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GERALD OLIVER FRANKLIN

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Signature of Author

March 14, 1974

Date

THE EFFECT OF ACUTE BLOOD PRESSURE ELEVATION BY ANGIOTENSIN AND NOREPINEPHRINE ON PROSTAGLANDIN OUTPUT IN THE RHESUS MONKEY

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To Leon Speroff, for whose encouragement, enthusiasm, inspiration and friendship I am deeply grateful

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Introduction and Background

THE KIDNEY: HYPERTENSIVE AND ANTI-HYPERTENSIVE ROLES

The importance of the kidney as a regulator of blood pressure has long been recognized. With Goldblatt's discovery in 1934 that unilateral nephrectomy and constriction of the contralateral renal artery resulted in the development of sustained hypertension in the dog[1] and the discovery of the renin-angiotensin system, investigational activity was directed toward elucidation of a possible renal pressor role in the pathophysiology of hypertension. At the same time, however, evidence began accumulating which pointed toward an anti-hypertensive role for the kidney as well and even called into question the supposed dominance of the renal pressor hypothesis in the pathogenesis of some forms of hypertension.

Of initial interest was Goldblatt's inability to produce hypertension in dogs in the presence of a normal kidney opposite the one in which renal artery stenosis was induced. In 1947 Braun-Menendez and Von Euler reported that hypertension could be induced in rats from which the kidneys had been entirely removed.[2] Two years later, using an electrolyte-free diet of casein, dextrose and lard to prolong the lives of their animals, Grollman et al.[3] were able to demonstrate a more prolonged elevation of blood pressure in totally nephrectomized dogs. In 1951, Grollman et al.[4] demonstrated that the hemodynamic state produced by renoprival hypertension in the rat was identical to that found in human malignant

hypertension. This state involved no change in blood volume, cardiac output or venous pressure from pre-hypertensive levels. Furthermore, they demonstrated identical tissue histopathology for the two hypertensive states, characterized by proliferative changes in the media of small and medium sized arterioles. Later that year, Turner and Grollman[5] were able to demonstrate the independence of renoprival hypertension from adrenal secretions, since bilateral adrenalectomy did not prevent elevation of arterial pressure.

To better define the mechanism by which hypertension occurred, Grollman et al.[3] also performed dog experiments in which both kidneys were left in place with the ureters ligated, and others in which one ureter was implanted into either the small bowel or abdominal vena cava and the contralateral kidney was removed. Finding that only in cases of bilateral nephrectomy did a sustained elevation of blood pressure occur, they concluded that intact renal tissue was essential for the maintenance of the normotensive state and that this function was independent from the organ's excretory function. Furthermore, they were the first to postulate that "hypertension of renal origin" must not be due solely to liberation of a pressure agent but must stem in part from the kidney's failure to perform some antihypertensive activity.

These observations were partially confirmed by the subsequent finding that renoprival hypertension could be dramatically reduced within hours by either isolated renal perfusion[6] or renal trans-

plantation[7], even in the presence of continued high sodium and water intake. Again it was noted that the blood pressure lowering effects of the kidney were not due merely to loss of water and salt by diuresis since adequate salt and water replacement of urinary losses was provided, no sudden change in animal body weight occurred, and radioactive labelling techniques failed to reveal a change in either extracellular fluid or blood volume.

In another series of experiments, Muirhead and co-workers[8] explanted whole kidney and medullary and cortical fragments to the peritoneum and lungs of dogs before and after renal ablation. In bilaterally nephrectomized control animals and those in which only renal cortex had been explanted, the expected rise in blood pressure was observed. In animals receiving explanted renal medulla, however, protection against renoprival hypertension was conferred. Subsequently, these same investigators demonstrated the ability of an intravenously administered saline extract of renal medulla to blunt the hypertensive effects of bilateral nephrectomy in dogs, as well as to lower blood pressure in dogs in which renoprival hypertension had already been established.[9] At the same time, extracts of spleen, lung, and erythrocytes failed to alter the course of renoprival hypertension.

Once the renal antihypertensive "factor" had been localized to the medulla, efforts were made to refine and characterize it. In 1964 Hickler and co-workers[10] reported that the "depressor activity" of crude medullary homogenates was localized in the

lipid phase following extraction from acidified renal medullary tissue. Following this work, Lee and co-workers[11] published a major paper reporting isolation and characterization of three acidic lipid vaso-depressor substances using column and thin-layer chromatographic techniques on a variety of solvent extractions of renal medullary tissue. Production of a sustained blood pressure depression in the normotensive vagotomized pentolinium-treated rat was the assay employed. While only two of the three acidic lipid substances were found to cause significant lowering of blood pressure, all three were found to stimulate non-vascular smooth muscle to varying degrees. Of a number of acidic lipid substances known to have such properties at the time, Lee's group selected prostaglandins as a broad series of compounds with similar characteristics with which to compare the newly isolated renomedullary compounds.

Chemically, Lee demonstrated strong similarities between the prostaglandins and his own renomedullary acidic lipids.[11] These were listed as follows:

1) Both were ethanol soluble and completely lipid extractable from an acid aqueous phase but only incompletely extractable from an alkaline aqueous phase.

2) Infrared spectroscopy revealed the presence of carbonyl and methylene groups "with evidence for the existence of hydroxyl groups and trans ethylene bonds" in both groups of compounds.

3) Two of Lee's three compounds ("Medullin" and "Compound 1" as opposed to "Compound 2") shared similar UV spectra with prostaglandins of the E series.

Lee also noted some differences in chemical properties between medullin and the PGE series of compounds, however, despite their apparent structural similarities. Among these were the following:

1) Medullin was found to be significantly more mobile than PGE compounds on thin layer chromatography.

2) Medullin stained more intensely with iodine vapors than equal amounts of PGE_1.

3) PGE_1 in ethanol possessed no characteristic absorption at higher wavelengths, whereas a methanolic solution of medullin did.

4) PGE_1 was found to be 50 times more potent in stimulating non-vascular smooth muscle than medullin.

In light of the above evidence, Lee concluded that medullin was a derivative of prostanoic acid with basic structural similarities to PGE_1 but was more unsaturated and had fewer polar components (hydroxyl groups). This compound was later identified by Lee as a new prostaglandin, PGA_2. At the same time, Lee identified his "Compound 2" as PGE_1 on the basis of thin layer chromatographic mobility similar to PGE_1 and the other properties listed above. Because Lee's "Compound 1" was less mobile than PGE and demonstrated strong non-vascular smooth muscle stimulating activity with absent or weak vaso-depressor properties--all characteristics common to PGF--Lee tentatively established the identity of "Compound 1" as PGF.[11]

PROSTAGLANDINS AS VASO-ACTIVE SUBSTANCES

Since the identification and characterization of the prostaglandins, a great deal of work has been done to elucidate their effects and roles in cardiovascular and renal physiology. In virtually every laboratory animal studied, prostaglandins of the E series have caused a lowering of arterial blood pressure following parenteral administration. Among the animals studied have been the dog,[12-15] cat,[16,17] monkey,[18] baboon,[18] rabbit,[19,20] and rat.[16,21]

In man, Bergstrom infused PGE intravenously at a rate of 13 - 47 μg/min with the production of facial flushing, intense headache, cardiac palpitation, increase in heart rate varying from 10 - 24 beats per minute, fall in systolic and diastolic blood pressure of from 15 - 27 and 15 - 31 mm Hg respectively, and the development of severe chest and gastrointestinal discomfort.[22] In addition, Bergstrom's group later demonstrated the ability of PGE_1 to blunt the pressor response to norepinephrine by as much as 50%.[23] More recently, Carlson and co-workers[24] performed dose response studies on 8 human volunteers and found that at PGE_1 infusion rates of 0.058 - 0.10 μg/kg/min cardiac output increased by a mean of 66%, heart rate by 27 beats per minute, and that mean arterial pressure fell by a mean of 8.0 mm Hg. At doses of 0.18 - 0.32 μg/kg/min pulse rate increased another 7 per minute, cardiac output fell slightly due to smaller stroke volume, and mean arterial pressure decreased another 9 mm Hg. Peripheral resistance at this point was calculated to be 33% lower than the

pre-infusion level.

In all the above studies, route and rate of administration of the E prostaglandins appeared to influence significantly the cardiovascular response to this group of compounds. In general, arterial, and particularly intrathoracic aortic injections of PGE produced more marked vaso-depression than intravenous administrations. Similarly, as the studies cited above indicate, a rapid intravenous injection of 50 μg of PGE_2 produced a fall in blood pressure and a tachycardia whereas slow intravenous infusions of doses of this magnitude per minute were without effect in man. Furthermore, in order to reproduce the effects of a rapid 50 μg intravenous administration of PGE_2, an intramuscular or subcutaneous dose 100 times as large was required.[25]

It was felt that the explanation for these differences might be related to rapid uptake, redistribution or metabolism of PGE compounds. Using autoradiographic techniques, Samuelsson[26] noted a high rate of uptake and concentration of labelled PGE_1 in rat lungs, a finding which prompted Anggard and Samuelsson[27] to investigate a possible role for the lung in prostaglandin metabolism. Using guinea pig preparations, they found that lung tissue contains a 15-hydroxy prostaglandin dehydrogenase which converts PGE and PGF compounds to two less polar inactive metabolites. Ferreira and Vane[28] later demonstrated the rapidity with which PGE can be removed from the circulation by perfusing intact liver and lungs with labelled PGE. They reported that in just one passage through

these tissues, 95% of an infused dose of PGE was removed and metabolized by the lungs, 70% by the liver. These findings were held to be consistent with evidence that PGE exerts greater effects when infused intra-arterially than intravenously.

A number of mechanisms have been advanced to explain the blood pressure lowering effect of the E prostaglandins. In light of the ability of PGE to increase cardiac output while at the same time lowering arterial blood pressure,[11,15,21,29] and in light of a large volume of evidence developed from peripheral perfusion studies in the dog,[15,30] cat,[16] rat,[11] and human[22-24,29,31] the most favored explanation centered on the ability of the E prostaglandins to decrease peripheral resistance. Of particular note is the work done by Smith and co-workers[32] using atropine, propranolol, and pyribenzamine in conjunction with PGE_1 to demonstrate PGE_1's direct vasodilatory action independent of acetylcholine, beta-adrenergic stimulation or histamine release. Similarly, it has been shown that neither pre-treatment with ganglionic blocking agents[14,30,33] nor reserpine[14] interferes with the ability of PGE_1 to lower arterial pressure, suggesting further the direct action of PGE on peripheral vascular smooth muscle.

In contrast to PGE compounds, PGF compounds show marked species related differences in cardiovascular effects. Anggard and Bergstrom[34] and Horton and Main,[20,35] for example, have demonstrated PGF compounds to be vaso-depressor in the cat and rabbit, whereas DuCharme and Weeks[36,37] and Nakano and co-workers[15,38]

demonstrated them to be vasopressor in rats and dogs. In humans, intravenous infusion of 4.0 μg/kg/min of $PGF_{2\alpha}$ for 60 minutes and intramuscular and subcutaneous doses of 20 mg were without effect on blood pressure or heart rate.[25,39] Rapid single intravenous injection of 500 mg of $PGF_{2\alpha}$, however, has been shown to elevate blood pressure.[36]

As shown by peripheral perfusion studies, the mechanism of $PGF_{2\alpha}$'s vascular effect once again appeared to involve largely direct peripheral vasoconstriction or dilatation rather than centrally mediated phenomena. Perfusion of the hind limb of a dog with $PGF_{2\alpha}$, for example, demonstrated that $PGF_{2\alpha}$ has a venoconstrictor action versus an arteriolar smooth muscle stimulatory action, and it has been suggested that $PGF_{2\alpha}$ exerts its blood pressure elevating effect by increasing venous return and cardiac output.[36,37] In these studies, pre-treatment with hexamethonium and reserpine also failed to block the $PGF_{2\alpha}$ effect. Another mechanism has been advanced by Nakano and Cole,[38] who attribute at least part of $PGF_{2\alpha}$'s blood pressure elevating effect to a direct increase in myocardial contractility. As for $PGF_{2\alpha}$'s depressor qualities in some species, a number of investigators have shown $PGF_{2\alpha}$ to directly dilate blood vessels in muscle but not those of the kidney or skin in the cat.[34,35]

Prostaglandins of the A series, while much less active than PGE in stimulating non-vascular smooth muscle, have substantial vaso-depressor activity, as demonstrated by the work of Lee's

group,[11] Weeks,[31] and others. As is the case with PGE, PGA produces a decrease in blood pressure with a concomitant increase in cardiac output in all laboratory animals studied.[15,31,40-42] Once again decrease in peripheral resistance has been implicated as the mechanism involved.

A AND E PROSTAGLANDINS AS ANTI-HYPERTENSIVE HORMONES

Perhaps because PGA_2 was isolated and characterized by Lee in the search for a renal anti-hypertensive "factor", much of the investigational interest in the PGA compounds has been directed toward determining a possible role for the renal prostaglandins in the physiology of hypertension. Lee found that a single intravenous injection of 50 μg of PGA_2 into a 25 year old hypertensive subject transiently lowered the blood pressure from 185/116 to 160/95 mm Hg within 18 seconds.[11] At the same time, heart rate reflexly increased from 102 to 114, cardiac output increased from 8.4 to 9.5 liters/min and calculated peripheral resistance fell from 17.5 to 14.1 peripheral resistance units. With prolonged infusion of 382 μg/min in the same subject, blood pressure fell from 186/114 to 165/97 mm Hg and was maintained at this level for the duration of the infusion. A marked diuresis occurred during the infusion, but side effects such as headache, chest oppression and gastrointestinal cramping, which had occurred in experiments with PGE_1 infusion, were absent. These findings have been corroborated in other studies as well.[39,43]

More recently, evidence has been presented which indicates that E and A prostaglandins have actions which may influence blood pressure in ways other than by simple vaso-depression as well. In both dogs[44-48] and man[49,50] PGE and PGA have been shown to increase renal blood flow, urine volume, free water clearance, and sodium and potassium excretion. Using graduated doses of PGA, Westura[49] and Carr[50] both demonstrated that the earliest effect of PGA is a rise in sodium excretion and urinary flow, which is then followed by the expected blood pressure lowering response at higher doses. Furthermore, Fichman and co-workers[51] have presented data suggesting that PGA increases aldosterone secretion in man independent of changes in serum electrolytes, renin, or ACTH levels. Thus mechanisms have been discovered by which PGE and PGA may influence both immediate and longer term regulation of blood pressure.

With evidence revealing the anti-hypertensive properties of PGA and PGE continuing to grow, Lee in particular has been a proponent of the theory that these prostaglandins, and PGA in particular, may play active physiological roles in blood pressure and sodium homeostasis as circulating anti-hypertensive hormones. As Lee and others have pointed out, in contrast to many other anti-hypertensive agents, PGE and PGA not only spare renal function but increase renal blood flow, urine formation, and salt excretion. Thus a balance is established between the hormones' blood pressure lowering effect and renal perfusion. Secondly, evidence has been presented which indicates that PGE and PGA compounds cause a

redistribution of blood flow from renal medulla to cortex, an action which would tend to counter stimulation of renin release from cortically located juxtaglomerular apparatuses at times of abnormal renal cortical ischemia.[52,53] Thirdly, of particular significance was the finding that PGA compounds, unlike PGE and PGF, are relatively unaffected by the 15-hydroxy prostaglandin dehydrogenase present in lung tissue and are more slowly metabolized in the liver of most species.[42,54] As Lee points out, slower metabolism could also explain why PGA, despite its presence in smaller amounts in renal medulla than PGE,[33,55] has greater cardiovascular effects than the E prostaglandins when administered intravenously[42,49] and why it is therefore a more likely candidate as a possible circulatory anti-hypertensive hormone. Finally, Lee has proposed that in some forms of hypertension, if his theory is correct, a deficiency in the body's ability to synthesize, release, and maintain adequate levels of circulating prostaglandins might be expected.[48]

Factual support for Lee's concept of PGA as a circulating natriuretic anti-hypertensive hormone awaited the development of rapid sensitive assay techniques for the prostaglandins. Among the most successful of these have been the radioimmunoassay techniques developed by Caldwell et al.[56,57] Armed with these techniques, Zusman demonstrated that in normal humans subjected to dietary salt restriction and loading, PGA levels respectively rise and fall in parallel with plasma renin levels.[58] In so doing,

he refuted Lee's concept of PGA as a circulating natriuretic hormone and Lee's prediction that a high sodium intake would be associated with an elevation of PGA to facilitate sodium excretion.[48] Rather, just the reverse relationship was found to hold true. To explain this finding, Zusman postulated that sodium restriction would lead to an elevation in renin levels and angiotensin production as discussed by Mulrow and Goffinet.[58] Angiotensin, which has been shown to stimulate release of prostaglandin-like material into renal venous effluent,[59] would result in elevation of prostaglandin output, which would then tend to moderate the hypertensive effect of the elevated angiotensin levels, as demonstrated by Herbaczynska-Cedro and Vane.[60] Furthermore, the elevated prostaglandin levels would tend to stimulate secretion of aldosterone as demonstrated by Fichman,[51] thus tending to stimulate sodium reabsorption and a return toward normal sodium levels.

After establishing 1.60 ng/ml as the approximate level of circulating PGA in venous blood of normal humans, Zusman then found that in both patients with essential and renovascular hypertension, PGA levels were markedly lower than in normal humans, averaging in the range of 0.60 - 0.70 ng/ml.[61] Furthermore, in these hypertensive patients no correlation could be made between PGA and plasma renin activity levels. This finding suggested to Zusman that perhaps "the normal interplay between these two vasotonic substances had been disturbed." In addition, in those

patients with renal artery stenosis, he was able to demonstrate significantly higher levels of PGA in renal venous blood than in peripheral venous blood, supporting the contention that the kidney is a major source of circulating prostaglandins. In his work Zusman was unable to demonstrate variations in the levels of circulating PGE or PGF, but as he rightfully points out, metabolism of these compounds by the lungs is in all likelihood so rapid as to preclude meaningful measurement of them in peripheral plasma. Furthermore, with the recent discovery of enzyme systems in human kidney and plasma with the capability of converting PGE to PGA,[62,63] Zusman suggests that enzymatic conversion of PGE, which has been found to account for 60% of renal medullary prostaglandin content versus 15% for PGA,[64] may contribute importantly to circulating PGA levels.

Zusman's contribution to our understanding of prostaglandin physiology was his demonstration of the interdependence of sodium intake and PGA levels, presumably in the maintenance of sodium homeostasis, and his finding of abnormalities in PGA synthesis or release mechanisms in essential and renovascular hypertension. He has summarized his concept of a possible role for PGA in sodium and blood pressure homeostasis in Figure 1. If, as evidence suggests, this conception is an accurate one, investigational attention must be focused on those specific stimuli that may cause prostaglandin synthesis and release, for it is presumably some defect in these mechanisms that underlies the abnormalities Zusman

and others have found associated with essential and renovascular hypertension.

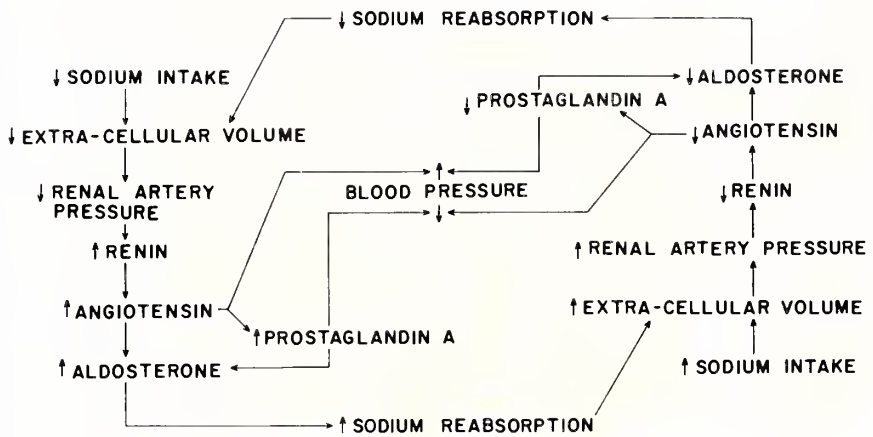

Figure 1. Zusman's conception of the role of PGA in sodium and blood pressure homeostasis. (With permission of the authors).

PURPOSE OF THIS INVESTIGATION

It is the purpose of this investigation to explore in greater depth some of the specific stimuli that have been implicated in causing elevation of circulating prostaglandin levels in blood pressure homeostasis. As noted previously, McGiff and co-workers have demontsrated that prostaglandin-like material appears in the venous effluent of the isolated perfused dog kidney in response to renal artery infusion of angiotensin II.[59] Such a stimulus-response relationship has never been demonstrated in the intact animal, however, particularly under conditions in which blood pressure is abnormally elevated. Furthermore, no effort has as yet been made to clearly differentiate whether this stimulus-response relationship is specific for the renin-angiotensin system or whether it obtains as well for other stimuli tending to elevate blood pressure--catecholamines, for example.

To better define this relationship, experiments were designed in which angiotensin II and norepinephrine were infused into normal anesthetized animals to cause blood pressure elevation on an acute basis. Before, during, and after infusion, blood samples were drawn for the determination of renin activity and prostaglandin levels.

In addition, similar procedures were carried out on monkeys in the third trimester of pregnancy to examine stimuli for the release of prostaglandins in the uteroplacental circulation, a

deficiency of which, Speroff has suggested, may be one of the mechanisms involved in toxemia, a hypertensive disease of pregnancy.[65] Speroff bases his hypothesis on the following historical findings:

1) Young[66,67] first postulated uteroplacental ischemia as a cause of toxemia, a postulate consistent with the increased incidence of toxemia noted in women with vascular disease, fibroids, anemia, twin pregnancy, or primigravid uterus. Beker (68) confirmed the larger caliber of multiparous uterine arteries by barium injection of bovine and human specimens.

2) Experimental compromise of uterine circulation in a variety of animals by a variety of techniques produced blood pressure elevation in pregnant animals but did not do so in non-pregnant animals. (69-74)

3) Pressor activity has been found in placental and uterine extracts by a number of investigators who have compared or identified the substance as renin. (75-78).

4) Renin release by the uteroplacental unit has been found to behave independently from renal output of renin. (79,80).

5) Toxemia has been found to be associated with a 40-60% reduction of blood flow through the uteroplacental unit (81,82), a reduction which has been found by dehydroepiandrosterone sulfate clearance studies to begin some time before the clinical appearance of hypertension (83,84).

6) Ferris and co-workers (85) have demonstrated in the rabbit that a reduction in uterine blood flow causes an outpouring of renin in the uterine vein, whereas administration of angiotensin increases uterine blood flow.

7) Ryan and co-workers (86) have found that acidic extraction of placental tissue yields a substance with blood pressure lowering and smooth muscle stimulating effects similar to PGE. Furthermore, they found that toxemic placentas contained less of this substance than normal placentas.

Putting this picture together, Speroff has suggested that

during pregnancy increased uterine pressure, twin pregnancy, the primiparous state, or a variety of other factors predisposing to uteroplacental vascular insufficiency may result in liberation of increased levels of renin. This renin would increase levels of circulating angiotensin which would result in increased blood flow through the uteroplacental bed and stimulate the output of prostaglandins by the placenta to help reduce resistance to blood flow through the placental bed. The hypertension of the toxemic state, Speroff suggests, may be characterized in part by defective prostaglandin production, loss of response to prostaglandin, or a combination of these.

This investigation was conceived to study the interaction of certain blood pressure elevating stimuli and prostaglandin output responses in blood pressure homeostasis. Angiotensin and norepinephrine infusion were the stimuli employed in normal rhesus monkeys from which plasma samples were obtained at various times before, during, and after infusion for assay of the prostaglandins and renin activity. A more extensive investigation involving angiotensin infusion in rhesus monkeys in the third trimester of pregnancy was also carried out to determine whether elevated angiotensin levels do in fact stimulate prostaglandin output in uterine venous blood, to determine the relationship between renin activity and prostaglandin levels in these samples, and to study the effect of indomethacin, a prostaglandin synthesis inhibitor, and P_{113}, an

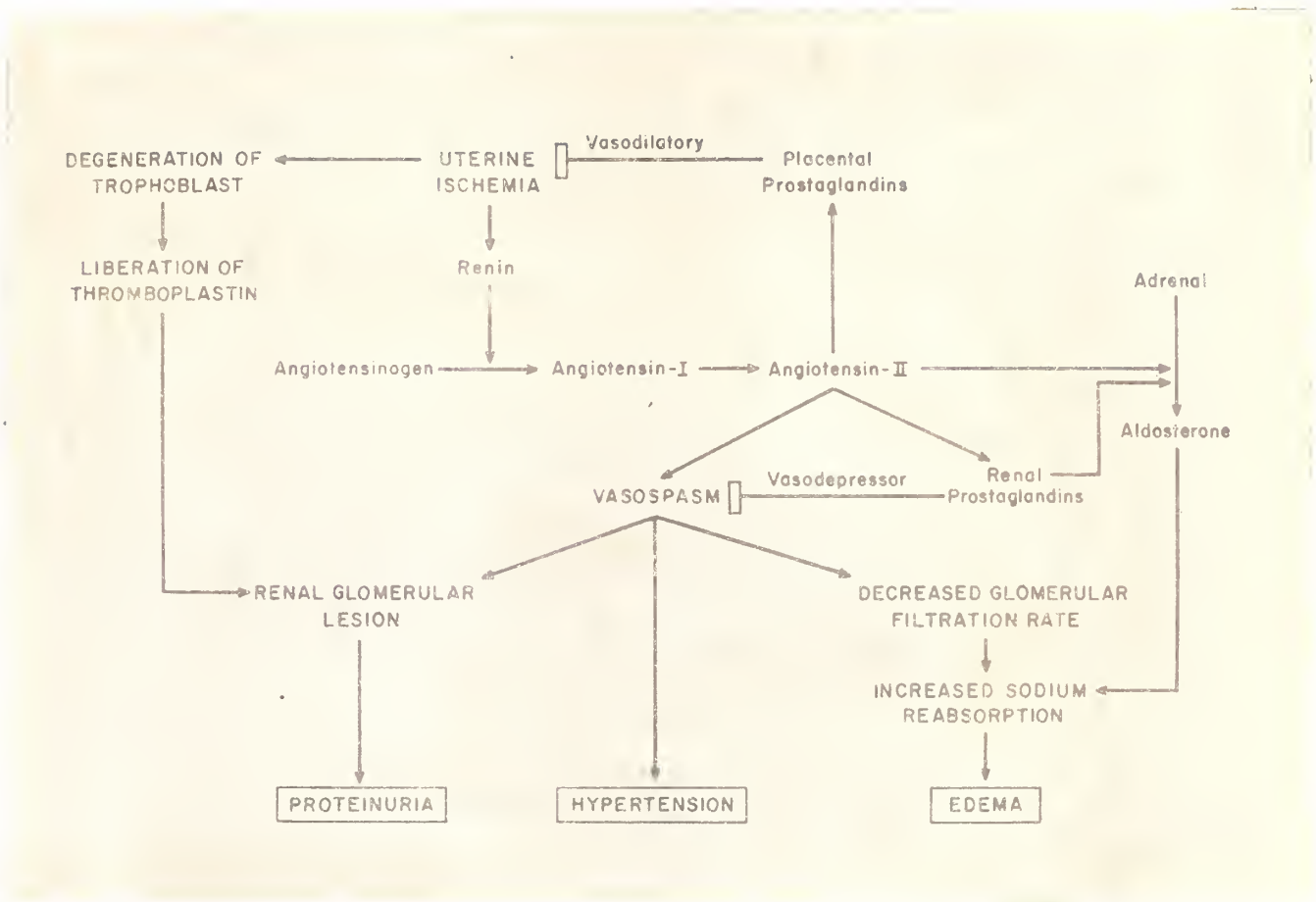

Figure 2. Speroff's Hypothesis for the Role of Prostaglandins in the Development of Toxemia of Pregnancy. (With permission of the author)

angiotensin analogue, on this system. It is hoped that by elucidating some of the mechanisms of blood pressure homeostasis in pregnant and non-pregnant states a broader understanding of the pathophysiology of hypertension in these states will be achieved.

EXPERIMENTAL STUDIES

RENIN ACTIVITY AND PROSTAGLANDIN A AND E LEVELS IN RESPONSE TO ACUTE BLOOD PRESSURE ELEVATION IN NON-PREGNANT MONKEYS

Materials and Methods

Experiments were carried out in normal non-pregnant female Rhesus Macaca mulatta monkeys ranging in weight from 5.0 to 6.5 kg. Only monkeys with a blood pressure no higher than 110/70 mm Hg at the start of the experiment were used. Monkeys inparticular were selected as experimental animals for the following reasons. First, being primates, they are as close to a human physiologic model as could reasonably be found. Secondly, the existence of a plasma isomerase spontaneously converting PGA to physiologically inactive PGB has been demonstrated in the rabbit, cat, dog and rat.[87] In humans, however, there is strong evidence that this isomerase does not exist.[88] Because of the possibility of cross reactions between the PGA antiserum employed in our radioimmunoassay and PGB, we felt it might be difficult to derive statistically significant results from animals known to harbor the isomerase. No isomerase has as

yet been identified in the monkey.

The monkeys were first tranquilized with 8.0 mg of Sernylan intramuscularly and then brought to the operating room where an intravenous infusion of 5% dextrose in water was begun in the left forearm at a rate just sufficient to keep the vein open. The monkeys were then either anesthetized with intravenous pentobarbital or intubated and anesthetized with halothane. Different methods of anesthesia were employed to insure that a given method of anesthesia did not introduce what would otherwise have been unrecognized effects upon the data.

After the monkeys were adequately anesthetized, the right antecubital fossa was prepared and a cut-down was performed. The brachial artery was isolated and cannulated with a polyethylene cannula attached to a Corometrics pressure transducer to produce a continuous tracing of systolic and diastolic blood pressure. Either inguinal region was then prepared and a cut-down on the femoral vein performed. A polyethylene cannula was then inserted into the femoral vein and advanced far enough to insure that its tip was at or beyond the level of the entrance of the renal veins into the inferior vena cava. This cannula was used to obtain blood samples for the duration of the experiment.

After the cannulas had been placed and the condition of the monkey had stabilized, two 10 ml blood samples were drawn at fifteen minute intervals for control purposes and to establish a baseline for renin activity and prostaglandin levels. A slow

infusion of either angiotensin II (Beckman) in four monkeys, or norepinephrine (Winthrop) in three monkeys, was administered by Harvard constant infusion pump into the peripheral intravenous line at starting doses of 0.06 μg/min and 1.6 μg/min respectively. In all cases blood pressure began to rise within five minutes of the start of infusion. Dosage of both drugs was titrated empirically using as endpoints either an approximate 75% increase in systolic or diastolic pressure or the development of irregularities in the blood pressure tracing judged to be indicative of a cardiac arrhythmia. If a cardiac arrhythmia was judged to have developed, dosage was reduced just enough to cause its disappearance. In all cases at least a 30 mm Hg increase in systolic and diastolic pressure was achieved. Maximal doses averaged 0.24 μg/min of angiotensin and 6.4 μg/min of norepinephrine.

When blood pressure had stabilized at a satisfactory level, usually within 10 - 15 minutes after starting infusion, three 10 ml blood samples were drawn from the femoral vein cannula at approximately 10 minute intervals, after which the infusion of angiotensin or norepinephrine was discontinued. When blood pressure had dropped to and stabilized at pre-infusion levels (or below in some cases), usually within 10 minutes, two more 10 ml blood samples were obtained at 15 minute intervals to establish a post-infusion baseline. In addition, one or two simultaneous arterial blood samples were drawn from the brachial artery cannula during and after infusion for arterio-venous comparison of renin activity and prosta-

glandin levels. The cannulas were then removed, the wounds closed with subcuticular chromic sutures, and the animal returned to its cage after intramuscular injection of 1.0 cc of Combiotic, a veterinary preparation of of penicillin and streptomycin.

Once obtained, each 10 ml blood sample was divided into a 7.0 ml aliquot put into a heparinized conical centrifuge tube for prostaglandin assay and a 3.0 ml aliquot put into a tube containing EDTA for assay of renin activity. Both aliquots were immediately centrifuged and the plasma decanted and rapidly frozen in dry ice and methanol for storage until assay. The remaining red cells, approximately 5 cc for every 10 cc of blood drawn, were reinfused via the femoral vein cannula immediately after the following 10 cc blood sample had been collected to avoid the development of anemia. During the experiment each animal received approximately 50 cc of its own packed red cells and at least 50 cc of fluid in the form of D5W and heparinized 0.9% saline flushes to keep the cannulas open, thereby approximately replacing the 90 cc of blood drawn during the experiment.

Prostaglandin Radioimmunoassay

The methodology employed for the radioimmunoassay, which was carried out for PGA and PGE in this series of experiments, is essentially as reported by Caldwell et al.[56] and Zusman et al.[57]

EXTRACTION was carried out as follows: Tritium-labelled prostaglandin, approximately 1000 cpm, was added to 0.5 ml of

plasma to serve as a tracer for recovery. Recovery throughout the assay averaged 60% for PGA and 85% for PGE. The plasma was acidified to pH 3.5 - 4.0 with 0.1 ml of HCl. The samples were extracted with redistilled ethyl acetate, 7 ml for each 0.5 ml of plasma extracted, dried under air, and re-dissolved in a solvent system consisting of a 60/40/2 ratio of benzene/ethyl acetate/methanol in preparation for column chromatography.

CHROMATOGRAPHY was performed using silicic acid minicolumns (0.5 gm silicic acid in a column 1.0 x 15 cm). The elution pattern obtained is diagrammed in Figure 3 along with the solvent systems employed. It should be noted that although the various classes of prostaglandins can be separated, the technique cannot distinguish between prostaglandins within a family, e.g. PGE_1 and PGE_2. Therefore results are reported as concentrations of PGA and PGE.

RADIOIMMUNOASSAY followed the procedures described in the references cited above using PGA antiserum prepared by covalently linking PGE_2 to bovine serum albumin by reaction with carbdiimide reagent as described by Caldwell et al. for $PGF_{2\alpha}$.[56] The resulting compounds were then injected into rabbits from which the antibodies were later obtained. Cross reactions for our PGA antiserum are illustrated in Figure 4. The antiserum used in measuring the E prostaglandins was obtained from Dr. Robert Skarnes of the Worcester Foundation. Full characterization has been published by Dr. Skarnes.[89] Studies from our own laboratory reveal that 50% binding of the E antiserum occurs with 0.5 ng/ml of PGE_1, 80 ng/ml

of $PGF_{2\alpha}$, 93 ng/ml of PGA_2, and 650 ng/ml of PGB_2. Furthermore, all three of our antisera (PGA, PGE, and PGF) have been tested with the 15-keto and 13,14-dihydro,15-keto derivatives of the E, F, and A prostaglandins. The cross reaction with all derivatives and each antiserum is no greater than 0.1%.

VALIDATION OF THE ASSAYS for precision has been carried out by measuring increasing amounts of prostaglandin added to a known low level plasma pool as illustrated in Figure 5. For assessment of reproducibility, plasma pools containing low and high prostaglandin concentrations were run with each assay. The coefficient of variation for between (inter) and within (intra) assay reproducibility has been approximately 10% for each assay.

Radioimmunoassay for Plasma Renin Activity

Plasma renin activity was determined by measurement of the angiotensin I generated in vitro. The pH of 1.0 ml aliquots of each sample was adjusted to 6.0 with 0.2M maleate buffer, pH 6.0. Separate aliquots of the samples were then incubated in the presence of inhibitors of the converting enzyme and angiotensinase for one hour at $37^{o}C$ or $4^{o}C$. At the end of the incubation period, all samples incubated at $37^{o}C$ were matched against their $4^{o}C$ control in an ice bath and the angiotensin I measured by radioimmunoassay. The angiotensin I assay was carried out by a modification of the Haber method[90] using the radioimmunoassay materials (anti-angiotensin I serum, angiotensin I (^{125}I), and angiotensin standard)

reagents and protocol supplied in the Angiotensin I (^{125}I) Radioimmunoassay Kit distributed by the New England Nuclear Corporation (Boston, Mass.). Separation of antibody-bound and free angiotensin I (^{125}I) was achieved by differential adsorption of the free antigen on activated charcoal followed by centrifugation.

The results were calculated using the logit transformation,[91] and plasma renin activity was expressed as nanograms of angiotensin I formed per ml per hour (ng/ml/hr). All values were corrected for the "angiotensin I" measured in the control samples incubated at $4^{o}C$. To eliminate the influence of assay to assay variability, all samples from a given experiment were measured in the same assay.

Using this radioimmunoassay system, the lower limit of sensitivity for the assay was found to be 0.01 - 0.02 ng angiotensin I (unpublished results). The intra-assay coefficient of variation for 7 replicate samples from a plasma standard with a mean renin activity of 7.85 ng/ml/hr was 4.8%. The quantity of angiotensin I generated in vitro was found to be proportional to the time of incubation for at least 1.5 hr. After addition of charcoal there was no significant stripping of the antigen-antibody complex for a period of at least 20 minutes.

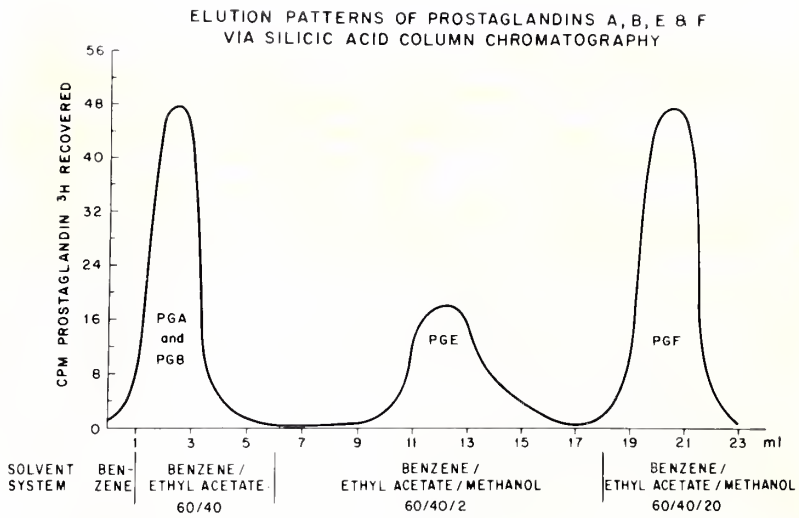

Figure 3. Elution Patterns of Prostaglandins A, B, E, & F via Silicic Acid Column Chromatography.

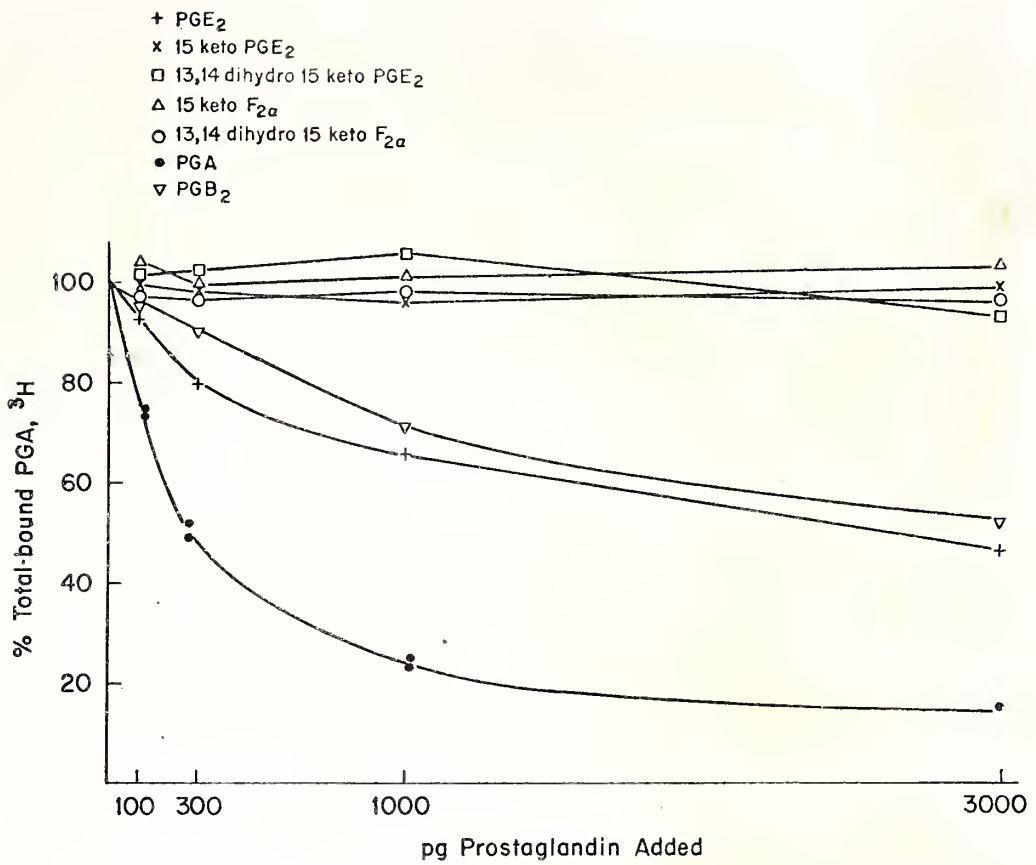

Figure 4. Cross Reactions for the PGA Antiserum

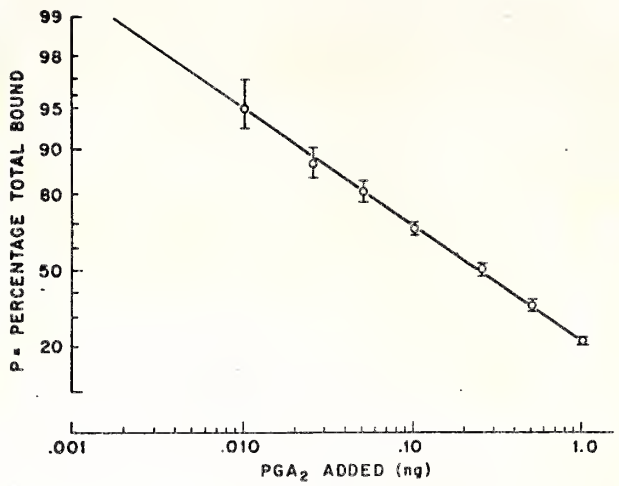

Figure 5a. Addition of Known Amounts of PGA to a Low Level Plasma Pool.

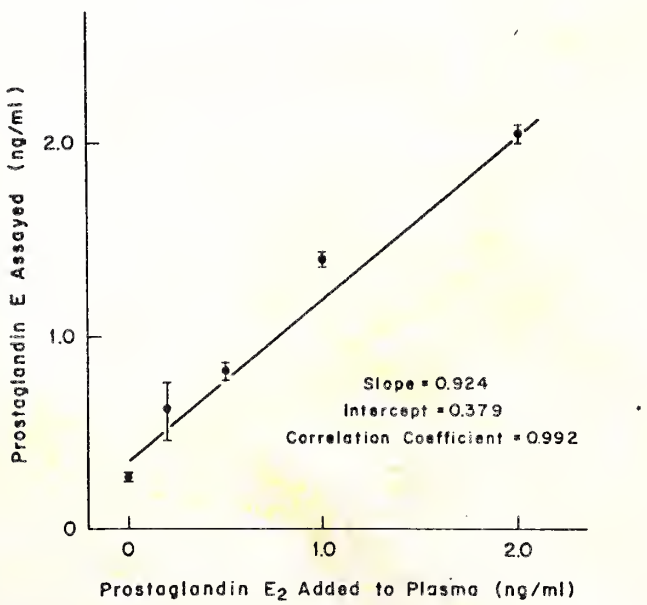

Figure 5b. Addition of Known Amounts of PGE to a Low Level Plasma Pool.

Results--Non-Pregnant Monkeys Receiving Angiotensin

A summary of radioimmunoassay results for PGA, PGE, and plasma renin activity for this series of monkeys will be found in Tables 1 - 3. From this data an average pre-infusion level for each substance was determined from the mean of the two pre-infusion blood samples from each monkey. This level was considered as baseline for comparison with hormone levels obtained during the period of angiotensin infusion. The results were expressed as per cent change from baseline during infusion and were then pooled for purposes of statistical analysis. Mean per cent change for each substance from baseline and standard error of the mean were then calculated for all observed points during infusion with "P" values determined by T_{N-1} test.

Prostaglandin A

In all four monkeys, regardless of anesthesia used, acute elevation of blood pressure with angiotensin II was associated with an average 26% decrease in inferior vena caval levels of PGA. This change was significant to $P < .001$. It was found in addition that baseline levels of PGA varied markedly from monkey to monkey, with two point pre-infusion averages for each monkey ranging from 0.17 to 1.03 ng/ml. When plotted against time, the PGA levels displayed peaks and depressions which fell at random and could not be correlated with concurrent renin or PGE data or with subtle changes in blood pressure recorded before, during, or after angiotensin infusion.

Prostaglandin E

A 29% decrease in inferior vena caval levels of PGE, significant at $P < .001$, was associated with blood pressure elevation with angiotensin II. In general, PGE levels were markedly higher than PGA levels in any given monkey, often by a factor of 5 or more. As with PGA, however, no correlation could be made between random peaks and depressions of PGE levels in individual monkeys and concurrent renin data or subtle changes in blood pressure recorded before, during, or after angiotensin infusion.

Plasma Renin Activity

Plasma renin activity consistently fell from baseline levels by approximately 45% in response to angiotensin infusion. This change was significant at $P < .001$. The decline was most marked during the first 10 minutes of angiotensin infusion, after which PRA levels either continued a slow decline or plateaued for the duration of the infusion. Following cessation of the infusion, a sharp rise in plasma renin activity back to pre-infusion levels was noted for all four monkeys. These patterns held true irrespective of type of anesthesia used and appeared to reflect more the dose of angiotensin infused at a given time than the simultaneous blood pressure response.

Results--Non-Pregnant Monkeys Receiving Norepinephrine

A summary of radioimmunoassay results for PGA, PGE, and plasma renin activity for this series of monkeys will be found in Tables 4 - 6.

Prostaglandin A

In all three monkeys studied, no significant change in circulating venous PGA levels could be demonstrated in response to acute blood pressure elevation by norepinephrine infusion. Baseline levels of PGA ranged from 0.18 to 0.50 ng/ml with little variation during or after norepinephrine infusion. In general, blood pressure could not be elevated as high with norepinephrine as with angiotensin because of the increased incidence of cardiac arrhythmias produced by norepinephrine, but in all cases elevation of both systolic and diastolic blood pressure by approximately 30% above baseline was achieved.

Prostaglandin E

As with PGA, no significant change in inferior vena caval levels of PGE could be demonstrated in response to norepinephrine infusion. In contrast to the PGA findings, however, baseline levels of PGE varied widely from monkey to monkey, ranging from 0.36 to 3.33 ng/ml. In addition, although there was no statistically significant change in PGE levels overall, the data indicate that PGE output from one blood drawing to the next was quite irregular, with many random peaks and depressions that could not be correlated in time with specific simultaneous changes in PGA, plasma renin activity, or blood pressure.

Plasma Renin Activity

No significant change in plasma renin activity was associated with acute blood pressure elevation by norepinephrine infusion in

TABLE 1

ANGIOTENSIN II INFUSION

EFFECTS ON PROSTAGLANDIN A--NON-PREGNANT MONKEYS

Monkey: X Halothane	ng/ml	Monkey: P Pentobarb.	ng/ml	Monkey: E Pentobarb.	ng/ml	Monkey: T Pentobarb.	ng/ml
Pre-infusion--	1.38	Pre-infusion--	0.58	Pre-infusion--	0.15	Pre-infusion--	0.46
	0.69		0.32		0.18		0.30
Infusion------	0.72	Infusion------	0.29	Infusion------	0.12	Infusion------	0.47
	0.53		0.48		0.12		0.24
	0.78		0.28		0.12		0.24
	(0.62		0.24		0.14		
Art.--(0.86							
Post-infusion--	0.67	Post-infusion--	0.31	Post-infusion--	0.19	Post-infusion--	0.35
	(0.80		0.41				0.23
Art.--(0.66							
	0.79						

%Change from baseline during infusion $\pm$ SEM.....-26.5 $\pm$ 5.0

N = 15 P < .001

TABLE 2

ANGIOTENSIN II INFUSION

EFFECTS ON PROSTAGLANDIN E--NON-PREGNANT MONKEYS

Monkey: Xenia Halothane	ng/ml	Monkey: Polyhymnia Pentobarb.	ng/ml	Monkey: Euterpe Pentobarb.	ng/ml	Monkey: Terpsichore Pentobarb.	ng/ml
Pre-infusion--	1.76	Pre-infusion--	4.25	Pre-infusion--	1.56	Pre-infusion--	6.96
	0.93		3.80		2.56		5.07
Infusion------	1.44	Infusion------	2.87	Infusion------	1.03	Infusion------	4.67
	1.11		2.52		0.79		4.20
	0.98		2.48		1.07		3.86
	(1.95		1.48		0.92		
Art.--(1.17							
Post-infusion--	0.95	Post-infusion--	1.80	Post-infusion--	1.01	Post-infusion--	2.34
	(1.20		1.92				5.44
Art.--(0.89							
	1.04						

%Change from baseline during infusion $\pm$ SEM.....-29.5 $\pm$ 7.0

N = 15 P < .001

TABLE 3

ANGIOTENSIN II INFUSION

EFFECTS ON RENIN--NON-PREGNANT MONKEYS

Monkey: Xenia Halothane	ng/ml/hr	Monkey: Polyhymnia Pentobarb.	ng/ml/hr	Monkey: Euterpe Pentobarb.	ng/ml/hr	Monkey: Terpsichore Pentobarb.	ng/ml/hr
Pre-infusion--	23.76	Pre-infusion--	29.29	Pre-infusion--	16.05	Pre-infusion--	29.90
	27.53		34.22		10.86		33.30
Infusion------	13.89	Infusion------	21.47	Infusion------	8.38	Infusion------	18.71
	13.12		18.78		9.50		16.35
	11.64		19.20		6.99		18.20
	(11.01		24.16		3.53		
Art.-	(12.46						
Post-infusion--	14.11	Post-infusion--	44.08	Post-infusion--	8.90	Post-infusion--	17.09
	(24.67		39.34		14.77		29.80
Art.-	(19.26						
	30.28						

%Change from baseline during infusion $\pm$ SEM.....-44.2 $\pm$ 3.1

N = 15 P < .001

TABLE 4

NOREPINEPHRINE INFUSION

EFFECTS ON PROSTAGLANDIN A--NON-PREGNANT MONKEYS

Monkey: Urania Pentobarb.	ng/ml
Pre-infusion--	0.27
	0.22
Infusion------	0.18
	0.20
	0.21
Post-infusion--	0.18
	0.16

Monkey: Ariadne Pentobarb.	ng/ml
Pre-infusion--	0.23
	0.20
Infusion------	0.34
	0.53
	0.26
Post-infusion--	0.29
	(0.15
	Art.(0.22
	0.19

Monkey: Lysistrada Pentobarb.	ng/ml
Pre-infusion--	0.92
	0.83
Infusion------	0.78
	0.81
	0.40
Post-infusion--	0.59
	0.29
	0.36
	0.38

%Change from baseline during infusion $\pm$ SEM.....+8.7 $\pm$ 16.0

N = 9 P = NS

TABLE 5

NOREPINEPHRINE INFUSION

EFFECTS ON PROSTAGLANDIN E--NON-PREGNANT MONKEYS

Monkey: Urania Pentobarb.	ng/ml
Pre-infusion--	0.98
	0.69
Infusion------	0.98
	1.13
	1.17
Post-infusion--	0.89
	1.78

Monkey: Ariadne Pentobarb.	ng/ml
Pre-infusion--	0.50
	0.21
Infusion------	0.75
	1.32
	0.67
Post-infusion--	0.44
	(0.25
	Art.(0.27
	0.40

Monkey: Lysistrada Pentobarb.	ng/ml
Pre-infusion--	3.30
	3.36
Infusion------	2.58
	3.41
	1.44
Post-infusion--	1.54
	0.97
	1.11
	0.52

%Change from baseline during infusion $\pm$ SEM.....+75.2 $\pm$ 43.6

N = 9 P = NS

TABLE 6

NOREPINEPHRINE INFUSION

EFFECTS ON RENIN--NON-PREGNANT MONKEYS

Monkey: Urania Pentobarb.	ng/ml/hr
Pre-infusion--	13.60
	10.18
Infusion------	10.59
	11.30
	18.28
Post-infusion--	18.48
	21.91

Monkey: Ariadne Pentobarb.	ng/ml/hr
Pre-infusion--	7.34
	10.00
Infusion------	8.77
	9.74
	9.92
Post-infusion--	13.45
	(11.91
	Art.(12.73
	12.89

Monkey: Lysistrada Pentobarb.	ng/ml/hr
Pre-infusion--	8.04
	7.16
Infusion------	7.76
	9.27
	8.32
Post-infusion--	16.96
	(12.08
	Art.(11.31
	12.27

%Change from baseline during infusion $\pm$ SEM.....+10.9 $\pm$ 6.4

N = 9 P = NS

the three monkeys studied. Levels of renin activity at baseline were fairly consistent from monkey to monkey within a range of approximately 8 to 12 ng/ml/hr and remained within that range in almost all instances for the duration of the norepinephrine infusion. Following cessation of the infusion, levels of renin activity rose from 30 to 50% above baseline and either remained at this level or slowly declined for the duration of the experiment.

Discussion

McGiff and co-workers have demonstrated that prostaglandin-like material is released into the renal venous effluent in response to arterial angiotensin infusion in the isolated perfused dog kidney.[59] The present series of studies, however, was unable to demonstrate such an output of either prostaglandin A or E in response to infusion of either angiotensin or norepinephrine in the intact anesthetized monkey; in fact, angiotensin infusion was associated with statistically significant decreases or approximately 25 and 30% for PGA and PGE respectively. These findings must be weighed in light of the following considerations.

It is entirely likely that the kidney might behave differently in the isolated perfused state than in the living animal. It is almost certain that the intact animal would exert a variety of compensatory influences tending to moderate not only an initial stimulus, but perhaps even some of the physiological responses to that stimulus. Although it is premature to attribute a great deal

of importance to the role of compensatory influences in the intact animal, the current study suggests that the role of prostaglandin output as an antihypertensive mediator in response either to angiotensin specifically or to acutely elevated blood pressure in a more general sense may be less obvious than originally inferred from McGiff's work with isolated perfused dog kidneys.

On the other hand, the current study involved infusion of quantities of angiotensin and norepinephrine producing acute changes in blood pressure and vascular tone vastly greater than seen in any physiological or pathological condition with the possible exception of the hypertensive crisis of pheochromocytoma. It is possible that such intense degrees of renovascular vasoconstriction were achieved that the kidneys were unable to effect or maintain adequate prostaglandin output secondary to inadequate perfusion. In this case, one might have expected to see an initial burst of prostaglandin output followed by a decline and plateau of output at relatively low levels. Since the first blood sample obtained following the start of infusion was taken only after blood pressure had stabilized at the desired hypertensive level, it is conceivable that an initial burst of prostaglandin output might have been missed. Future experiments will have to be arranged so that blood samples can be obtained continuously from the start of infusion. At present, work is also being done in pregnant monkeys to establish dose-response data to determine at what dose of angiotensin prostaglandin output, if any, will be at a maximum.

Unfortunately the current study can give no precise information as to the source of the prostaglandins being measured. Although the likelihood is great that this source is the kidney, this expectation can only be confirmed by direct sampling of renal venous blood, as was done by Zusman in his study of prostaglandin levels in hypertensive humans.[61] Early in the course of the current series of experiments, renal vein cannulation was attempted using Hypaque and fluoroscopy, but this technique was soon abandoned because of the poor tolerance of the monkeys to this procedure in our hands. The remaining experiments of this series were therefore carried out with the blood sampling cannula placed in the inferior vena cava at or above the entrance of the renal veins, and the blood sampled was thus an admixture of renal and vena caval blood. As such, it is possible that lower levels of the prostaglandins than expected were observed secondary to a decrease in the proportion of renal venous to vena caval blood in the samples obtained during the periods of renal vasoconstriction, which would tend to decrease the flow of renal venous effluent and even mask elevations in the concentration of prostaglandins being released by the kidney. Future experiments using renal vein cannulation will hopefully clarify this issue.

UTERINE VENOUS RENIN ACTIVITY AND PROSTAGLANDIN LEVELS IN RESPONSE TO ACUTE BLOOD PRESSURE ELEVATION IN PREGNANT MONKEYS

Materials and Methods

These experiments were carried out in normal female Rhesus Macaca mulatta monkeys in the third trimester of pregnancy with weight ranging from 5.55 to 9.0 kg and day of gestation from 117 to 140.

In three monkeys, the procedure was as follows: Ketamine, 2 mg/kg, was administered intramuscularly, and the monkeys were intubated and brought to the operating room. Under general anesthesia achieved with halothane (50 ml/min) and oxygen (3 liters/min), an intravenous infusion of D5W was begun in the right forearm and the left brachial artery was cannulated with a polyethylene cannula attached to a Corometrics pressure transducer to produce a continuous tracing of systolic and diastolic blood pressure. The abdomen was then prepared and laparotomy performed to isolate either major uterine vein. The uterine vein was then cannulated and blood drawn and angiotensin infused as described in the previous experiments. Renin activity and prostaglandin radioimmunoassay were carried out as described above. At two times during each run, simultaneous arterial samples were drawn as well.

In one monkey the same procedure was carried out except that following procurement of baseline bloods, angiotensin was infused for 20 minutes followed by a 20 minute return to baseline blood

pressure. Angiotensin infusion was then started again for a 30 minute period, after 15 minutes of which a solution of 80 mg of indomethacin, a potent inhibitor of prostaglandin synthesis, was administered intravenously to determine its effect on blood pressure and plasma renin activity. The indomethacin solution was prepared by the method of Preston[92] as follows: The required amount of indomethacin was suspended in 3.0 cc of 0.9% saline and pH adjusted to 11 to produce a clear yellow solution using 1 normal NaOH. The pH was then titrated back to pH 7.4 - 8.0 using 1 normal HCl. The final amount of solution amounted to approximately 5 cc. During the course of the experiment, blood samples of 10 ml each were drawn at 15 - 20 minute intervals as previously described.

In two monkeys exactly the same procedures were carried out as for the first three pregnant monkeys except that D5W was infused instead of angiotensin for control purposes. Near the end of these two experiments, a bolus of indomethacin solution was administered intravenously to each monkey, 20 mg to one and 40 mg to the other, in order to observe the effect of this drug on blood pressure and plasma renin activity. In addition, in the second control monkey, all blood samples were drawn slowly over a ten minute period, approximately 1.0 ml/min, rather than all 10 ml at once to make certain that no abnormal deviation in renin activity or prostaglandin levels occurred as a result of rapid withdrawal of a large amount of blood from the monkey within a short period of time.

Results--Pregnant Monkeys Receiving Angiotensin

A summary of radioimmunoassay results for PGA, PGE, PGF, and plasma renin activity for this series of monkeys will be found in Tables 7 - 12.

Prostaglandin A

In the first three monkeys receiving angiotensin as described in "Materials and Methods" above, no statistically significant effect of angiotensin-induced systemic hypertension on PGA levels in uterine venous blood could be demonstrated. In contrast to the inferior vena caval blood samples of the non-pregnant monkeys, uterine venous PGA levels in pregnant monkeys were fairly consistent from monkey to monkey at a baseline of approximately 0.50 ng/ml. Throughout the experiments, PGA levels fluctuated between levels of approximately 0.35 and 0.60 ng/ml in such a fashion that no correlation could be made between PGA levels and levels of the other prostaglandins, plasma renin activity, or subtle fluctuations in systemic blood pressure recorded before, during, or after angiotensin infusion. Because no statistically significant correlation could be made between angiotensin infusion and changes in PGA levels, PGA was not assayed in the control monkeys.

Prostaglandin E

In the same three pregnant monkeys reported above, a mean increase of nearly 50% above pre-infusion baseline levels was demonstrated for uterine venous PGE levels in association with angiotensin-induced systemic hypertension. This change was signifi-

cant at $P < .05$. Uterine venous baseline PGE levels averaged approximately 1.0 ng/ml within a range of 0.67 to 1.64 ng/ml. In one instance the increase in PGE was gradual during the course of the infusion, but in the other two the increases were characterized by rather sharp spikes followed by declines to near baseline levels. The timing of the spikes during the course of the angiotensin infusion was variable, however, and seemed to bear no consistent relationship to changes of blood pressure, renin, or the other prostaglandins during the course of the experiment.

In one pregnant monkey submitted to a 20 minute angiotensin infusion, then allowed to restabilize, and then submitted to another angiotensin infusion at the end of which 80 mg of indomethacin was administered intravenously, no significant elevation of uterine venous PGE levels occurred during the first period of infusion (Table 11). During the second infusion period there was again no elevation in PGE levels, which fell promptly to almost unmeasurable levels following indomethacin administration. Concurrent with indomethacin administration, a further elevation of systolic and diastolic blood pressure of approximately 15 mm Hg was observed. Of particular interest, however, was the fact that following the final angiotensin infusion, Caesarian section was performed on this monkey revealing a twin pregnancy. Both infant monkeys were alive at birth but were of low apgar, most likely secondary to maternal anesthesia. Unfortunately it was impossible to tell whether they shared a common placenta.

Two other pregnant monkeys were also infused with D5W followed by intravenous administration of 20 and 40 mg of indomethacin for control purposes. This data is presented in Table 12. In one monkey a rise in uterine venous PGE levels comparable to that observed in the three experimental animals was noted during D5W infusion. Near the end of D5W infusion the PGE level began to fall and continued to do so for approximately 15 minutes, after which it again rose without immediately apparent cause to 1.35 ng/ml from a baseline of 0.40 ng/ml. Following administration of 20 mg of indomethacin the PGE level fell to 0.15 ng/ml in association with a 15/15 mm Hg elevation of blood pressure. In the second control monkey, baseline uterine venous PGE levels averaged 1.0 ng/ml and rose during the course of D5W infusion to 3.30 ng/ml and to 5.60 ng/ml immediately following cessation of the infusion. Immediately prior to administration of 40 mg of indomethacin, PGE levels dropped again to 1.83 ng/ml, and following indomethacin administration a further drop to 0.15 ng/ml was noted. Drawing of blood samples slowly over a 10 minute period versus all at once did seem to provide a smoother curve with fewer random peaks and depressions of prostaglandin levels observed.

Prostaglandin F

In the original three pregnant monkeys submitted to angiotensin infusion, a slight but statistically insignificant elevation of PGF levels was noted in association with angiotensin-induced systemic hypertension. Pre-infusion baseline levels of PGF averaged

0.63 ng/ml, and the pattern of PGF output was once again noted to be one of random peaks and depressions. Because no significant correlation could be made between angiotensin infusion and changes in PGF levels, PGF levels were not assayed in the control monkeys.

Plasma Renin Activity

In all four pregnant monkeys receiving angiotensin infusion, an average decrease in uterine venous plasma renin activity of 24% was noted in association with angiotensin-induced systemic hypertension. This change was significant at $P < .05$ and was similar to the pattern of change observed in the non-pregnant monkeys, although not as dramatic. In the two pregnant control monkeys, uterine venous plasma renin activity was observed to increase moderately during D5W infusion. The effect of indomethacin on plasma renin activity was variable. In one monkey during angiotensin infusion, plasma renin activity rose from 3.32 to 25.67 ng/ml/hr in response to 80 mg of indomethacin. In the two control monkeys, 20 and 40 mg of indomethacin produced no significant change in levels of plasma renin activity.

TABLE 7

ANGIOTENSIN II INFUSION

EFFECTS ON PROSTAGLANDIN A--PREGNANT MONKEYS

Monkey: Pat Halothane	ng/ml
Pre-infusion--	0.56
	0.32
Infusion------	0.57
	0.47
	0.34
Post-infusion--	0.34
	0.22

Monkey: Tricia Halothane	ng/ml
Pre-infusion--	0.44
	0.49
Infusion------	0.48
	(0.50
Art.	(0.43
	0.56
	0.41
Post-infusion--	0.34
	(0.37
Art.	(0.55

Monkey: Julie Halothane	ng/ml
Pre-infusion--	0.54
	0.46
Infusion------	0.53
	(0.62
Art.	(0.51)
	0.42
	0.45
Post-infusion--	0.54
	(0.45
Art.	(0.43

%Change from baseline during infusion $\pm$ SEM.....+3.6 $\pm$ 5.3

N = 11 P = NS

TABLE 8

ANGIOTENSIN II INFUSION

EFFECTS ON PROSTAGLANDIN E--PREGNANT MONKEYS

Monkey: Pat Halothane	ng/ml
Pre-infusion--	1.64
	1.52
Infusion------	3.52
	1.70
	2.11
Post-infusion--	1.73
	1.08

Monkey: Tricia Halothane	ng/ml
Pre-infusion--	0.67
	0.80
Infusion------	0.89
	(0.88
	Art.(0.75
	1.29
	1.02
Post-infusion--	1.32
	(1.30
	Art.(0.97

Monkey: Julie Halothane	ng/ml
Pre-infusion--	0.91
	0.87
Infusion------	1.13
	(1.96
	Art.(1.33
	1.32
	1.02
Post-infusion--	0.95
	(1.64
	Art.(1.35

%Change from baseline during infusion $\pm$ SEM.....+48.6 $\pm$ 11.7

N = 11 P < .01

TABLE 9

ANGIOTENSIN II INFUSION

EFFECTS ON PROSTAGLANDIN F--PREGNANT MONKEYS

Monkey: Pat Halothane	ng/ml
Pre-infusion--	0.48
	0.83
Infusion------	0.91
	0.35
	0.33
Post-infusion--	0.69
	0.53

Monkey: Tricia Halothane	ng/ml
Pre-infusion--	0.74
	0.66
Infusion------	0.78
	(0.71
Art.	(0.70
	0.64
	0.73
Post-infusion--	1.17
	(0.84
Art.	(0.74

Monkey: Julie Halothane	ng/ml
Pre-infusion--	0.51
	0.44
Infusion------	0.73
	(0.81
Art.	(0.52
	0.74
	0.52
Post-infusion--	0.66
	(0.91
Art.	(0.60

%Change from baseline during infusion $\pm$ SEM.....+11.8 $\pm$ 11.3

N = 11 P = NS

TABLE 10

ANGIOTENSIN II INFUSION

EFFECTS ON RENIN--PREGNANT MONKEYS

Monkey: Pat Halothane	ng/ml/hr
Pre-infusion--	27.50
Infusion------	21.56
	18.54
	23.15
Post-infusion--	23.43
	20.97

Monkey: Tricia Halothane	ng/ml/hr
Pre-infusion--	12.11
	11.33
Infusion------	18.30
	(8.15
	Art.(8.61
	12.36
	6.62
Post-infusion--	7.94
	(9.03
	Art.(9.84

Monkey: Julie Halothane	ng/ml/hr
Pre-infusion--	22.07
	17.19
Infusion------	13.71
	(11.27
	Art.(12.28
	7.56
	11.23
Post-infusion--	9.95
	(13.30
	Art.(11.27

%Change from baseline during infusion $\pm$ SEM.....-23.7 $\pm$ 9.1

N = 11 P < .05

TABLE 11

ANGIOTENSIN AND INDOMETHACIN

EFFECTS ON UTERINE VENOUS PGE AND RENIN IN A PREGNANT MONKEY

	PGE ng/ml		Plasma Renin Activity ng/ml/hr
Pre-infusion--	0.78	Pre-infusion---	6.90
	(1.02		(17.91
Art.	(1.06	Art.	(6.33
	0.40		8.07
Angiotensin---	(0.44	Angiotensin----	5.42
Art.	(0.45		
Angio. Off----	1.08	Angio. Off-----	4.48
Angio. On-----	0.68	Angio. On------	3.32
Angio + Indo--	0.12	Angio + Indo---	25.67

NOTE: This monkey was found at Caesarian section to be carrying a twin pregnancy. Both infant monkeys were alive at birth.

TABLE 12

D5W CONTROL INFUSION

EFFECTS ON UTERINE VENOUS PGE AND RENIN--PREGNANT MONKEYS

Monkey: Tara
Halothane

	PGE ng/ml		Plasma Renin Activity ng/ml/hr
Pre-infusion--	0.39	Pre-infusion---	12.91
	0.42		21.49
D5W infusion--	0.65	D5W infusion---	21.24
	(1.18		(19.18
	Art.(0.99		Art.(21.94
	0.77		16.98
Infusion Off--	0.47	Infusion Off---	37.06
	(1.34		(18.59
	Art.(0.54		Art.(16.79
Indomethacin-- (20mg)	(0.15	Indomethacin--- (20mg)	(19.37
	Art.(0.11		Art.(14.36

Monkey: Okemo--(Bloods drawn slowly over 10 min)
Halothane

	PGE ng/ml		Plasma Renin Activity ng/ml/hr
Pre-infusion--	1.24	Pre-infusion---	12.47
	(0.73		(11.42
	Art.(0.66		Art.(12.81
	1.88		16.08
D5W infusion--	2.90	D5W infusion---	16.76
	(3.34		(14.64
	Art.(1.81		Art.(13.14
Infusion Off--	5.63	Infusion Off---	14.64
	1.85		18.51
Indomethacin-- (40mg)	0.19	Indomethacin--- (40mg)	16.08
	0.16		13.20

This study demonstrates that acute elevation of systemic blood pressure by angiotensin infusion is associated with a significant increase in the release of prostaglandin E in the uterine venous blood of rhesus monkeys in the third trimester of pregnancy. Levels of prostaglandins A and F, however, remain unchanged. These findings suggest that prostaglandin E may play an important role in the regulation of vascular resistance in the utero-placental bed. The responsiveness of PGE rather than PGA also serves to underline the likelihood that the major effect of the response is directed locally rather than systemically.

As previously noted, Ferris and co-workers[85] have demonstrated in the rabbit that a reduction in uterine blood flow causes an outpouring of renin in the uterine vein and that administration of angiotensin increases uterine blood flow. In addition, Ryan and co-workers[86] have found that acidic extraction of placental tissue yields a substance with blood pressure lowering and smooth muscle effects similar to PGE. Results from the present investigation would seem to support the possibility that the mechanism by which angiotensin increases uterine blood flow is through release of prostaglandin E, although proof of this hypothesis awaits further investigation employing direct flow measurements across the utero-placental bed. The observation of a consistent arterio-venous difference in levels of prostaglandin E, with greater levels observed in uterine venous versus brachial arterial blood, points strongly to the utero-placental unit as the source of the PGE elevation.

Two pregnant monkeys in this study were treated as controls and received an infusion of 5% dextrose in water instead of angiotensin II. In these monkeys sugnificant elevations of PGE were also noted to occur, but in contrast to the experimental animals, these elevations were associated with simultaneously rising levels of plasma renin activity. Because of this it is suspected that endogenously produced angiotensin may have been responsible in part for the rise in PGE levels that was observed, although the role of anesthesia and surgical stress cannot be overlooked. Experiments are currently underway using the angiotensin II analogue P_{113}, which competes with angiotensin at its binding sites but exerts only slight hypertensive effects of its own, to elucidate further the role of exogenous and endogenous angiotensin II stimulation of prostaglandin production by the utero-placental unit.

During three pregnant monkey experiments inhibition of prostaglandin synthesis was achieved with varying doses of imdomethacin. In all cases, a rise in systolic and diastolic blood pressure of approximately 15 mm Hg was observed within five minutes of indomethacin administration. This blood pressure increase was most marked in an animal receiving simultaneous angiotensin infusion but was also observed in the D5W control monkeys, suggesting again that suppression of prostaglandin synthesis and release mechanisms may be an important factor in this and other hypertensive states. Since the data for this thesis was tabulated, preliminary evidence has been produced by the use of Statham flow-measuring instruments

which does in fact demonstrate a decrease in uterine artery blood flow associated with the observed increase in blood pressure in response to indomethacin administration. This evidence tends to support the suggestion that prostaglandins may play an important role in maintaining decreased vascular resistance in the utero-placental bed.

CONCLUSION

The results of this study of non-pregnant monkeys do not tend to support the conception that prostaglandins, and particularly PGA, are secreted by the kidney in response to the pressor effect of elevated levels of either circulating angiotensin or norepinephrine. Despite this fact, however, and in light of the overwhelming evidence in support of an antihypertensive role for the renal prostaglandins, the hypothesis that a defect in prostaglandin synthesis and release mechanisms is associated with many forms of spontaneously occurring hypertension remains an attractive one. This is particularly true in light of Zusman's finding of decreased levels of circulating PGA in humans with essential and renovascular hypertension.[61] The key questions in need of further clarification are what blood pressure related stimuli are directly responsible for influencing prostaglandin output and what defects in the stimulus-response pattern arise that may result in or stem from

the development of hypertension.

One possibility that must be considered is that the renal prostaglandins, rather than responding to elevated angiotensin levels, increase in direct response to elevated renin levels. Although renin infusion, as such, has never been attempted, such information can be derived from studies using the angiotensin analogue P_{113} to determine whether salt loading and depletion studies such as that undertaken by Zusman[58] will yield similar results in the absence of angiotensin II binding sites.

The response of PGE in some but absence of PGA response in all of our studies suggests the possibility that PGA may not play an important role in modulating acute hypertensive stimuli. In view of the observation that 60% of the renomedullary prostaglandin content is in the form of PGE versus only 15% for PGA, there is a definite possibility that the first response to an acute hypertensive stimulus may be the output of PGE into local tissues to exert local versus systemic vasopressor and natriuretic effects. It is also likely that for the maintenance of a refined day to day balance between hypertensive and antihypertensive influences in the normal animal, local renal adjustments in vaso-motor tone and sodium excretion might play a more important role than systemic vasodepression. In the face of chronic hypertensive stimuli, however, PGA may be called upon to play a longer term systemic role in maintaining normotension. Such a hypothesis is consistent with the unresponsiveness of PGA observed in our acute experiments,

and it may be that a longer term hypertensive model will be required if elevations in PGA are to be observed. In addition, this hypothesis is consistent with the depressed levels of PGA observed by Zusman in humans with spontaneous endogenous hypertension, in whom chronic stimulation of PGA output may have led to a "burning out" of renal medullary interstitial cell ability to produce the substance.

Of interest throughout the entire series of infusions in both non-pregnant and pregnant monkeys was the pattern of prostaglandin output observed. Rather than remaining constant or smoothly rising or falling in response to the hypertension-producing infusions, PGA and PGE output tended to occur in a pattern of pulses followed by lulls or depressions in output until the next pulse. A similar pattern of output has also been observed by McGiff and co-workers.[59] Palsrud[93] has suggested an interesting scheme of enzyme kinetics for the prostaglandin synthetase enzyme to explain this phenomenon. According to this scheme, prostaglandin synthetase may only be able to undergo a limited number of active enzyme-substrate turnovers before becoming permanently inactive and requiring resynthesis. Although there is no factual support for this concept as yet, it would explain the lull following pulses of prostaglandin output, during which resynthesis of prostaglandin synthetase itself would be required.

In the experiments on pregnant monkeys, it was especially exciting to find that the one pregnant monkey whose uterine venous

PGE levels did not respond to angiotensin infusion was carrying a twin pregnancy. The finding of an unresponsive pattern of utero-placental PGE output in a monkey with a type of pregnancy suspected of being associated with utero-placental vascular insufficiency strengthens the possibility that chronic uterine ischemia may ultimately be associated with decreased prostaglandin output by the utero-placental unit. As previously mentioned, Ryan et al.[86] have found that human toxemia placentas contain less of of a PGE-like substance than normal placentas. Although spontaneous development of the true toxemia state has never been reported in laboratory animals and has been produced only with considerable difficulty in pregnant dogs[94] and baboons[95] by compromising uterine blood flow, the results of the present study are consistent with the contention that the hypertension of the toxemic state may be characterized in part by defective prostaglandin production.

The pathophysiology underlying such a defect in prostaglandin production is as yet unknown, however data concerning the behavior of renin and angiotensin during normal and toxemic pregnancies permit the following speculation. In normal human pregnancy, it is nowgenerally recognized that circulating levels of renin and angiotensinogen, the plasma precursor of angiotensin, are frequently elevated above levels found in non-pregnant normotensive women. In a recent prospective study, pregnant women who subsequently developed toxemia had significantly higher plasma renin levels than normal pregnant women in mid-trimester[96] whereas another study

has demonstrated that by the third trimester, women with frank toxemia have markedly decreased levels of circulating renin, angiotensinogen, angiotensin II, and aldosterone.[97] This pattern suggests that an underlying lesion of some sort, probably uteroplacental vascular insufficiency, provides a stimulus for increased renin output, angiotensin production, and, according to the current study, increased output of prostaglandin E. Over a period of time the chronicity of the underlying vascular insufficiency and perhaps its increasing severity during the course of pregnancy might tax these homeostatic mechanisms to the point of exhaustion. Apparent exhaustion of renin output by the juxtaglomerular apparatuses of the kidneys in pregnant rats has already been induced by restriction of sodium intake.[98] A similar line of reasoning has also been used to explain the diabetogenic effects of growth hormone-secreting pituitary adenomas, in which increased levels of blood sugar secondary to the effects of the hormone are felt to cause overstimulation and subsequent "burn out" of the pancreatic islet cells.[99] Factual support for such a model of toxemia awaits investigation of PGE levels in the second and third trimesters of human pregnancies at high risk for the eventual development of toxemia, as well as further animal experimentation.

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