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STUDIES ON A PLACENTAL FACTOR IN SPECIFIC HYPERTENSIVE DISEASE OF PREGNANCY AND ITS EFFECTS IN RATS

The Ohio State University

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STUDIES ON A PLACENTAL FACTOR IN
SPECIFIC HYPERTENSIVE DISEASE OF PREGNANCY
AND ITS EFFECT IN RATS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Silvana Beatriz Brianceschi, A.B., M.S.

* * * * *

The Ohio State University
1982

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This work is dedicated to Jesus Christ, whose unconditional love rejoices in my success and continues despite my failures. It is also dedicated to my mother and father, who sacrificed many of their dreams so that I might attain mine, to my husband Phil, whose love encouraged me through my despair and to my inspiration and joy Isaac, our son.
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INTRODUCTION

General Considerations

Specific Hypertensive Disease of Pregnancy (SHDP) can develop after the 24th week of gestation (i.e. in the third trimester of pregnancy). It occurs more frequently in primigravida who are either under seventeen or over thirty years old, and more frequently in lower income groups. The American Committee on Maternal Welfare has classified SHDP in terms of its severity as mild or severe pre-eclampsia or eclampsia.

The criteria for mild pre-eclampsia include the following:
1. an increase in systolic blood pressure to 140 mmHg or to 30 mmHg above previous usual level
2. an increase in diastolic blood pressure to 90 mmHg or to 15 mmHg above previous usual level
3. proteinuria
4. pitting edema of hands, face and legs

Also associated with pre-eclampsia are a decreased plasma volume, pulmonary edema, oliguria, hepatic and renal lesions, headaches and other CNS manifestations. Eclampsia is accompanied by convulsions, which may lead to coma and then death (Zuspan, 1975).

The renal lesions seen in SHDP have been described by Heptinsall (1974) and Tribe, et al., (1979). The changes seen by light microscope are usually confined to the glomeruli and include:

1. Frequently total glomerular involvement, although usually
only a small percentage of the affected areas displays the readily identifiable pattern.

2. Glomerular enlargement.

3. The number of cells in the tuft increase and in the more severe cases form lobules. Endothelial cell cytoplasm swells which leads to reduction of the capillary lumen, and in very severe cases, may result in total occlusion of the capillaries and bloodless glomeruli.

4. Small amounts of fibrin have been reported in very severe cases.

5. In severe cases it has also been found that there is profound thickening of the capillary basement membrane. These lesions are reversible and usually disappear 2-4 weeks post partum.

Humoral Factors Implicated in SHDP

Various workers have implicated a humoral factor in the pathophysiology of SHDP. Vasopressor activity not found in the normal parturients, has been demonstrated in the urine (Goretzlehner and Riethling, 1968) plasma, (Mauad-Fihlo and Meirelles, 1978; Tatum and Mule, 1962), decidual extract (Hunter and Howard, 1960), placental extract (Page, 1939) and amniotic fluid (Hunter and Howard, 1960) of those with SHDP. The characterization of the humoral factor has been highly disputed. Hunter and Howard (1960) found it to be heat stable in a pH range from 3.3 to 8.3, inactivated by proteolytic enzymes. The humoral factor's effect could not be blocked by phentolamine methanesulfonate, an α-adrenergic blocking agent or by lysergic acid diethylamide, a serotonin blocker. In addition, it did not demonstrate the antidiuretic effect of ADH or oxytocin, nor did it demonstrate many chemical characteristics similar to angiotensin.
However, Dhall et al., (1979) found it to be heat labile and to have a pressor activity similar to angiotensin II.

Amniotic fluid, placental and decidual extracts from women with SHDP were added to the bathing media of rabbit aortic strips, and to uterine muscle strips from virgin guinea pigs (Hunter and Howard, 1960). They demonstrated pressor activity in the amniotic fluid and in the decidual extract, but none in the placental extract. No pressor activity was found in similar preparations from normotensive pregnant women. When decidual extracts from women with SHDP superimposed on a molar pregnancy were (Hunter and Howard, 1961) added to the rabbit aortic strip media, pressor activity was again demonstrated. A decidual extract from normotensive molar pregnancy did not show pressor activity. When plasma from women with SHDP was injected into pithed, nephrectomized cats, a transient increase in blood pressure was seen.

In attempts to explain the failure of some investigators to demonstrate the pressor activity in the plasma from women with SHDP, Tatum and Mule (1962) collected 500 milliliters of whole blood from toxemic women whose blood pressure was elevated. When their blood pressure returned to normal, reinfusion with their own toxemic blood resulted in a rise in their blood pressure. Increased blood pressure was not seen in either normotensive women receiving their own blood or toxemic women receiving normal blood.

Mauad-Fihlo and Meirelles (1978) further supported the theory of a humoral factor in SHDP. These investigators measured the perfusion pressure, at a constant flow rate, of plasma from SHDP parturients through umbilical cords from toxemic women and from normal parturients. They
detected a greater vasoconstrictive effect in the plasma from SHDP, than that from normotensive donors.

Using the contraction of the rat colon as a bioassay for the constrictor ability of amniotic fluid, Dhall et al. (1979) found a heat labile, low molecular weight substance, whose effect was blocked by cinnarizine, an antihistamine. This substance was present to a greater concentration in the amniotic fluid of those women with SHDP. In addition although they found no difference in the plasma angiotensin II levels in SHDP as compared to the normal parturient, they reported an increase in the angiotensin II-like activity in the plasma and amniotic fluid of the SHDP donors as compared with their controls. Based on all their findings they concluded that the pressor activity in the amniotic fluid of SHDP was due to an angiotensin II-like substance.

**Schematic of the Renin-Angiotensin System**

Many investigators have implicated the renin-angiotensin system in the pathogenesis of pre-eclampsia. There is much discrepancy in changes that occur in SHDP, as compared to the normotensive pregnant state. The renin-angiotensin system is greatly altered in the normotensive pregnancy from the normal non-pregnant subject. In normal pregnancy there is a rapid rise in the plasma renin activity during the first three months, which increases more slowly thereafter, whereas, renin substrate concentration increases progressively throughout the pregnancy (Symonds et al., 1976). As a result angiotensin II and aldosterone are greatly elevated (Skinner et al., 1975; Weir et al., 1975; Skinner et al., 1972; Helmer and Judson, 1967;
Tapia et al., 1972; Massini et al., 1967). In SHDP renin substrate concentration was reported to be higher (Tapia et al., 1972), the same (Helmer and Judson, 1967) or lower (Weir, 1973) than in the normotensive pregnancy. Renin activity were reported to be higher (Symonds et al., 1976) or lower (Helmer and Judson, 1967; Chesley, 1978; Weinberger et al., 1976). Plasma angiotensin levels, were reported to be higher (Symonds et al., 1976), the same (Massani et al., 1967) or lower (Weir, 1973; Chesley, 1978). Plasma aldosterone levels were cited to be the same (Hulk, 1979) or lower (Weir, 1973; Chesley, 1978). Renin concentrations were reported to be higher (Symonds et al., 1976) or lower (Weir, 1973; Chesley, 1978). Reasons which were supportive of an imbalance in the renin-angiotensin system's involvement in the etiology of SHDP were:

1. the presence of hypertension (Zuspan, 1975)
2. Na retention (Zuspan, 1975)
3. edema (Zuspan, 1975)
4. vascular spasm (Zuspan, 1975)
5. an increased granularity of the juxtaglomerular cells (Pollak and Nettles, 1960; Altchek et al., 1968)

If the plasma renin substrate levels are higher than in normal pregnancy, this could be explained by the action of the levels of progesterone and estrogens seen in SHDP. In normal pregnancy estrogens and progesterones, which are high relative to the non-pregnant state, increase sodium excretion, in opposition to the action of aldosterone, thus stimulating the secretion of renin, angiotensin II and consequently, aldosterone. Angiotensin II is believed to be the direct stimulus for
aldosterone secretion. Therefore the higher level of progesterone found by some (Christensen, 1974) could explain increases of the angiotensin system seen by Symonds et al. (1976), and others.

Others believe the source of renin to be extra-renal. Although Pollak and Nettles (1960) found an increase in the granularity of the juxtaglomerular apparatus from the kidneys of eclamptic and pre-eclamptic women as compared to the normotensive pregnant woman, Altchek et al. (1968) found hyperplasia of the juxtaglomerular cells and atrophy of the macula densa in SHDP. Altchek's finding would imply that the macula densa is not involved in the mechanism to stimulate the juxtaglomerular cells. Renin has been found in the placenta (Hodari et al., 1967; Skinner et al., 1968), chorion (Skinner et al., 1968; Symonds et al., 1968), uterus (Gross et al., 1964; Ferris et al., 1967) and in the fetal kidneys (Geelhoed et al., 1968). Any combination or all of these could be the extra-renal source. Symonds (1979) found that in ten normotensive women, levels of angiotensin II in the blood leaving the placenta were higher than those entering the placenta, implying that at least angiotensin I had been converted to angiotensin II, and perhaps that the entire renin-angiotensin II cycle had occurred.

Converting enzyme activity was found in cord arterial and venous blood, (Oparil et al., 1976) and in placental tissue (Oats et al., 1980). All the constituents of the renin-angiotensin system can be found in the fetal-placental unit.

A consistent difference between normotensive pregnant women and pre-eclamptic women is their vascular reactivity to pressor agents.
Pressor response to a crude vasopressin extract, (Dieckman and Michel, 1937), to vasopressin (Browne, 1946) and to catecholamines (Raab et al., 1956) is greater in SHDP than in normal pregnancy. However no significant difference was found between the non-pregnant controls and the normotensive pregnant subjects. Pressor response to angiotensin II in normal pregnancy is decreased compared to the normal non-pregnant subject (Gant et al., 1974). Chesley et al. (1965) found 2.3 times as much angiotensin was needed in order to give similar mean pressor response in normotensive pregnant women as non-pregnant subjects. This resistance to angiotensin in pregnancy disappears in the immediate post partum period (Abdul-Karim and Assali, 1961).

It was shown by Conn et al. (1968) that patients with primary aldosteronism had an increased vascular reactivity to angiotensin II infusions. In other conditions associated with secondary hyperaldosteronism, similar to pregnancy (cirrhosis with ascites, congestive heart failure, nephrosis)(Laragh, 1962; Johnston and Jose, 1963) there is a decreased pressor response to angiotensin II. In the first cases there are decreased plasma renin levels and in the second case increased plasma renin levels. Therefore it seems that those conditions associated with decreases in plasma renin levels would have increased pressor responses to angiotensin II infusion, while the opposite would be true for conditions associated with low plasma renin levels. Since normotensive pregnant women require 2.3 times more angiotensin II than those with SHDP, similar to the normal non-pregnant subject (Chesley et al., 1965), and though the renin system may be depressed in SHDP, there is obviously another factor involved in the increased in vascular reactivity
of those with SHDP. It seems that SHDP is associated with a vascular hyperreactivity to pressor agents.

Volume deficits have been known to increase pressor resistance, or the refractoriness to angiotensin II in non-pregnant subjects. For this reason a possible explanation for the response of the normotensive pregnant woman was the result of a relative volume deficit, in other words the underfilling of the vascular tree. However, a rapid volume expansion in normotensive women did not result in a change in the vascular responsiveness to the infusion of angiotensin II. There was, however, a decrease in the renin activity in all of these women (Gant et al., 1974). Normal pregnancy is therefore an unusual condition in that although there may be an increase in plasma volume and a decrease in plasma renin activity, the vascular reactivity to angiotensin II is not affected. Therefore, in the normotensive pregnant woman the pressor responsiveness is determined by the degree of vascular smooth muscle resistance, whereas in the non-pregnant subject the pressor responsiveness to angiotensin II is mainly dependent on the circulating angiotensin II levels (Chinn and Dusterdieck, 1972).

Changes in intracellular sodium content and/or the number of angiotensin II binding sites can alter a vessel's responsiveness. It was also demonstrated by Gant (1974) that infusions of 5% NaCl altered the pressor responsiveness to angiotensin II in normotensive pregnant women. This finding provided a possible mechanism of action for the humoral factor(s) implicated by others in SHDP. If the factor(s) increase the vessel's angiotensin II binding sites, the normal balance between high levels of angiotensin II seen in normotensive pregnancy
and the reduced responsiveness of angiotensin II is disturbed, resulting in SHDP.

**Uterine Ischemia in the Etiology of SHDP**

The development of uteroplacental ischemia as a cause for toxemia was first proposed by Young (1914) as reported by Speroff (1975). Berker (1929) postulated that SHDP resulted from an increased resistance to blood flow in the uterus. By injecting barium into the uterine arteries of primigravid and multiparous cows, two months pregnant, he found the size of arteries of the multiparous cow to be much larger. When Berker (1948) examined human subjects using the same technique, he found similar results. These findings he believed to be an explanation of the higher incidence of SHDP in the primigravida and concluded that the hypertension seen in SHDP was a compensatory mechanism to maintain blood flow to the uterus.

Page and Ogden (1939) proposed that placental ischemia resulted in the release of a substance responsible for producing toxemia. Based on these theories and findings many of the following animal models were proposed.

**Animal Models for Studies of SHDP**

Hypertension in pregnant non-human primates and rodents is found very infrequently. In addition, no well defined clinical syndrome comparable to SHDP has been found. Experimental inductions of SHDP in animals have been attempted for the past fifty years. These have been attempts to mimic experimentally the clinical characteristics of SHDP, especially the hypertension. The animal which has most frequently been used is the rat. In an attempt to create an animal model using the rat,
Douglas and Langford (1965) gave uninephrectomized rats daily injections of desoxycorticosterone acetate while increasing their salt intake (DCA-NaCl rats). Even though this model succeeded in reproducing hypertension, proteinuria and renal lesions similar to those of SHDP, the investigators did not wish to imply that excess mineralocorticoid production was the cause of SHDP.

Others have taken the route of injuring the rats placenta in order to produce an animal model. Langford et al. (1967) and Smith et al. (1967) attempted to induce SHDP by immunological means. Placenta in Freund's adjuvant was injected into pregnant and non-pregnant rats. In the pregnant animals an increased blood pressure and proteinuria were found. In another immunological attempt to induce a SHDP like condition, rabbit anti-rat placenta serum was injected into pregnant and non-pregnant rats. This treatment resulted in hypertension. It was suggested by these authors that in toxemia placental damage could result in the release of a substance into the circulation causing the hypertension.

The study of Goldblatt hypertensive rats in relation to pregnancy was examined by Douglas (1976). Rats were bred after applying a Goldblatt clamp to one kidney and removing the other kidney. Because they found that during pregnancy the blood pressure of these rats actually dropped, returning to pre-pregnancy, post clamp hypertensive levels following delivery, they concluded that the hypertension seen in the rat by the Goldblatt clamp is not of the same type as that seen in pre-eclampsia.

The association of poor nutrition with pre-eclampsia has led many investigators to attempt to create toxemic symptoms in animals fed
restrictive diets. Douglas and Langford (1966) attempted to produce pre-eclampsia in the rat using a vitamin E deficient diet described by Stamler (1959). They produce morphologic lesions in the pregnant rat similar to those seen in SHDP. However, this was in the absence of hypertension, edema and proteinuria. Seidl et al. (1979) compared a naturally occurring ketotic syndrome resembling SHDP found in the pregnant guinea pig, to a ketosis induced by fasting pregnant guinea pigs. They found these two conditions differed in that the "toxemic" guinea pig's ketosis was induced by uteroplacental ischemia. However, even the naturally occurring toxemia in the guinea pig did not show edema or hypertension, though it too showed the morphologic lesions seen in SHDP, as did rats on the vitamin E deficient diet.

The most common method to use to produce pre-eclampsia is via uterine ischemia. Douglas and Langford (1967) attempted this by restricting the enlargement of the uterus by a cellophane wrapping. Although they succeeded in producing mild blood pressure elevation, they reported that their model had failed to produce a true model of SHDP. In a different and by far the most successful attempt to produce experimental toxemia in a laboratory animal, Abitbol (1980) severely constricted the abdominal aorta, and thus reduced the blood flow to the pregnant uterus of dogs. In these animals he found an increased blood pressure, proteinuria, and morphologic lesions like those seen in SHDP. Although this technique has met with fair success in the dog (Abitbol et al., 1976a), in the rabbit (Abitbol et al., 1976b) and in the monkey (Abitbol, 1977), as yet no successful rat animal model using this technique has been reported.
Prostaglandins have been implicated in the regulation of blood flow to the pregnant uterus (Terrago et al., 1974). In another attempt to imitate the uterine ischemia seen in SHDP in an animal model (Parks et al., 1980) indomethacin, a prostaglandin inhibitor, was given orally to DCA-NaCl hypertensive rats and to spontaneously hypertensive rats (SHR) during the last week of pregnancy. They found the DCA-NaCl rats treated with indomethacin had a 3% increase in blood pressure at term, whereas those not treated had a 6% decrease at term. The SHR treated with indomethacin showed a 9% decrease in arterial pressure compared to 16% decrease in the untreated animals. The level of significance was not reported nor were any other of the clinical signs of SHDP examined.

**Purpose of Study**

The present experiments were undertaken for the following reasons:

1. In order to demonstrate the presence of a pressor factor(s) in the placenta, placental extracts from normal and toxemic women were infused acutely into female rats, and systolic (and diastolic) recorded pressure.

2. In order to determine whether this extract creates a clinical picture similar to SHDP, kidneys were examined histologically, urine output and protein excretion were measured.

3. In order to further characterize the humoral factor, normal and toxemic placental extracts were subjected to ultrafiltration and acutely infused into rats.

4. In order to initiate the creation of a chronic animal model using toxemic placental extracts, extracts were chronically infused using Alzet osmotic minipumps into rats and caudal arterial pressure measurements were taken daily.
(5) in order to investigate possible interactions between placental extracts and hormones of pregnancy, extracts were infused acutely and chronically into pregnant rats, and systolic pressure was measured.

(6) in order to further compare the clinical signs of SHDP with the effect of chronic infusion of placental extracts, kidneys of treated rats were examined histologically, and in pregnant animals fetal weights were recorded and compared.

(7) in order to test interaction of placental extracts with the renin-angiotensin system, plasma-angiotensin I level, plasma aldosterone and plasma renin activity were measured in the chronically infused pregnant and non-pregnant animals.

(8) in order to test interaction of toxemic placental extract, with angiotensin II binding sites, saralasin, a competitive blocker of angiotensin II binding sites, was injected into acutely infused, TPE pre-treated animals.
I. Extraction Procedure

Term placentas obtained from normotensive pregnant women and from women with SHDP were frozen immediately after delivery. Both groups were para 1, gravida 0. Placentas were transported within twenty-four hours and kept for later extraction.

Each placenta was washed in 2 liters of cold 0.9% NaCl by gently stirring frozen placenta, until placenta was thawed and most of the residual blood was removed. This was repeated in 2 more liters of cold saline until the tissue was pink. Umbilical vessels, amniotic sac, and other placental vessels were removed from the placental tissue. The remaining spongy placental tissue was washed again in 2 liters of cold saline, blotted dry and weighed. The tissue was then placed in a beaker on ice and cut into small pieces. The tissue was extracted in cold 1 M acetic acid (1 gm of placental tissue/4 ml of acetic acid). Small pieces of tissue were placed in a glass homogenizer with approximately 20 ml of cold 1 M acetic acid. The tissue was homogenized with a Polytron homogenizer (Brinkman Instruments, Inc.). The polytron homogenizer was washed with a portion of the remaining 1 M acetic acid (approximately 20-40 mls), and added to pooled placental homogenate. The homogenate was then centrifuged at 4000 x g for 30 minutes at 4°C.
The supernatant was pooled and the pellets were rewashed with the remaining 1 M acetic acid (approximately 500 mls), recentrifuged under the previous conditions. The supernatant from this centrifugation was added to the first. The pH of this solution was adjusted to 7.4 by dropwise addition of 2 M NaOH, via titration column. The placental homogenate was placed on ice, with constant stirring. In order to inactivate all remaining proteolytic enzymes the homogenate was placed in a boiling water bath for 5-7 minutes, and immediately cooled on ice. Removal of the precipitate which resulted from the addition of the NaOH was accomplished by centrifugation at 4000 x g for 30 minutes at 4°C. The supernatant was decanted into flasks, lyophilized and stored in covered test tubes at -20°C until experimental use, at which time lyophilizate was redissolved in phosphate buffered saline (PBS) at a concentration of 0.1 g lyophilizate/ml and centrifuged at 4000 x g at 4°C for 10 minutes to remove any undissolved particles.

Some of the lyophilizate was redissolved in PBS at a concentration of 0.1 g/ml and subjected to ultrafiltration using an Amicon Ultrafiltration unit. The extract was first filtered using a membrane having a molecular weight cutoff of 500 daltons using a driving force of 30-40 psi nitrogen. When 80% of the solution was filtered, the volume was restored to 100% of initial volume by addition of the solvent. This process was repeated when 80% of this volume was filtered, thus insuring that 99% of the material recovered had a molecular weight of greater than 1500 daltons. The filtrate was then subjected to a filter with 500 dalton cutoff, using the same procedure as above, with the
exception that this time the retentate was collected. On completion of this filtration the filter was washed in 20 mls distilled water, lyophilized, and stored in covered test tubes at -20°C until experimental use. At which time lyophilizate was redissolved in PBS (0.1 g lyophilizate/ml). This extract was then infused into the animals represented in Figure 6.

II. Animals

All rats used in these studies were of the Sprague-Dawley strain. They were virgin females weighing 200-300 grams. Rats were housed in windowless quarters maintained at 68°C and artificially illuminated to provide 14 hrs of light and 10 hrs of darkness per day. Food (standard pellet diet) and water were available ad libitum. All animals were acclimated to this environment at least one week prior to experiment.

For the study using pregnant rats, estrous cycles were followed by vaginal lavage for at least two complete cycles. Females were placed with stud males on the afternoon of proestrus. The occurrence of mating was verified by the presence of sperm in the vaginal smear and/or the presence of a sperm plug on the following morning, which was considered day 1 of pregnancy. These rats continued to receive daily vaginal lavage until the day of experiment. Pregnancies were verified by laparotomy.

III. Procedures for Acute Experiment

On the day of the experiment animals were anesthetized with sodium pentobarbital (35 mg/kg body weight) and then fastened to a surgical board, ventral side up. The femoral vein on the left side was isolated, cleared and cannulated with a catheter made of Tygon Microbore (15 mm x .020 in. x .060 in., the Norton Co.) and teflon (55 mm x 28 ba., Small
Parts, Inc.) tubing. The catheter was connected to a Deseret infusion set, which was connected to a 5 cc syringe mounted on a Sage Model 341 infusion pump. The syringe and tubing were filled with either one of three test materials, toxemic placental extract (TPE), normal placental extract (NPE), or saline.

The arterial pressure was measured near the end of the abdominal aorta through a left femoral artery catheterization. The left femoral artery was isolated, cleared and cannulated with a catheter tip made identical to the femoral vein catheter tip. This catheter tip was connected to a Konigsberg P4 transducer kindly modified for this purpose by Dr. Heinz Pieper. The transducer was permanently cemented into a plexiglas housing with a removable cannulating tip and a sidearm connected to a 1 cc syringe for flushing. This system was hydraulically coupled to the arterial system with heparinized saline (75 U/ml). The pressure signal was amplified and recorded on a Grass polygraph.

The arterial pressure was then monitored for at least a 10 minute period or until pressure was stabilized. Infusion of test material was then started at a rate of .005 ml/gm rat weight/hr for 1 hour. Urine was collected for measuring protein concentration and for volume determination, by placing a clean test tube at urethra to collect spontaneous flow and on completion of experiment, by expression of the contents of the bladder by gentle compression. Protein determination was done using Coomassie Blue technique. Upon completion of the experiment the kidneys were removed via a ventral incision and stored in 10% buffered formalin until histological processing.
The mean systolic and diastolic pressure for each 10 minute segment of the polygraph record was determined directly using a Hewlett Packard Model 9874A digitizer connected on-line to a Minc-ll Computer (Digital Equipment Corp.) using a program designed by Dr. John Curry.

IV. Procedure for Chronic Experiments

Rats were trained to sit quietly in plexiglas restraining cages, placed on a heat pad at 40°C, with two surgical lights shining on them. A narrow, 1 cm wide inflatable cuff (Narco Bio Systems), was fastened around the proximal part of the tail and connected via a T-tube to a P4 Konigsberg transducer and to a calibrated air manometer with an attached blood pressure cuff pump. The signal from the transducer was amplified and recorded on a Grass polygraph. An E and M Pneumatic Pulse Transducer (MK 111 Ser 901) was fastened 1 cm distal to the cuff. The output from the pneumatic pulse transducer went to another channel of the polygraph. The output from the pressure transducer was calibrated with an air manometer. At least two measurements were made on each rat each day, or as many as needed to obtain readings without large discrepancies. The onset of pulsation occurred when the cuff pressure fell below the rat's systolic pressure (see Figure 1).

Chronic administration of test materials was accomplished by use of the Alzet Osmotic Minipump, which delivers test substance at a rate of 1.07 μl/hr for 7 days. Minipumps filled with TPE, NPE or PBS were implanted into non-pregnant or pregnant rats on day 14 of pregnancy. Intravenous infusion of test substance was accomplished using minipumps
Figure 1. Recording of caudal arterial pressure from a rat. Arrow in lower trace indicates systolic pressure concurrent with initial pulsation recorded from pneumatic pulse transducer.
implanted into the right external jugular vein. On the day of implantation animals were anesthetized with 40 mg/kg body weight sodium pentobarbital and fastened ventral side up on a surgical board. The external jugular was located and cleaned for a distance of 1.5 cm. All large branches were tied off. A catheter 2.5 cm (PE 60) long attached to a minipump, both filled with test substance, was inserted into the vein and advanced to the level of the heart. The catheter was secured with two ties. A pocket was made in the side of the neck, under the skin, into which the minipump was inserted, the incision was closed with wound clips. The day following implantation was arbitrarily designated day 1. Daily recordings of blood pressure were made for one week prior to implantation and the seven days following implantation. On day 7 of implantation the osmotic minipumps were removed under ether anesthesia, and residual volume recorded. Animals were then decapitated and blood collected for determination of renin activity and aldosterone levels. Kidneys were then removed and stored in 10% formalin for histological examination. Fetuses and placentas of pregnant rats were removed, weighed and stored in Bouin's fixative containing acetic acid.

V. Angiotensin System Evaluation

Acute experiments as described in section II were performed on pregnant rats between day 14-22 of pregnancy. Following infusion of TPE or NPE each animal received a 10 mg/kg injection of saralasin, a competitive blocker for AII binding sites. The blood pressure was monitored for an additional ½ hr after infusion in these animals.
VI. Histological Examination of Kidneys

Kidneys were stored for at least one week in 10% neutral buffered formalin.

Tissues were double embedded in paraplast and celloidin, but prior to this, they were progressively dehydrated in a series of alcohol followed by a clearing in methyl benzoate and infiltration of parlodion. This was accomplished by placing the tissues in marked plastic containers and immersing them in the solvents with gentle agitation. The schedule was as follows:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Ethanol</td>
<td>3 hrs</td>
</tr>
<tr>
<td>70% Ethanol</td>
<td>4 hrs (vacuumed 8-10 PSI for 10 min)</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>3 hrs</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>3 hrs</td>
</tr>
<tr>
<td>Methyl Benzoate</td>
<td>12 hrs</td>
</tr>
<tr>
<td>2% Parlodion</td>
<td>24 hrs</td>
</tr>
<tr>
<td>3% Parlodion</td>
<td>24 hrs</td>
</tr>
<tr>
<td>4% Parlodion</td>
<td>24 hrs</td>
</tr>
<tr>
<td>Benzene</td>
<td>2 hrs then (vacuumed 7-8 PSI for 15 min)</td>
</tr>
</tbody>
</table>

After clearing in the benzene, the tissues were infiltrated with paraplast by immersion into three consecutive 1 hour changes into a paraplast solution. During the third change the tissue was subjected to 7-8 PSI of vacuum at 56-60°C in a vacuum oven. Tissue was then embedded in blocks, allowed to cool at room temperature for 24 hours and serially sectioned at 10 μm. The resulting ribbons were placed
in water bath maintained at 40°C. When the surface tension of the
water caused the sections to spread, the parafin sections were mounted
on glass slides which had been lightly coated with 30% bovine serum
albumin (BSA). The mounted tissues were placed in an incubating oven
at 37°C for 24 hrs.

Prior to hematoxylin and eosin staining, the mounted tissues
were cleared in xylene and rehydrated in decreasing ethanol concentra-
tions according to the following schedule:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylene</td>
<td>10</td>
</tr>
<tr>
<td>Xylene</td>
<td>10</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>3</td>
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<tr>
<td>100% Ethanol</td>
<td>3</td>
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<tr>
<td>95% Ethanol</td>
<td>3</td>
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<tr>
<td>95% Ethanol</td>
<td>3</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>3</td>
</tr>
<tr>
<td>80% Ethanol</td>
<td>3</td>
</tr>
<tr>
<td>70% Ethanol</td>
<td>15</td>
</tr>
</tbody>
</table>

The hematoxylin-eosin stain was done according to the procedure
of Ehrlich (1886) as described by Mallory (1938). Tissues were
stained in Ehrlich's hematoxylin for 2 minutes, blued in tap water
and then stained 1 minute in 0.5% aqueous eosin A. After rinsing
tissues in distilled water they were dehydrated in alcohol and cleared
in xylene according to the following procedure:
<table>
<thead>
<tr>
<th>Solution</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% Ethanol</td>
<td>5</td>
</tr>
<tr>
<td>80% Ethanol</td>
<td>5</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>3</td>
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<td>95% Ethanol</td>
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<tr>
<td>100% Ethanol</td>
<td>3</td>
</tr>
<tr>
<td>Xylene</td>
<td>10</td>
</tr>
<tr>
<td>Xylene</td>
<td>10</td>
</tr>
</tbody>
</table>

All slides were then coverslipped with Gold Seal Cover Glass coverslips and permount (Fisher Scientific Co.). All microscopic examinations were done using an Olympus EH Microscope. Photomicrographs were taken using a Polaroid camera attached to the microscope. Detailed morphometric analysis of changes in glomerular size and Bowman’s capsule size were carried out on camera lucida drawings of renal glomerulia using a Kuffler & Essig polar planimeter.

VII. Hormone Determination

1) Plasma Renin Activity

Plasma renin activity was determined by the quantitation of generated angiotensin I using a radioimmunoassay kit obtained from New England Nuclear Co. The procedure is as follows:

(a) Generation of Angiotensin I

(1) frozen plasma were thawed in an ice bath

(2) 1 ml of each plasma was added to 10 ul of dimercaprol
solution and 10 μl of 8-Hydroxyquinoline solution in polystyrene tubes

(3) add 2.0 ml of pH 6.0 0.2 M maleate buffer to each tube and vortex

(4) 1 ml of each tube is transferred to similarly labeled polystyrene tube, which is then incubated for 1 hour at 37°C, while keeping remaining samples on ice

(b) Radioimmunoassay Protocol

(1) pipet 500 μl of 0.1 M tris-acetate buffer at pH 7.4 into blank tubes

(2) pipet 100 μl of 5% BSA into blank tubes and into zero standard

(3) pipet 100 μl of each standard into the appropriate tube

(4) pipet 100 μl of each sample generated at 37°C and its 4°C counterpart into well-marked consecutive tubes

(5) add 100 μl of angiotensin I (5-L-isoleucine) (tyrosyl-^{125}I) (angiotensin I tracer) to all tubes

(6) add 500 μl of angiotensin I rabbit antiserum to all tubes except blank

(7) vortex all tubes for 5 seconds

(8) incubate all tubes at 4°C for 18-24 hrs

(9) actively stir charcoal suspension

(10) transfer 1.0 ml of charcoal suspension to each tube
(11) vortex all tubes and centrifuge at 1200 kg for 15-20 minutes
(12) decant the supernatant fraction to appropriately numbered polystyrene tube and count radioactivity for 5 minutes

2) Aldosterone Concentration

Plasma aldosterone concentrations were determined using a radioimmunoassay kit obtained from Pantex, Inc. The procedure is as follows:

(a) Extraction Protocol
   (1) pipet 1.0 ml of serum into a 16 x 125 mm screw-capped culture tube with a Teflon-lined cap
   (2) add 50 μl of recovery aldosterone - H³, vortex and allow to sit at room temperature for 10 minutes
   (3) add 5.0 mls of dichloromethane to each tube and mix on a rotator for 10-15 minutes
   (4) centrifuge at 2500 RPM for 10 minutes
   (5) aspirate off the mat and the aqueous layer
   (6) transfer 4 ml aliquots of extract into LSC vials in a 50°C water bath and evaporate off the solvent with the aid of air
   (7) add 1.0 ml of diluent into each vial, mix, cap and place into a 37°C water bath for 30 minutes

(b) Radioimmunoassay Protocol
   (1) pipet 500 μl of standard or sample into appropriately
labeled 10 x 75 mm glass tube

(2) pipet 100 μl of tracer aldosterone $^{125}$I to each tube

(3) pipet 100 μl of 1st antiserum, mix gently

(4) incubate all tubes for 1 hour at 37°C

(5) place all tubes in an ice bath for 15 minutes

(6) pipet 200 μl of dextran coated charcoal and vortex all tubes

(7) incubate in an ice bath for 20 minutes

(8) centrifuge at 2500 RPM for 10 minutes

(9) decant the supernatant fraction into appropriately numbered polystyrene tube and count the radioactivity for 5 minutes
RESULTS

I. Pressor activity determination of extracts from placentas of women with SHDP.

In order to assess the pressor activity of the placental extracts prepared from toxemic patients, extracts were infused into rats while monitoring femoral arterial pressure. The results of these experiments (Figure 2) showed a rise in the systolic arterial pressure from pre-infusion values, with a wide variation of the effect on individual rats. Infusion of the vehicle showed no increment in the arterial pressure during the same hour period. The change in arterial systolic pressure was found to be significantly different (p < .05) between the saline and the toxemic extract infusion in every time interval except the second. Infusion of normal placental extract did not show a tendency to an increase in systolic pressure, and the second, third, fourth and sixth intervals were significantly (p < .05) different from the toxemic placental infusion. The normal placental extract infusion was different from the saline infusion (p < .05) only in the first time interval.

Examination of diastolic pressure during the same time intervals of these experiments showed a similar tendency to rise as seen in the systolic pressure (Figure 3). Diastolic pressures during NPE were
Figure 2. Mean arterial systolic pressure expressed as percent of mean pre-infusion value in virgin rats during 10 minute intervals during a 1 hr infusion of saline, normal placental extract (NPE) or toxemic placental extract (TPE). Mean ± S.E. ★, p < .05 for TPE vs. NPE.
Figure 3. Mean arterial diastolic pressure expressed as percent of mean pre-infusion value in virgin rats during 10 minute intervals during a 1 hr infusion of saline, normal placental extract (NPE) or toxemic placental extract (TPE). Mean ± S.E. ★, p < .05 for TPE vs. NPE.
significantly different (p < .05) from that of the TPE infusion in the first, fourth and fifth time intervals. There was no increment seen in the diastolic pressure with infusion of the normal placental extract or infusion of the vehicle.

II. Effects of the infusion of placental extracts on urine flow and urinary protein excretion.

There was no significant difference between those animals receiving NPE or saline infusions. The mean protein excreted in μg/hr for TPE, NPE and saline (Figure 4) were 1167.4 ± 247.0, 171.4 ± 50.6, and 126.5 ± 12.8 respectively. The lowest value for protein excreted in the toxemic group was 444.4 μg/hr and the highest value for the normal placental infusion was 250.1 μg/hr.

There was a significant increase in the urine output of those rats infused with TPE over both those infused with NPE or with saline (p < .05) (Figure 5). There was no significant difference between the effects of infusion of NPE or saline. The mean urine volumes in milliliters for TPE, NPE and saline infusions were 3.38 ± .69, 1.37 ± .54 and 0.50 ± .13, respectively.

III. Further characterization of placental extracts using ultrafiltration.

The results of these pilot experiments (Figure 6) showed an even greater increase in systolic pressure following TPE administration limited to molecular weights of 500 to 1500 daltons. The same molecular weight fraction of NPE showed a tendency to decrease the systolic pressure.
Figure 4. Protein excretion by non-pregnant female rats during a 1 hr infusion of saline, NPE or TPE. Mean ± S.E. ★, p < .01 TPE vs. NPE.
Figure 5. Urine production of non-pregnant female rats during a 1 hr infusion of saline, NPE or TPE. Mean ± S.E.
★, p < .05 for TPE vs. NPE.
Figure 6. Mean arterial systolic pressure change as percent of mean pre-infusion control value during a 1 hr infusion of NPE or TPE subjected to ultrafiltration to exclude molecules having molecular weights of less than 500 daltons and greater than 1500 daltons.
IV. Effect of chronic infusion of placental extracts into rats using implantable osmotic minipumps on caudal arterial systolic pressure.

Mean systolic pressures of rats chronically infused with TPE showed a slight increase from day one to day seven (Figure 7). However there was no significant difference between any of the groups, on any day, nor were there any significant changes in systolic blood pressure between day one and day seven in any of the groups.

V. Interaction of placental extracts with hormones of pregnancy.

(a) Effect of placental extracts in chronically infused pregnant rat.

In the first of these experiments thirty-four rats were mated and on the fourteenth day of pregnancy implanted with a minipump containing either TPE, NPE, or PBS. The mortality was high especially in the toxemic group. Daily measurements of mean systolic pressures of TPE-treated rats a significant increase of 38 mmHg by day 5 from day 1 of implantation (Figure 8). Mean systolic pressure of TPE infused animals was also significantly greater than those of NPE and PBS infused animals by day 5. NPE and PBS infused animals were not significantly different from each other on any day. All groups showed the usual drop in blood pressure which occurs in pregnant rats two days before delivery.
Figure 7. Mean caudal arterial systolic pressure during a one week infusion of TPE, NPE or PBS in virgin rats using Alzet osmotic minipumps. Mean ± S.E.
Figure 8. Mean caudal arterial systolic pressure during a one week infusion of TPE, NPE or PBS in pregnant rats using Alzet osmotic minipumps. Mean ± S.E. ★, p < .05 of TPE vs. NPE or PBS.
Figure 9.  
(a) Femoral arterial pressure record of a pregnant animal during an acute infusion with TPE.  
(b) Femoral arterial pressure record of a pregnant animal during an acute infusion with NPE.
Figure 10. Mean caudal arterial systolic pressure in pregnant and non-pregnant animals during a one week infusion of PBS via Alzet osmotic minipumps. Mean ± S.E. ★, p < .05 between pregnant and non-pregnant PBS treated groups.
Figure 11. Mean caudal arterial systolic pressure in pregnant and non-pregnant animals during a one week infusion of TPE via Alzet osmotic minipumps. Mean ± S.E.
When pregnant and non-pregnant animals chronically infused with PBS were compared (Figure 10), significantly different mean systolic pressures were found for days 4, 6, and 7. These results are consistent with the widely accepted finding that blood pressure is decreased during pregnancy in the rat. However when non-pregnant and pregnant animals chronically infused with TPE were compared (Figure 11), no significant difference was found on any day, including days 6 and 7, where the precipitous drop in blood pressure was seen in the pregnant group.

(b) Effect of placental extracts in acutely infused pregnant rat.

In the second part of this experiment TPE or NPE was acutely infused into nine pregnant rats, six and three in these respective groups. Three of the TPE infused rats died during the experiment. The remaining three rats showed vasospasms (Figure 9a) making accurate estimation of mean systolic arterial pressure for ten minute segments virtually impossible. Vasospasm was increased in animals close to term as compared with animals in the second trimester of pregnancy. There were no vasospasms seen in the animals infused with NPE (Figure 9b).

VI. Effect of infusion of placental extracts to pregnant animals upon fetal weights.

On the last day of pregnancy, rats which had been infused with either TPE, NPE, or PBS were sacrificed and their fetuses were weighed. Significantly lower birth weights have been recorded in children of women with SHDP, as compared with normal parturients (Zuspan, 1975). Consistent with this finding those animals receiving
TPE had fetus with significantly lower weights \((5.5 \pm 0.14 \text{ g})\) than those receiving PBS \((6.1 \pm 0.11 \text{ g})\) (Figure 12). However, those animals receiving NPE infusion also showed decreased fetal weights \((5.4 \pm 0.09 \text{ g})\) as compared to the PBS, but no significant difference as compared to those receiving TPE infusions.

VII. Evaluation of the effect of chronic infusion of placental extracts on the renin-angiotensin system.

In order to examine the effect of chronic infusion of the test substances on the renin-angiotensin system, plasma levels of angiotensin I, aldosterone and plasma renin activity were determined on blood samples collected after decapitation of pregnant and non-pregnant rats following a week infusion with TPE, NPE or PBS using osmotic minipumps. The results of the plasma angiotensin measurements are shown in Figure 13. Although the mean values for the non-pregnant TPE, NPE and PBS infused were lower than those of the pregnant TPE, NPE and PBS infused, no significant difference was found (Table 5).

Similar results were found in both the plasma aldosterone levels and the renin activity in these chronically infused animals. Aldosterone levels (Figure 14) and mean plasma renin activities (Figure 15) showed no significant differences among any groups (Table 5).
Figure 12. Weight of fetuses from rats chronically infused for one week with TPE, NPE or PBS. Mean ± S.E. ★, p < .01 vs. PBS treated animals.
Figure 13. Plasma angiotensin I levels (ng/0.1 ml) in non-pregnant and pregnant rats chronically infused with TPE, NPE or PBS. Mean ± S.E.
Figure 14. Plasma aldosterone levels (ng/ml) in non-pregnant and pregnant rats chronically infused with TPE, NPE or PBS. Mean ± S.E.
Figure 15. Plasma renin activity (ng/ml/hr) in non-pregnant and pregnant rats chronically infused with TPE, NPE or PBS. Mean ± S.E.
VIII. Effects of saralasin upon the vascular effects of TPE.

To test the hypothesis that the toxemic extract might in some way be acting via angiotensin II binding sites, pregnant animals that had been acutely infused with TPE or NPE for 1 hour, received a 10 mg/kg dose of saralasin, a competitive blocker for angiotensin II binding sites. As reported above, the pregnant animals that received TPE developed vasospasms. The administration of saralasin succeeded in both reducing the vasospasms and decreasing the blood pressure as shown in Figure 16.

IX. Quantitation of Bowman's space in glomerular units from preparations of kidneys of chronically infused animals.

Examination of typical glomeruli of kidneys of pregnant and non-pregnant rats chronically infused with TPE, NPE and PBS showed that Bowman's space in TPE infused rats was significantly decreased in both the non-pregnant and pregnant groups as compared to the NPE and PBS infused rats (Figure 17).
Figure 16. Femoral arterial pressure recorded from a pregnant (third trimester) rat. The recording shows pre-infusion record, record after 50 minutes acute infusion of TPE, record 10 minutes after administration of saralasin.
Figure 17. Bowman's space expressed as percent of glomerular unit in non-pregnant and pregnant rats treated with PBS, NPE and TPE. Mean ± S.E. ★, p < .05 for TPE vs. NPE. ○, p < .05 for TPE vs. PBS.
Figure 18. Photomicrographs of glomeruli taken from (a) a rat chronically infused with TPE and (b) a rat chronically infused with NPE. Note the decreased size of Bowman's space (2) in the glomerular unit from the rat infused with TPE.
DISCUSSION

The results of these experiments are in support of the theory of Page and Ogden (1939) that the placenta releases a substance which produces toxemia. The effect of the acute infusion of the toxemic extract into rats mimicked some of the characteristics of the clinical picture of SHDP. Administration of TPE resulted in a rise in systolic pressure, significantly different from controls in four time intervals, a rise in diastolic pressure, significantly different from controls in three time intervals, an increased excretion of protein in the urine and an increased urine output, which are all consistent with the clinical picture of SHDP and thus with the thesis that the placenta may be the source of the factor(s) responsible for the clinical signs of SHDP. Since the increase in blood pressure seen following the infusion of TPE is not apparent after the administration of NPE, it would seem that the factor(s) responsible for increased blood pressure is not present to any functional degree in the normal placenta. It would also appear that the factor(s) responsible for increased proteinuria and polyuria are not present in the normal placenta.

It also appears evident that mild uterine ischemia alone would probably not result in the clinical symptoms of true toxemia. Immuno-histologic examination of toxemic and normal placentas by Kaltenbach
(1980) showed a relationship between the proliferation of the cyclo-
trophoblast layer and of the placenta, and the severity of hypertension
in SHDP. This finding and the pressor effect which we found with TPE
would indicate the placentas from toxemic women are not the same as
those from normal women and the lack thereof in the normal placental
extract is in agreement with observations of Goretzlehner and Riethling
(1968), Mauad-Fihlo and Meirelles (1978), Tatum and Mule (1968), Page
(1939) and Hunter and Howard (1960, 1961) on the pressor activity of
urine, plasma, placental extract, decidua and amniotic fluid from women
with SHDP and on the absence of such activity in similar preparations
from normotensive women.

The wide effect of the toxemic extract on the blood pressure of
acutely infused non-pregnant rats reflect the wide variation of blood
pressure increases seen in women affected with SHDP (Zuspan, 1975).
The systolic pressure in women diagnosed as having pre-eclampsia can
vary from 130 mmHg to 220 mmHg. Another possible explanation for the
varied effect is the hormone levels of the rats on the particular day
of the experiment. In view of the relative success in the development
of hypertension in the chronically infused pregnant rats as compared
with the non-pregnant rats, it could be speculated that the hormonal
milieu is important for the hypertensive action of the toxemic extract.
This is supported by the increased susceptibility to the TPE infusion
of the rats later in their gestational period.

As to the chemical characteristics of the factor(s) involved, our
conclusions are based predominantly on the extraction we used in
preparation of the extract. It/They is probably not a large protein
since nearly all large proteins would be destroyed by boiling. It/They appear(s) to be heat stable, and pH stable from 2.3 to 7.4. From the pilot study using the molecular exclusion ultrafiltration, it would appear that the molecular weight(s) of the factor(s) responsible for the hypertension is between 500 and 1500 daltons, although no definite conclusion may be drawn from this study alone. Also, the factor is probably not lipid in nature since lipids tend to adhere to the ultrafiltration membranes. All of these facts are in agreement with the observations of Hunter and Howard (1960). The conclusion drawn by these researchers, that there were two factors present in toxemia; one a heat stable peptide and another larger protein, is not in disagreement with our findings. Their inability to demonstrate pressor activity in their placental preparation, may be explained by the dose of extract they used as was the case of our non-pregnant chronically infused animals which will be discussed later.

The vasospasms characteristic of toxemia (Zuspan 1975) were observed during acute infusion of the toxemic extract into three pregnant rats. The vasospasms were never observed in the acutely infused non-pregnant animals, nor were they observed in pregnant NPE infused animals. The vasospasms observed in the rats later in their gestational period were more severe than those seen in a 14 day pregnant rat. It appears that the rat's susceptibility to the infusion of the toxemic extract was altered by the physiological state of pregnancy. It would also appear that the factor(s) responsible for the vasospasms is not present in the normal placentas.
Chronic infusion of the toxemic placental extract in the non-pregnant animal at the rate of 0.107 µg/hr did not increase the systolic pressure. The failure of TPE to elicit a pressor response in the chronically infused non-pregnant animal may reflect the lower dose infused 0.107 µg/hr as compared with at least 0.1 g/hr in the acutely infused. It is evident, at least at this rate of infusion, that the chronically infused non-pregnant animal is not a good animal model in relation to the production of hypertension.

Chronic infusion of TPE into pregnant (third trimester) animals significantly increased the systolic pressure by the fifth day of implantation. Although this difference was obliterated by the customary decrease in blood pressure two days before parturition, hypertension had developed in this animal model up to this point. Comparison of the chronically infused PBS pregnant and non-pregnant animals showed the decrease in systolic which is normally seen in pregnancy (Figure 10). This decrease was significant in three days. A similar comparison of the pregnant and non-pregnant animals chronically infused with TPE showed that this infusion eliminated that difference.

Although, as was discussed earlier, some have succeeded in creating hypertensive pregnant rat models (Douglas and Langford, 1965, Langford et al., 1967 and Smith et al., 1967). Others have produced animal models with morphologic lesions similar to those seen in SHDP (Douglas and Langford, 1966 and Seidl et al., 1979). Until the use of pregnant rats chronically infused with TPE no model showed both hypertension and renal effects. The renal effect of chronically infused TPE in the pregnant and non-pregnant rats showed a decreased
Bowman's space, i.e. a glomerular enlargement in proportion to the glomerular unit. Another characteristic of SHDP, poor intrauterine growth (Salvadore et al., 1980), was observed in both the TPE and NPE infused animals but not in the PBS infused animals. The placenta, both normal and toxemic, evidently contains something that when infused into pregnant rats retards intrauterine growth. These/This factor(s) is probably not the same as the factor(s) which have/has the pressor effect in the TPE extract, since this effect was absent in the NPE extract. Since infusion of the normal extract demonstrated the same effect as the toxemic extract with respect to the intrauterine growth, this may be reflective of the degenerative state of toxemic placenta releasing its constituents into the system of pregnant woman, as opposed to the normal situation, in which a barrier to these substances is maintained between mother and placenta. Another common occurrence in pre-eclampsia is abruptio placenta, or pre-mature separation of the placenta. The pregnant rats chronically infused with TPE which died before the seventh day of implantation showed vaginal bleeding two days prior to death. Although the dead animals were not examined for the presence of abruptio placenta, it seems likely that this had occurred.

The renin-angiotensin system has been implicated in the pathogenesis of SHDP by many investigators. However the wide discrepancy found by these investigators in the direction of the changes of this system (if any) make a difficult comparison of the parameters of the angiotensin system in the TPE rat and the woman with SHDP. It would appear from the results of the plasma levels of angiotensin I, aldosterone and
renin activity, that the hypertension which developed by day 5 in the pregnant rat chronically infused with TPE was not due to changes in the level or activity of these components. Although the levels of these hormones were not measured on day 5, at which time the animals were detectably hypertensive, hormone levels were probably the same. In a study by Parks et al. (1980) it was shown that indomethacin was able to obliterate pre-parturional decrease in blood pressure in two types of hypertensive rats; the Spontaneously Hypertensive (SHR) and the Desoxycorticosterone Acetate Treated, NaCl loaded (DCA-NaCl). They report that the renin activity was undetectable in the DCA-NaCl rats which had been treated with indomethacin, and that the mechanism for this pre-parturional decrease in blood pressure must be something other than the activity of the Renin-Angiotensin system.

In light of our findings of no changes in the Renin-Angiotensin system in response to infusions of the extracts, the consistent difference between normotensive pregnant women and SHDP women, in their vascular reactivity to challenges of AII was still puzzling. Gant's (1974) finding that infusions of 5% NaCl into normotensive women resulted in altering their vascular responsiveness to AII infusions. Because the effect of infusion of 5% NaCl results in changes in the angiotensin II binding sites, it appears likely that the effect of the infusions of TPE is to do just this, change the angiotensin II binding site. In support of this theory is our finding that saralasin, a competitive angiotensin II binding site blocker succeeded in eliminating the TPE-induced vasospasms and reducing the blood pressure in the pregnant TPE acutely infused rats.
CONCLUSIONS

1. The factor(s) responsible for producing the clinical signs of Specific Hypertensive Disease of Pregnancy appears to be of placental origin.
2. Infusion of this material into rats appears to produce a syndrome consistent with those seen SHDP.
3. These studies have provided the first true rat animal model for SHDP. This model is potentially valuable for studies of the mechanisms and treatment of the disease.
APPENDIX
Table 1. Mean arterial systolic pressure expressed as percent of mean pre-infusion value in virgin rats during 10 minute intervals during a 1 hr infusion of saline, normal placental extract (NPE) or toxemic placental extract (TPE).

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Saline</th>
<th>NPE</th>
<th>TPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>96.1 ± 1.2</td>
<td>100.7 ± 1.0</td>
<td>106.0 ± 3.7</td>
</tr>
<tr>
<td>10-20</td>
<td>97.1 ± 1.2</td>
<td>98.0 ± 3.0</td>
<td>109.5 ± 5.4</td>
</tr>
<tr>
<td>20-30</td>
<td>95.3 ± 3.9</td>
<td>96.7 ± 2.9</td>
<td>115.3 ± 7.9</td>
</tr>
<tr>
<td>30-40</td>
<td>97.0 ± 1.8</td>
<td>94.9 ± 3.3</td>
<td>116.3 ± 1.8</td>
</tr>
<tr>
<td>40-50</td>
<td>95.3 ± 3.9</td>
<td>97.0 ± 2.9</td>
<td>111.8 ± 7.9</td>
</tr>
<tr>
<td>50-60</td>
<td>95.9 ± 1.4</td>
<td>97.2 ± 3.4</td>
<td>117.1 ± 10.6</td>
</tr>
</tbody>
</table>

The values indicated are mean arterial systolic pressures expressed as percent of mean pre-infusion values ± SE.
Table 2. Mean arterial diastolic pressure expressed as percent of mean pre-infusion value in virgin rats during 10 minute intervals during a 1 hr infusion of Saline, Normal Placental Extract (NPE) or Toxemic Placental Extract (TPE).

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Saline</th>
<th>NPE</th>
<th>TPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>99.3 ± 1.5</td>
<td>99.5 ± 0.9</td>
<td>108.6 ± 5.0</td>
</tr>
<tr>
<td>10-20</td>
<td>97.3 ± 3.0</td>
<td>99.6 ± 5.3</td>
<td>107.0 ± 9.5</td>
</tr>
<tr>
<td>20-30</td>
<td>96.4 ± 5.4</td>
<td>93.8 ± 5.8</td>
<td>109.7 ± 9.6</td>
</tr>
<tr>
<td>30-40</td>
<td>95.1 ± 4.1</td>
<td>90.6 ± 3.3</td>
<td>112.8 ± 6.5</td>
</tr>
<tr>
<td>40-50</td>
<td>93.0 ± 3.5</td>
<td>92.6 ± 4.5</td>
<td>113.3 ± 6.5</td>
</tr>
<tr>
<td>50-60</td>
<td>94.0 ± 3.1</td>
<td>94.5 ± 5.8</td>
<td>114.4 ± 9.7</td>
</tr>
</tbody>
</table>

The values indicated are mean arterial diastolic pressures expressed as percent of mean pre-infusion values ± SE.
Table 3. The effect of a one week infusion of PBS, NPE or TPE in non-pregnant or pregnant rats on caudal arterial systolic pressure

<table>
<thead>
<tr>
<th>Number of Days After Implantation</th>
<th>Non-Pregnant Treatment</th>
<th>Pregnant Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBS</td>
<td>NPE</td>
</tr>
<tr>
<td>1</td>
<td>107.0±7.1</td>
<td>115.2±3.9</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(6)</td>
</tr>
<tr>
<td>2</td>
<td>117.9±2.9</td>
<td>115.5±4.3</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(4)</td>
</tr>
<tr>
<td>3</td>
<td>115.6±4.4</td>
<td>121.2±3.5</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(6)</td>
</tr>
<tr>
<td>4</td>
<td>120.7±4.1</td>
<td>118.0±6.6</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(6)</td>
</tr>
<tr>
<td>5</td>
<td>122.4±3.8</td>
<td>111.7±4.7</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(6)</td>
</tr>
<tr>
<td>6</td>
<td>117.5±6.5</td>
<td>118.7±8.7</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(6)</td>
</tr>
<tr>
<td>7</td>
<td>121.2±2.8</td>
<td>120.2±5.0</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

The values indicated are mean systolic pressures (mmHg) ± SE with the numbers in the parentheses indicating the number of animal/group.
Table 4. Hormone levels in non-pregnant and pregnant rats chronically infused with PBS, NPE or TPE.

<table>
<thead>
<tr>
<th></th>
<th>Non-Pregnant Treatment</th>
<th>Pregnant Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBS</td>
<td>NPE</td>
</tr>
<tr>
<td>Aldosterone (ng/ml)</td>
<td>0.110±0.05</td>
<td>0.076±0.04</td>
</tr>
<tr>
<td>Renin Activity (ng/ml/hr)</td>
<td>3.9 ±0.7</td>
<td>3.7 ±0.7</td>
</tr>
<tr>
<td>Angiotensin I (ng/.1 ml)</td>
<td>0.17 ±0.03</td>
<td>0.10 ±0.03</td>
</tr>
</tbody>
</table>

The values indicated are hormone levels ± SE.
BIBLIOGRAPHY


Chinn, R.H. and Disterdieck, G. The response of blood pressure to infusions of angiotensin II: Relationship to plasma concentrations of renin and angiotensin II. Clinical Science 42:489, 1972.


