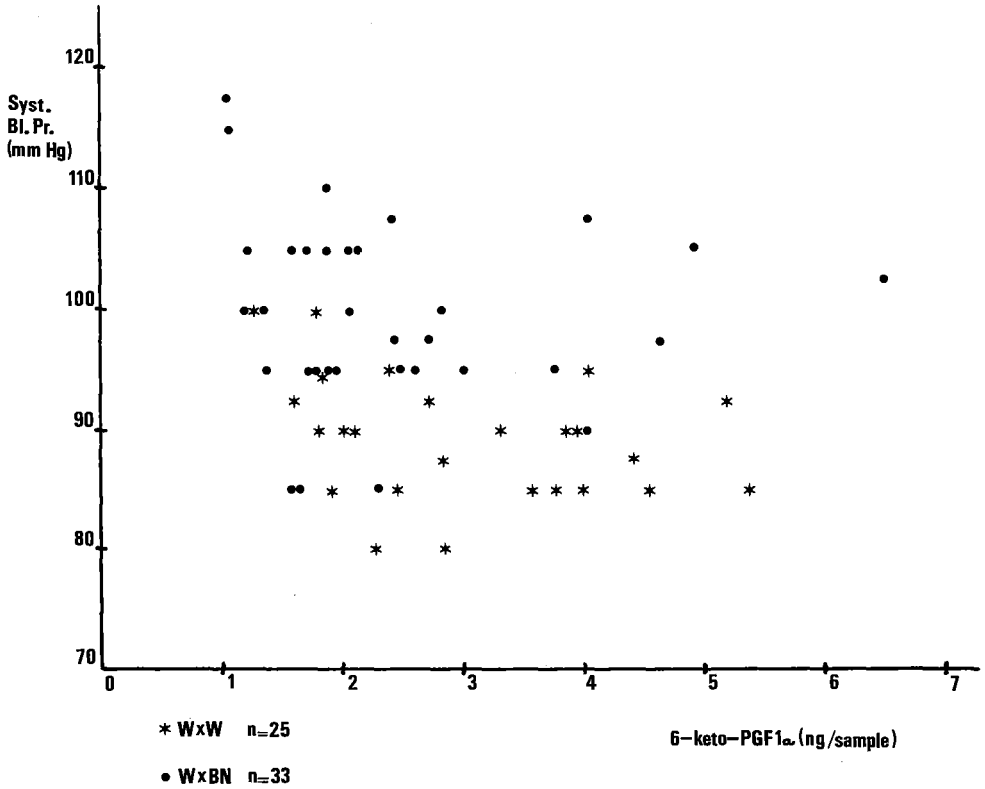
* litter size = number of fetuses alive

W x BN	6-keto-PGF1α (ng/sample)	syst.BP (mmHG)	proteinuria	litter size *	fetal death	fetal weight (gram ± SD)
1	1.61	85	+	11		3.55 ± 0.13
2	2.08	100	+	5	2	3.77 ± 0.18
3	4.03	90	-/±	12		3.63 ± 0.26
4	2.44	97.5	±	11		3.52 ± 0.24
5	1.87	105	±	11		3.70 ± 0.26
6	1.87	110	±	11		3.67 ± 0.10
7	2.29	85	±/+	10		3.65 ± 0.30
8	2.99	95	±	11		3.42 ± 0.15
9	1.82	95	+	8		3.63 ± 0.17
10	2.60	95	+	10		3.43 ± 0.15
11	2.47	95	+	13		3.43 ± 0.45
12	4.03	107.5	+	2	1	4.05 ± 0.64
13	2.29	105	±	11		3.50 ± 0.25
14	2.73	97.5	±	7	1	3.57 ± 0.16
15	1.95	95	+	9		3.67 ± 0.22
16	1.72	105	+/++	6		3.59 ± 0.13
17	2.42	107.5	+	10		3.81 ± 0.28
18	1.59	105	++	11		3.71 ± 0.23
19	2.86	100	++	10		3.83 ± 0.23
20	6.50	102.5	+	5		4.09 ± 1.18
21	3.77	95	±	10		3.65 ± 0.12
22	2.21	105	+	8		3.72 ± 0.24
23	4.94	105	+	2	1	3.78 ± 0.04
24	4.62	97.5	+	12		3.81 ± 0.20
25	1.38	95	+	9		3.57 ± 0.18
26	1.22	105	+	6		3.66 ± 0.37
27	1.20	100	±	10		3.68 ± 0.32

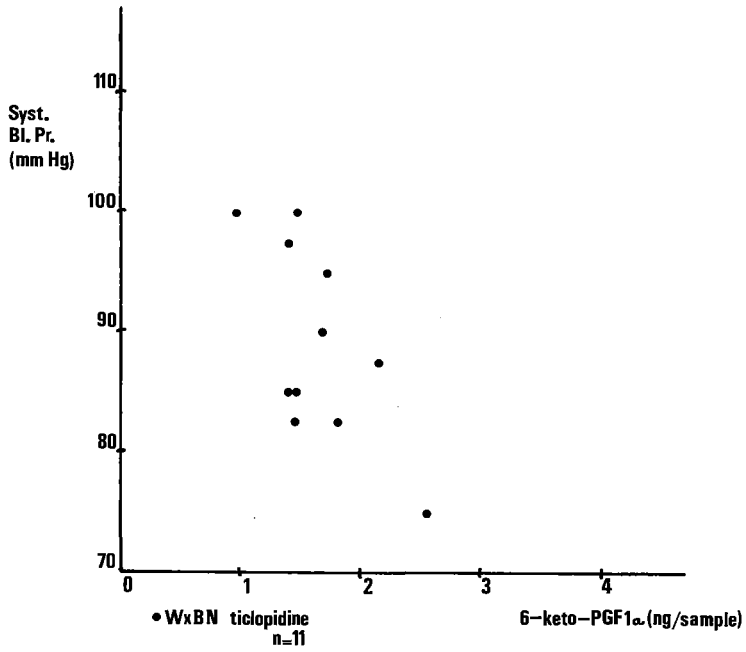
28	1.61	85	±	8		3.78 ± 0.15
29	1.35	100	±	9		3.22 ± 0.34
30	1.66	95	+	6	1	3.81 ± 0.59
31	1.04	117.5	+	3		3.75 ± 0.26
32	1.09	115	±	11		3.45 ± 0.32
33	1.74	95	±/+	10		3.77 ± 0.18

* litter size = number of fetuses alive

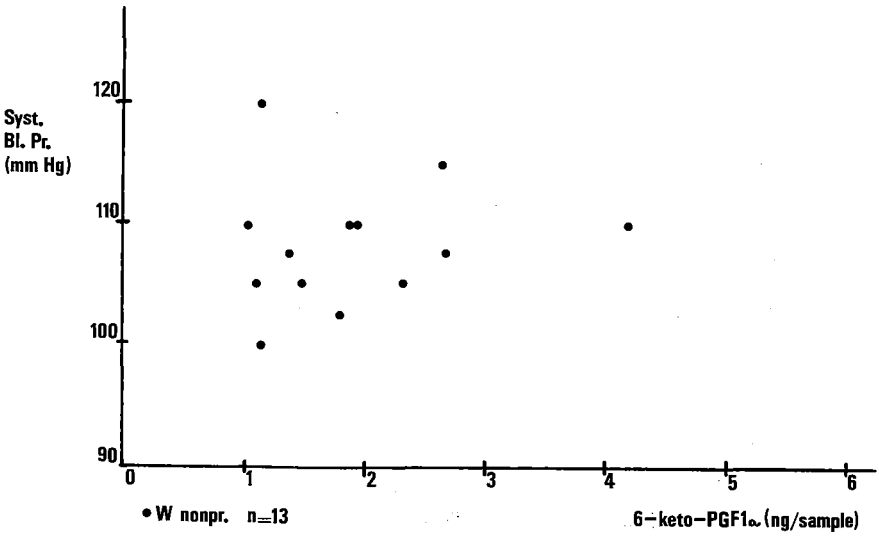
APPENDIX 2



Scatterdiagram 6-keto-PGF1α / systolic blood pressure
Wistar x Wistar and Wistar x Brown Norway
Correlation coefficient r=-0.27 n=58 p < 0.025



Scatterdiagram 6-keto-PGF1α / systolic blood pressure
Wistar x Brown Norway Ticlopidine
Correlation coefficient r=-0.65 n=11 p < 0.025



Scatterdiagram 6-keto-PGF1α / systolic blood pressure Wistar females nonpregnant. Correletion coefficient r=0.14 n=13 N.S.

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