EFFECTS OF THROMBOXANE SYNTHETASE INHIBITION ON BLOOD CELL FUNCTION AND MORPHOLOGY DURING OVINE PREGNANCY-INDUCED HYPERTENSION

by

Kevin William Miller

Thesis submitted to the Faculty of the
Virginia-Maryland Regional College of Veterinary Medicine,
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

VETERINARY MEDICAL SCIENCE
Physiology

APPROVED:

James C. Keith, Jr., B.S., D.V.M., Ph.D.,
Chairman

Craig D. Thatcher, D.V.M., M.S., Ph.D.

W. Kent Scarratt, D.V.M.

Maurice K. Eggleston, M.D.

October, 1987
Blacksburg, Virginia

EFFECTS OF THROMBOXANE SYNTHETASE INHIBITION ON BLOOD CELL FUNCTION AND MORPHOLOGY DURING OVINE PREGNANCY-INDUCED HYPERTENSION

by

Kevin William Miller
Committee Chairman: James C. Keith, Jr., D.V.M., Ph.D.
Department of Veterinary Biosciences

(ABSTRACT)

Scanning and transmission electron microscopy and platelet aggregometry were employed to study erythrocyte morphology and thrombocyte morphology and function during ovine pregnancy-induced hypertension (OPIH). Nine multiparous gravid ewes were observed during normal pregnancy, OPIH, and thromboxane synthetase inhibitor administration.

Blood pressure, plasma thromboxane B_2, serum chemistries and electrolytes, fibrin/fibrinogen degratory products, and total platelet count were monitored throughout the investigation to document the circulatory environment during this syndrome.

Arterial blood pressure, plasma thromboxane B_2 levels, and serum chemistries and electrolytes were significantly altered ($p \leq 0.005$) during hypertension. However, no change in total platelet count or fibrin/fibrinogen degratory product levels were detected. Collagen-induced platelet aggregation also became abnormal during this time, while ADP-induced aggregatory response remained essentially unchanged. Platelet aggregation changes seemed to correspond to degranulation and swelling of the canalicular tubule system of these cells. Echinocytosis was present during baseline measurement and persisted throughout hypertension. However, changes in shistocyte numbers were not detected.

Administration of the thromboxane synthetase inhibitors CGS13080 or CGS12970 lowered blood pressure ($p \leq 0.005$) and serum thromboxane B_2 levels ($p \leq 0.005$), and normalized serum chemistries and electrolytes ($p \leq 0.005$). Echinocyte numbers decreased ($p \leq 0.05$) and discocyte numbers increased ($p \leq 0.005$) after treatment. Platelet counts decreased after drug therapy, but normal collagen-induced aggregation was restored. No ultrastructural abnormalities were observed in thrombocytes after treatment.

There is good evidence that thromboxane synthetase inhibition is appropriate and effective treatment for pregnancy-induced hypertension. These data suggest that such therapy is especially indicated in cases complicated by hematologic disorders as evidenced in an ovine model of this syndrome.

ACKNOWLEDGEMENTS

I found a great community at Virginia Tech. This manuscript is the fruit of that community's labor and selfless support.

I would like to thank the Laboratory Animal Resource Staff, especially , for the quality care they provided the sheep, and for putting animation into every hour I spent in lab. I also thank who monitored fetuses at night and throughout the treatment period so that my hours in lab weren't always consecutive.

provided advice and assistance on statistical analysis, computing, and typing. Figures were constructed by

provided technical assistance with radioimmunoassays.

The technical expertise of

in electron microscopy was paramount to the successful completion of this project. They took considerable care of my blood cells and of me. I am grateful for both.

was an endless source of lab and library assistance, organizational skill, faith, and companionship. I cannot thank her enough for the things she did, or for the love with which she did them.

The members of my graduate committee, Drs. James C. Keith, Jr., Craig D. Thatcher, Maurice M. Eggleston, and W. Kent Scarratt, are most deserving of my appreciation for their critical evaluation of my work and review of this manuscript.

I am most deeply indebted to Dr. James C. Keith, Jr., friend and

mentor, for taking me as his first graduate student. He sharpened my skills of observation, imparted peerless professional and ethical standards, and helped me to cultivate a life's interest in eicosanoid metabolism. Sincere thanks and respect to his wife, , for her encouragement and kind nature, and their children and who, daily, allowed us all to see the exciting possibilities beyond the problems of PIH.

The ewes of this study were good friends. They gave me a more gentle, respectful attitude toward research animals.

This project was funded by a grant from the Clinical Biology Section of the Ciba Giegy Corporation, Pharmaceuticals Division, Summit, New Jersey. The thromboxane synthetase inhibitors used in this study were procured through Dr. Frank Douglas, Director, and Dr. John Turk, Associate Director. Their interest in, and support of this project is graciously acknowledged.

TABLE OF CONTENTS

I. INTRODUCTION

Preeclampsia is a hypertensive disorder of human pregnancy. It usually occurs in late gestation, and is a major cause of fetal and maternal mortality and morbidity. Diagnosis of the disease is often confused as its pathophysiologic mechanism is not well understood. Preeclampsia is currently thought to result from an error in the normal mechanism which produces generalized relaxation of uteroplacental and peripheral vascular smooth muscle during pregnancy. Increased whole blood viscosity and decreased blood flow are characteristic of preeclampsia. Functional and morphologic changes in blood cells contribute to this hemostatic compromise. Coagulopathy and reduced oxygen delivery to the placenta present additional dangers to both mother and fetus.

A hypertensive disorder similar to that seen in humans can be induced in gravid ewes. The morphology and aggregatory behavior of ovine blood is similar to human blood, so the sheep is particularly suited for the study of the hematologic response to preeclampsia.

This investigation was conducted to characterize thrombocyte function and morphology, and erythrocyte morphology before and after thromboxane synthetase inhibition in an ovine model of preeclampsia. The following review of literature is divided into three sections. The first section contains an overview of preeclampsia in both humans and sheep. Eicosanoid metabolism is discussed in the second segment. The third portion reviews platelet function and morphology and red blood cell morphology in both normal and hypertensive states.

II. REVIEW OF LITERATURE

A. Pregnancy-induced hypertension

1. Human pregnancy-induced hypertension

Human pregnancy-induced hypertension (PIH), or preeclampsia, is a hypertensive disorder of pregnancy which occurs in 5-10% of all pregnancies in the United States per year.[1] The disease usually occurs in late gestation, after the 20th week, and is a major cause of fetal and maternal mortality and morbidity. The incidence of PIH may approach 20% in primiparous women. Multiparous women with multiple fetuses or with gestational complications such as trophoblastic disease are also susceptible.

Eclampsia, the most fulminating stage of this hypertensive syndrome, is marked by convulsions and coma. Convulsion, a result of abnormal motor excitation of the brain, may be caused by acute vasospasm and cerebral ischemia. Cerebral hemorrhage may be seen in these cases. It is estimated that 10% of those affected by preeclampsia progress to eclampsia.[1]

Metabolic and hormonal disturbances, nutritional excesses or deficiencies, and idiosyncratic features of vascular reactivity have all been implicated as possible causes of PIH. Preeclampsia is now thought to result from an error in the normal mechanism which produces the generalized relaxation of uteroplacental and peripheral vascular smooth muscle during pregnancy.

Preeclampsia is marked by severe vasoconstriction, contraction of intravascular volume, proteinuria, altered renal, hepatic, and maternal neurologic function, edema and, at times, coagulation abnormalities.[2] The hallmark signs of the disease are hypertension, proteinuria, and generalized edema.[1,2]

Decreased vascular reactivity is normally seen in pregnancy, with blood pressure declining through the first half of gestation, and then rising gradually to pre-pregnant levels near term. This corresponds to an increase in the stable metabolite of prostacyclin, 6-keto-Prostaglandin F1 alpha, in both fetal and maternal circulations during the first half of pregnancy, and its subsequent decline near term.[3] The normal placenta has a slow circulatory rate. However, it exhibits a great circulatory capacity which exceeds that of either the vessels supplying or draining it. Changes in maternal blood pressure, therefore, normally exert only a slight effect on placental intervillous pressure. Uterine blood flow and placental perfusion are aided in pregnancy by an increase in plasma volume. Under normal circumstances, adequate uterine perfusion is maintained.

Placentas from preeclamptics demonstrate reduced formation of prostacyclin (PGI_2),[4,5,6] a vasodilatory, antiaggregatory prostaglandin; and increased production of thromboxane A_2 (TxA_2),[7] a vasoconstrictive, aggregatory prostaglandin. Levels of thromboxane may also be elevated without a decrease in prostacyclin in placentas from hypertensives.[8]

Hypertension is characterized by increased peripheral resistance, decreased uteroplacental blood flow, and platelet consumption.[1] It is confirmed by documentation of sudden and significant elevations in

systolic (≥140mmHg or 30mmHg increase), mean arterial (≥105mmHg or 20mmHg increase), and diastolic (≥90mmHg or 15mmHg increase) blood pressures from pre-pregnant or early pregnant levels.[1] Severe cases of preeclampsia have been reported with systolic blood pressures of ≥ 170mmHg (≥ 60mmHg increase), and diastolic blood pressures of ≥ 110mmHg (≥ 30mmHg increase).[1] In rare instances, the entire eclamptogenic episode has been seen to occur without systolic (≥ 140mmHg) or diastolic (≥ 90mmHg) pressure rises.[1]

Gestational hypertension may develop in previously normotensive women. Estrogen stimulates renin and angiotensin II which are vasoconstrictive.[9] Increased production of uterine or vascular prostacyclin during pregnancy is required to counteract the constrictive effects of angiotensin II.[10,11]

Hypertension may be present before pregnancy due to some preexisting disease. Hypertension complicated by pregnancy may be the result of a preexisting hypertensive disorder, renal or vascular disease, or diabetes mellitus. These confounding factors make the diagnosis of pregnancy-induced hypertension difficult, even when previous medical history is known. In women who are hypertensive before pregnancy, an additional elevation in blood pressure, together with proteinuria and edema, justifies diagnosis of superimposed preeclampsia.[12]

Proteinuria is generally defined by urine protein concentrations of at least 300mg/L in a 24hr collection, or 1g/L in each of 2 or more random specimens obtained at least 6hrs apart by clean catch or catheterization.[1] Protein concentrations may vary, however, from trace amounts to 1g/L as suggested.

Although globulins of all subgroups are excreted in proteinuria, albumin accounts for most of what is lost.[1] Angiotensinase, the protein enzyme which inactivates angiotensin II, is also lost.

2. Ovine pregnancy-induced hypertension

Ovine pregnancy-induced hypertension, more commonly called pregnancy toxemia, occurs after the 120th day of gestation (term = 146 days). It is precipitated by stress or acute nutritional deprivation.[13] Ewes carrying multiple fetuses, overfed ewes, and under and overconditioned ewes are most susceptible. Signs of the disease--proteinuria, uteroplacental ischemia, and hepatic and renal dysfunction--closely mimic the human disorder.[14-18] As in the human, uteroplacental perfusion and fetomaternal hemodynamics are gravely compromised.

Historically, hypertension has not been reported in pregnancy toxemic ewes.[18] Significant elevations in blood pressure have been documented recently, probably due to more frequent and continuous measurement.[19]

The pathophysiologic mechanism which induces pregnancy toxemia may be due to the development of an imbalance in the PGI_2:TxA_2 ratio. This imbalance is probably caused by the hyperketonemia which develops in food deprived, gravid ewes.[16,20] Increased levels of circulating free fatty acids decrease prostacyclin survival time in human plasma,[21] and this may also occur in the ovine. Prostaglandin imbalances induced by hyperketonemia may initiate a positive feedback cycle which then maintains and intensifies the PGI_2:TxA_2 imbalance of toxemia.

3. The Biochemical Basis of Ovine Pregnancy Toxemia

Ovine pregnancy toxemia (OPT) may be induced in ewes by making them energy deficient during the latter part of gestation; a time when

they have a great energy demand.[20] Glucose, the major substrate for the pregnant uterus and lactating mammary gland,[22] may only be synthesized in limited quantities in the fasting animal. In contrast to the monogastric, the rate of ovine gluconeogenesis decreases with fasting.[23] Body processes are maintained by the mobilization of free fatty acids from body stores. Excessive quantities of circulating fatty acids overwhelm beta-oxidative enzymatic capabilities. Excess fatty acids are shunted to ketone synthesis. The onset of clinical signs in ewes is preceded by hypoglycemia and hyperketonemia. However, these signs are not related to minimum glucose or maximum ketone levels.[24] Ketones may play a particularly important role in the pathogenesis of OPT, because they compete with prostacyclin for binding sites on albumin. Decreasing albumin levels due to proteinuria, combined with increasing levels of competitively binding ketones, further limit the effectiveness of prostacyclin.

Altered fatty acid composition of the blood changes blood cell membrane characteristics[25] as well as cellular function.[26] Elevated plasma platelet factor 4 levels, as well as platelet aggregates, may be seen within 72hrs of fast in humans.[27] Increased levels of fatty acids combined with low albumin levels cause hemolysis.[28]

B. Eicosanoid Metabolism

The eicosanoids are a group of C_{20} fatty acids. Many oxygenated eicosanoids are formed in animals from three commonly occurring C_{20} fatty acids. These precursors are 5-cis-,8-cis-,11-cis-,14-cis-eicoso-

tetraenoic acid (arachidonic acid), 8-cis-,11-cis-,14-cis-eicosatrienoic acid, and 5-cis-,8-cis-,11-cis-,14-cis,17-cis-eiosapentaenoic acid. Of these, arachidonic acid is most important.[29]

The oxygenated eicosanoids are separated into two groups according to the products they form. Prostanoids, prostaglandins and thromboxanes, comprise the first group. These are called cyclooxygenase products, as they are formed by the initial action of bisdioxygenase (formerly called cyclooxygenase). Prostanoids are autocoids, or local hormones, and act near the site of their synthesis.[29]

The second group, acted upon by any number of different bisdioxygenase enzymes, are called lipoxygenase products. These products are the hydroxy and hydroperoxyeicosaenoic acids and leukotrienes.[29]

The oxygenated eicosanoids are not stored, and their basal rate of synthesis is low. They are rapidly metabolized during their passage through the circulatory system.

Almost all animal organs synthesize prostaglandins,[30] but only certain cell types within a given organ form cyclooxygenase products. Generally, all smooth muscle and vascular endothelial cells, as well as blood platelets, form these products. Both the prostacyclin synthetic cascade of the vasculature and the thromboxane cascade of platelets occur in bursts, in response to certain stimuli.[29]

Arachidonic acid is esterified on membrane phosphoglycerides of the above cell types. External stimuli which induce prostaglandin formation cause the intracellular release of this C_{20} precursor. The result is an elevation in the cytosolic concentration of free arachidonate. Pathological stimuli cause the release of linoleate, oleate,

arachidonate, and other fatty acids from the membrane in amounts reflecting the proportion of fatty acids in the membrane.[29] The unstable endoperoxide, prostaglandin H_2 (PGH_2), is created by the oxygenation of arachidonic acid. PGH_2 then undergoes cell specific conversion to the major biologically active prostanoid forms (TxA_2, PGI_2, etc).[31,32] Blood platelets produce thromboxane A_2,[31,33-35] while vascular endothelial cells produce prostacyclin.[36]

C. Pregnancy-Induced Hypertension and Blood Cell Pathophysiology

Hemorrhage and coagulopathy are serious consequences of PIH. Current literature suggests that changes in thrombocyte and erythrocyte function and morphology are an integral component of the pathophysiology of PIH. A brief review follows.

1. Thrombocytes

Unactivated mammalian platelets are small, oval, discoid, anuclear blood cells which are derived from megakaryocyte cytoplasmic fragmentation.[37] Changes in size and shape are dependent upon age and species.[38,39]

Ultrastructural features of platelets have been divided into three zones.[40] The peripheral zone comprises the glycocalyx and cell membrane. The sol-gel zone includes the circumferential band of microtubules and microfilaments immediately beneath the cell membrane. The organelle zone contains major organelles of the platelet.

Platelet organelles include various granules, vesicles, mitochondria, tubule systems, and some glycogen.[41] The major granules are alpha, dense, and lysosomal granules. Alpha granules contain fibrinogen and

other coagulation factors. One such factor, beta-thromboglobulin, decreases endothelial cell production of PGI_2 in humans[42] and other species.[43] Dense granules, which have an eccentrically placed electron dense core, contain ADP, serotonin, and calcium. Lysosomal granules contain digestive enzymes.

There are two tubular systems of importance in platelets. The dense tubular system, derived from the smooth endoplasmic reticulum of the megakaryocyte, is involved in platelet activation.[37] It synthesizes and stores platelet products in young platelets[41] which has resulted in it being termed the sarcoplasmic reticulum of the platelet.[37] The open canalicular system functions in platelet granule release and transportation of plasma-borne substances to the center of the platelet.[37,44]

Platelets are paramount to the maintenance of normal vascular integrity and hemostasis.[45] In the production of a thrombus, normal platelets come in contact with an aggregating agent. They are activated when endothelial cells are damaged and form a hemostatic plug which covers the site of injury. Size of the plug is controlled by PGI_2 synthesis mobilized from membrane phospholipids in healthy vascular endothelial cells neighboring the injured site.[36] PGI_2 synthetase facilitates PGI_2 formation from cyclic endoperoxides.[37] PGI_2 induces elevations in platelet cAMP levels which inhibit aggregation by inactivating platelet phospholipase A_2 and cyclooxygenase.[36]

Pseudopods appear on the cell surface of platelets that become activated. These projections enhance the cell's ability to stick to surfaces or to one another. Cytosolic granules centralize, and nongranular cytoplasm and microtubules move to the peripheral areas of the

cell. This forms a "hyalomere" or clear zone. Coalescence of the outer membrane along the areas of surface contact follows. The aggregates that form are strengthened by a network of fibrin strands.

Platelets form thromboxane A_2 as their major cyclooxygenase product.[31,33-35] Many stimuli are responsible for TxA_2 release and subsequent aggregation in platelets. These include: collagen, arachidonic acid (though not as well in ruminants), prostaglandin endoperoxides, thromboxane intermediates, ADP, biogenic amines (ie: serotonin, epinephrine, and norepinephrine), proteolytic enzymes (ie: thrombin, trypsin, and plasmin), and particulate material (ie: bacteria or viruses).[37,39]

The primary stimulus for activation is exposure to type 1 and type 3 collagens.[46] Platelet exposure to collagen causes formation of diglyceride from phosphatidylinositol specific phospholipase C.[29,47] This phospholipase C is probably activated as a result of stimuli induced calcium mobilization. Newly formed diglyceride apparently serves two functions. Part of the diglyceride is cleaved by glyceride lipase(s) releasing arachidonate. The rest of the diglyceride may activate a diglyceride dependant protein kinase C, which may cause activation of phospholipase A_2 which cleaves arachidonate from phosphatidylcholine.[29,47] A normal lag time exists between cellular stimulation by collagen and aggregatory response. ADP, which also aggregates platelets <u>in vivo</u>,[48] follows a more direct, calcium independent route of aggregation. This classic agonist-receptor interaction[49] yields an immediate aggregatory response.

Platelet aggregatory function is highly dependent upon the metabolic function of the cell.[50] There are 2 phases of platelet aggregation.

Direct surface stimulation with the platelet receptor, induced by aggregating agents, is responsible for the first phase.[37,38] Thromboxane A_2 synthesis is responsible for the secondary wave of aggregation in human platelets[51] and in several animal species,[36] including the ovine. This phase is caused by the release of proaggregating factors, serotonin, and ADP,[52] and the activation of the prostaglandin synthetic pathway during the release reaction.[53] Both secondary aggregation and granule release are dependent upon the endoperoxides synthesized during the prostaglandin pathway which also produces thromboxane.[31,35,38] Secondary aggregation can occur due to the direct result of de novo thromboxane synthesis, independent of secretion.[51]

The platelet release reaction may be started by unstable prostaglandins, such as PGG_2 or PGH_2, TxA_2, or 15-hydroperoxy TxA_2.[32] In contrast, it is not initiated by stable prostaglandins, such as PGE_2, PGF_2 alpha, or PGD_2. These stable prostaglandins inhibit platelet aggregation by increasing cAMP which inhibits phospholipase A_2 and cyclooxygenase.

The activities of nearly all coagulation factors increase during gestation, reaching their peaks at the time of delivery,[54] but in preeclamptic patients clotting factor levels are decreased to levels far below those of nonpregnant women.[55] Changes in the chemical environment of circulating blood can make platelets hypersensitive,[56] due to alterations in the sensitivity of platelet eicosanoid receptors.[57] This phenomenon has been seen in thromboembolic disorders. Increased platelet sensitivity during atherosclerosis has been implicated as the cause of the coagulopathies that have accompanied this disorder.[49] In contrast, lack of

aggregation response to collagen but normal response to ADP, epinephrine, and ristocetin has been reported clinically.[58] Defective platelet receptors for collagen have also been seen by others.[59] Abnormal platelets may contain only a few or no granules, or in some situations, appear completely normal. Defects in function may or may not be accompanied by ultrastructural abnormality.[60]

The relationship that exists between percent aggregation of human platelets and the amount of thromboxane B_2 generated approximates a hyperbola with a roughly linear relationship from 0-70% aggregation and 0-50ng TxB_2 per ml of platelet rich plasma (PRP).[61] The amount of TxB_2 production may increase up to 500ng per ml of PRP with no clear relationship to disturbances in platelet function.[61]

Platelet survival in preeclamptic patients appears to be reduced even in the absence of thrombocytopenia, and platelet surface is altered in women with preeclampsia and their neonates, even in the absence of bleeding.[62] Platelet function has been restored in a patient with PIH after adrenergic receptor blockage with labetalol.[63] Lack of platelet function is thought to be due to platelet "exhaustion".[64] Normally, platelets will not aggregate in the presence of PGI_2. However, platelets have been reported to lose their sensitivity to PGI_2 without any other defect in platelet response.[65]

Addition of placental cytosol to PRP reduces or prevents aggregation by collagen. Reduced maximum aggregation and slope values at the inflection point, as well as increased lag times are characteristic of these aggregation curves.[66] Low dose aspirin also causes depression of aggregation curves which corresponds to prolonged bleeding times.[67]

2. Erythrocytes

Normal ovine erythrocytes are flat, discoid cells with little or no central depression as seen in human cells.[68] Slight anisocytosis is normal. Poikilocytosis, may be noted in renal disease, splenic disease, and blood loss anemias, but minor alterations in shape may be normal. Spherocytes--evenly stained, thickened cells with no central pallor--are not common in sheep. Crenation may result from normal aging or delayed drying, exposure to a lytic agent, presence of hypertonic solutions or when blood is allowed to stand. Acanthocytes are commonly seen in kidney disease and occasionally in liver disease.

During normal human pregnancy, erythrocytes become more deformable and flexible. Red cells from women with preeclampsia become more rigid and do not regain their shape upon removal of distorting circulatory forces.

Echinocyte membranes are rigid,[69-71] and are characterized by projections distributed over their surface.[72] Echinocytes demonstrate significantly impaired flow patterns in vitro.[70] Rigid sickle cells stay in the microvasculature much longer than cells with normal deformability and reduce blood flow to a greater extent than would be expected based on their percentage to the total RBC population.[73] This same behavior, if mimicked by echinocytes and shistocytes would increase whole blood viscosity and decrease blood flow.

Increased relative levels of thromboxane A_2 cause arteriolar sensitization and vasoconstriction. Vasospastic episodes in isolated resistance vessels of preeclamptic patients[74] promote microangiopathic hemolysis.[25] This is further enhanced with cellular deformities. Red cells subjected to shear stress release ADP which aggregates platelets

in citrated PRP *in vitro*.[75] Red cells leak ADP in concentrations capable of aggregating platelets even when shear stress is at a minimum, however.[76] Thus, the extent to which hemolysis may contribute to aggregation is debatable.

Elevated activity of the platelet TxA_2 metabolic pathway[77] and increased rates of platelet activation[1] and consumption[78] occur in pregnancy-induced hypertension. Cerebral, hepatic, renal, and pulmonary hemorrhage and hemorrhagic necrosis are all characteristic lesions of preeclampsia-eclampsia.[1] Extensive intervillous thrombosis is also a danger, as it may lead to premature placental aging (lesions associated with fibrosis and calcification) and separation. Disseminated intravascular coagulation, initiated by premature placental separation, may lead to a progressive consumptive coagulopathy. Fibrin microthrombi, together with systemic vasospasm, lead to local hemorrhage and necrosis.[79]

Diminution of maternal blood flow to the placenta leads to degeneration and necrosis of the organ. The slowing of blood flow, defects in vessel walls, and increased coagulation cascade activity fulfill Virchow's triad and thus allow for the formation of large mural thrombi.[80]

D. Treatment of PIH

1. Human Syndrome

Traditionally, hospitalization, strict bed rest, and magnesium sulfate administration for control of seizure activity have been the accepted treatments for PIH. Phenobarbital, oral beta-adrenergic receptor blockers, diuretics,[30,81] vasodialators, and blood volume

expanders[82] have also been used. These therapies, however, are of only limited benefit which leaves delivery as the only definitive treatment.

Few antiplatelet drugs have been studied in the treatment of PIH. Dipyridamole, a pyrimido-pyrimidine drug, inhibits phosphodiesterase which prevents breakdown of cAMP to AMP.[83] The nonsteroid antiinflammatory drugs such as aspirin, sulphinpyrazone, and indomethacin interfere with cyclooxygenase and prevent cyclic endoperoxide formation.[83] Low dose aspirin has been shown to prevent preeclampsia in angiotensin II-sensitive human primigravidae.[84] It decreases platelet TxA_2 production by acetylating cyclooxygenase, thus inactivating the enzyme.[46,85] Considerably greater quantities of cyclooxygenase inhibitors are needed to block enzyme activity in cells of the vessel wall than in blood platelets.[67,86,87] Similarly, adrenoceptor agonists have decreased platelet thromboxane production in vitro,[88] but these effects could not be demonstrated in human hypertensive pregnancies.[89] In contrast, cyclooxygenase inhibition, at high doses, decreases plasma renin levels[90] and decreases vascular refractoriness to infused angiotensin II in pregnant women.[91] These two events are also seen in women with PIH.

2. Ovine Syndrome

In the toxemic ewe, oral or intraveneous carbohydrate and glucocorticoids have traditionally been administered in the attempt to correct ketonemia. Such therapy, however, is unrewarding and often variable as glucocorticoid success is linked to early delivery.[92] The only effective therapy is early delivery. This generally results in death of the neonates due to immaturity.[92-94] U-63,557A, a thromboxane synthetase inhibitor, resolves the hemodynamic and coagulopathic abnormalities

associated ovine pregnancy toxemia.[19]

E. Potential Role of Thromboxane Synthetase Inhibition in the Treatment of PIH

Considerable work has been done to develop specific inhibitors of thromboxane synthetase, since abolition of thromboxane synthesis can be therapeutic in the prevention of coagulopathy specifically caused by TxA_2. Intravenous infusion of arachidonic acid produces intravascular platelet aggregation, pulmonary thrombosis, and sudden death.[95] However, prompt infusion with TxA_2 synthetase inhibitor prevents this mortality.[96] The potential role of these inhibitors in the treatment of preeclampsia is gaining importance as the benefit of altering PGI_2:TxA_2 ratios in preeclampsia is realized.[19]

Since preeclampsia involves the alteration of both vascular and platelet eicosanoid oxygenation pathways, it is of interest to note that there may be a differentiation between vascular and platelet receptors for thromboxane A_2.[48] Potency of thromboxane agonists is different in platelets than in vascular smooth muscle from the same species.[97]

Normally, TxA_2 is the only arachidonic acid metabolite released from blood platelets.[98] TxA_2 synthetase inhibition and subsequent treatment with aggregating agents, however, results in the secretion of prostaglandin endoperoxides (PGH_2 instead of TxA_2) into the circulation.[99-102] PGH_2 is then rapidly converted by the blood vessel endothelial cells to PGI_2.[98,103] Platelets are unable to synthesize new cyclooxygenase, and their ability

to synthesize prostaglandins is decreased throughout their remaining lifespan after aspirin administration.[104]

Thromboxane synthetase inhibitors, unlike aspirin, are reversible enzyme inhibitors. A single dose of imidazo (1,5-2) pyridine-5-hexanoic acid (CGS13080) causes a significant, reversible fall in TxA_2 after 6-12 hrs of dosing.[100,102,105] The biological half life of CGS13080 is approximately 6-8hrs with peak inhibition at 1hr.[100] Both no effect on bleeding times or platelet aggregation,[100] and prolongation of bleeding time and platelet aggregation inhibition[106] have been reported in response to CGS13080 administration. This latter instance may be related to increased vascular PGI_2 production from platelet endoperoxides since it occurred 2hrs after dosing.

It is hypothesized that thromboxane synthetase inhibitors, by boosting prostacyclin levels and disallowing intermediate metabolites to follow the thromboxane synthetic pathway, will restore normal fetomaternal homeostasis.

III. RESEARCH JUSTIFICATION AND HYPOTHESES

The significance of preeclamptic coagulopathy is quite controversial, and ideas about RBC changes are even less concrete. The arising disagreements over coagulation abnormalities stem from uncertainties as to how PIH itself should be managed.[2] The dangers of intravascular coagulation, especially during pregnancy, are clear. However, antithrombotic therapy with such agents as aspirin, dipyridamole, or heparin could be most devastating in hypertension complicated by hemorrhage.

To better understand the maternal hematologic abnormalities that occur with hypertensive pregnancy, erythrocyte morphology and thrombocyte function and morphology was assessed in an ovine model of preeclampsia.

The hemodynamics of the ewe has been studied for some time. Ovine vascular responses,[107] blood volume, and hemodynamic characteristics are similar to those of humans during pregnancy. The ewe has also been used to study vascular reactivity to angiotensin II during pregnancy.[108] PGI_2 infusion in nonpregnant and nonstressed pregnant ewes causes vasodilation and increased uterine blood flow.[109] Infused TxA_2, however, causes uterine vasoconstriction in pregnant sheep. Additionally, ovine platelets react similarly to those of humans in their adhesiveness to test plastics.

In this thesis, emphasis was placed on the relationships of arterial blood pressure and plasma thromboxane B_2, and the functional and morphological responses of blood cells to the changes in their circulating

environment. The following hypotheses were made:

A.) General Hypothesis:

Thromboxane synthetase inhibitors are therapeutic in the treatment of preeclampsia.

B.) Specific Hypotheses:

1.) Platelet ultrastructure changes with the occurrence of ovine pregnancy-induced hypertension. These ultrastructural changes correspond to abnormal in vitro aggregation responses; which are able to be quantitated by comparing aggregation curve characteristics (Chapter 4).

2.) Alterations in the circulating environment during pregnancy change RBC membrane characteristics, which may potentially alter their function (Chapter 5).

IV. MANUSCRIPT SUBMITTED TO THROMBOSIS AND HAEMOSTASIS ENTITLED "EFFECT OF THROMBOXANE SYNTHETASE INHIBITION ON PLATELET FUNCTION AND MORPHOLOGY DURING OVINE PREGNANCY-INDUCED HYPERTENSION."

Effect of Thromboxane Synthetase Inhibition on Platelet Function and Morphology During Ovine Pregnancy-Induced Hypertension

Kevin Wm. Miller, B.S. and James C. Keith, Jr., D.V.M., Ph.D.

Department of Veterinary Biosciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

This work was supported by the Ciba Giegy Corporation, Summit, New Jersey.

Reprint requests: Kevin Wm. Miller, Department of Physiology and Biophysics, 224 Basic Sciences Building, Georgetown University Medical Center, Georgetown University, Washington, D.C. 20057

Effect of Thromboxane Synthetase Inhibition on Platelet Function and Morphology During Ovine Pregnancy-Induced Hypertension

Kevin Wm. Miller, B.S. and James C. Keith, Jr., D.V.M., Ph.D.

Department of Veterinary Biosciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

Summary

Arterial blood pressure, serum fibrin/fibrinogen degratory products, plasma thromboxane B_2, in vitro platelet aggregation, and platelet ultrastructure were studied in nine multiparous gravid ewes during ovine pregnancy-induced hypertension and subsequent administration of the thromboxane synthetase inhibitors CGS13080 and CGS12970. During the hypertensive period, blood pressure ($P \leq 0.005$) and serum thromboxane B_2 levels ($P \leq 0.005$) were significantly altered. Collagen-induced platelet aggregation lag times increased ($p \leq 0.01$), and percent aggregation ($p \leq 0.05$), primary ($p \leq 0.01$), and secondary ($p \leq 0.005$) aggregatory slopes decreased. Collagen also failed to induce aggregation in some ewes. ADP-induced primary aggregation slopes decreased ($p \leq 0.01$) during hypertension. Degranulation and canalicular tubule swelling were observed in platelets which produced abnormal or no aggregation response. However, these ultrastructural abnormalities did not necessarily correspond to hypertensive periods.

Thromboxane synthetase inhibitor administration lowered blood

pressure ($p \leq 0.005$), serum thromboxane B_2 levels ($p \leq 0.005$), and total platelet count ($p \leq 0.01$). Abnormalities in collagen and ADP-induced platelet aggregation curves were also corrected, and ultrastructural abnormalities were not detected. Marked elevations in plasma thromboxane levels during ovine pregnancy-induced hypertension may have had an "exhaustive" effect on thrombocytes which was reversed by thromboxane synthetase inhibition.

Key Words

Thromboxane - Platelet Aggregation - Pregnancy-Induced Hypertension-Platelet Ultrastructure - Ovine Pregnancy Toxemia

Introduction

Preeclampsia (Pregnancy-induced hypertension or PIH), a hypertensive disorder of pregnancy which usually occurs in late gestation, is a major cause of fetal and maternal mortality and morbidity.[1] Changes in thrombocyte function are characteristic complications of preeclampsia, which may lead to disseminated intravascular coagulation. This change in function has been found to be accompanied by changes in the cell's ultrastructure.

The significance of preeclamptic coagulopathy is much debated. Therefore, the study of platelet function and ultrastructure in preeclampsia is important in determining the role of coagulopathy in this disease. However, little attempt has been made to characterize the response of thrombocytes to preeclampsia or its treatment in an animal model of pregnancy-induced hypertension.

A pregnancy-induced hypertensive disorder similar to that seen in

humans can be induced in gravid ewes.[2,3] The purpose of this investigation was to characterize thrombocyte function and morphology in an ovine model of pregnancy-induced hypertension during normal pregnancy and hypertension, and after thromboxane synthetase inhibition. Serum fibrin/fibrinogen degratory products and plasma thromboxane B_2 levels were determined to document changes in the circulatory environment of the thrombocyte during the experimental period.

Materials and Methods

Thirteen multiparous gravid ewes were obtained from a commercial breeder. Ewes were taken into the laboratory in pairs and placed in metabolism crates around the 120th day of gestation. Mixed hay, grain, water, and mineralized salt were offered ad lib. Each ewe was allowed an acclimation period of approximately 3-7 days. The laboratory environment was kept at constant temperature and photoperiod. During gestational days 127 through 134, measurements of maternal blood pressure, plasma thromboxane B_2 levels, and fibrin/fibrinogen degratory products (FDP's) were taken to establish baseline values for each animal. Platelet aggregation tests were performed, and aggregated and unaggregated thrombocytes were collected for ultrastructural examination.

On the 134th day of gestation, grain and hay were removed to induce ovine pregnancy-induced hypertension (ovine pregnancy toxemia or OPT). Water and salt were provided ad lib throughout the fast. Fasting periods varied for each ewe from 12 to 90hrs (the majority were fasted approximately 72hrs, days 134-137).

Of the thirteen animals fasted, nine became hypertensive. An animal was considered to have OPT when: 1.) a minimum rise in mean arterial pressure of 11mmHg was demonstrated; 2.) serum chemistries indicated hypoglycemia and renal dysfunction (proteinuria and ketonuria were also noted); and 3.) neurologic disturbances (ie: depression or muscle tremors) were observed.

At the onset of hypertension, the ewes were divided into three groups of three animals each: control - no treatment, CGS13080 treatment, or CGS12970 treatment (thromboxane synthetase inhibitors were

obtained from Ciba Geigy Corporation, Summit, N.J.). CGS13080 [N (1-carboxyheptyl) imidazole, imidazo 1,5-a pyridine-5-hexanoic acid] was administered at the rate of 0.1mg/kg/hr IV infusion. CGS12970 [3-methyl-2-(3-pyridyl)-1-idolectanoic acid] was administered at the rate of 1mg/kg IV bolus. All ewes were monitored up to 48hrs post treatment.

Maternal Blood Pressure:

While the ewes stood quietly in the metabolism crates, thirty minute recordings of systolic, mean, and diastolic maternal blood pressures were obtained at 12hr intervals throughout the experiment (gestational day 127 until approximately day 140), using the oscillometric method described previously.[4]

Fibrin/Fibrinogen Degratory Product Analysis and Thromboxane B_2 Determination:

10mls whole blood was obtained once a day by clean jugular venipuncture. 1ml was placed in a thrombin tube and allowed to clot for fibrin/fibrinogen degratory product (FDP) analysis. After the sample had clotted, the blood was centrifuged at 1,500rpm for 10min at room temperature. The thrombo-wellcotest (Wellcome Diagnostics) was performed on the serum for the detection of FDP's.

The other 9ml blood was thoroughly mixed with 1ml of 3.8% sodium citrate solution containing indomethacin (50µg/ml). The sample was immediately centrifuged at 1,500rpm for 10min at room temperature. Plasma was decanted from the cellular fraction, and immediately frozen at -70°C until analyzed. Radioimmunoassays were performed on 300µl aliquots of the unextracted plasma for thromboxane B_2, the stable metabolite of TxA_2, by commercially available (^3H) RIA kits (Amersham)

as previously described.[3]

Total Platelet Count and Platelet Aggregation:

Whole blood was obtained once a day by clean jugular venipuncture. Sodium citrate (3.2%) was used as the anticoagulant (1 part citrate : 9 parts blood). Total platelet counts were performed manually by the hemocytometer method (unopette 5453). The blood was centrifuged twice at 850rpm for 3min to harvest platelet rich plasma (PRP). Platelet poor plasma (PPP) was obtained by centrifugation at 1,500rpm for 10min.

Aggregation studies in PRP were performed according to the light transmission method of Born[5] with a dual channel platelet aggregometer (Payton Associates, Buffalo, NY). The PRP was incubated at 37.5°C and stirred at 800rpm. 2min of baseline recording was taken to test for spontaneous aggregation and baseline variability. Bovine collagen and ADP (Sigma) were added to induce aggregation at final concentrations of 10μg/ml and 8.5μg/ml, respectively. Aggregation curves were recorded until maximum aggregation had occurred. Aggregated and unaggregated samples of platelet rich plasma (PRP) were fixed in modified McDowell-Trumps fixative (pH 7.4) for at least 24hrs for transmission electron microscopic examination.

Thrombocyte and Aggregate Ultrastructure:

Fixed free platelets, collagen-induced platelet aggregates, and ADP-induced platelet aggregates were centrifuged at 1,500rpm for 3min at room temperature. The supernatant was discarded, and the platelets were washed 2x in Tyrode's buffer (pH 7.4). The cells were post fixed in 1% osmium tetroxide for 1hr, and again washed 2x in Tyrode's buffer. They were then embedded in 2% Nobel agar, and cut into 1mm cubes. After

dehydration in a series of ethanols and then propylene oxide, the agar cubes containing platelets were put in equal volumes of propylene oxide and Polybed 812 resin (Polysciences) for approximately 8-12hrs. Fresh resin was used to embed the platelet containing agar cubes in molds. These molds were allowed to stand for an additional 8hrs, and then oven cured at 60°C for 72hrs.

Sections of approximately 600-900 angstroms were cut from resin blocks using either a LRB IV or Sorvall MT2B ultramicrotome. Sections were placed on #200 mesh grids and stained with a 1:1 solution of uranyl acetate and acetone. A second staining was conducted with Reynolds lead citrate stain for 5 minutes.

Unaggregated and aggregated platelets were examined on a Jeol 100 CX-II scanning-transmission electron microscope.

Statistical Analysis:

Each ewe served as its own control and statistical analysis was performed by paired t test comparisons. Probability of ≤ 0.05 was considered to be significant. All results are reported as mean $\pm$ SE.

Results

Maternal Blood Pressure:

Baseline oscillometric arterial pressures from the nine animals were 131 ± 1.11mmHg (systolic), 90 ± 1.07mmHg (mean arterial), and 66 ± 1.03mmHg (diastolic) [n=356]. Blood pressure rose significantly ($p \leq 0.005$) during the toxemic period to 150 ± 1.24mmHg (systolic), 106 ± 1.37mmHg (mean arterial), and 80 ± 1.48mmHg (diastolic) [n=216]. 12hr after the administration of CGS13080 or CGS12970, systolic (144 ± 2.70mmHg), mean arterial (97 ± 3.15mmHg), and diastolic (74 ± 3.12mmHg) blood pressures fell to levels that were significantly different from those of OPT ($p \leq 0.005$) [n=60], but not of baseline.

Fibrin/Fibrinogen Degratory Product Analysis and Thromboxane B_2 Determination:

FDP's were present in a concentration of at least 10μg/ml in most animals for at least one of the three time periods. However, no consistent changes in the presence or absence of FDP's were noted at any time during the study, and no trends were able to be established. Therefore, FDP's did not prove to be a reliable indication of abnormal coagulation.

Plasma levels of thromboxane B2 rose significantly from 57.69 ± 3.1pg/ml [n=21] during baseline to 99.87 ± 8.28pg/ml during OPT [n=15] ($P \leq 0.005$). After CGS13080 or CGS12970 administration, thromboxane B_2 levels fell to 42.57 ± 5.21pg/ml, a level significantly lower than either OPT or baseline values ($P \leq 0.005$). The least detectable concentration of thromboxane B_2 was 5pg/tube. Interassay variation was ≤ 10%, and intraassay variation was ≤ 5%. Fifty percent binding of the standard curves for thromboxane B_2 was 27pg. Recovery rates for added thromboxane

B_2 ranged from 91-134%.

Total Platelet Count and Platelet Aggregation:

Total platelet counts, given in table I, remained constant throughout baseline and OPT. After thromboxane synthetase inhibition, they significantly decreased to levels lower than those of baseline or OPT, but still within the normal range for sheep.

ADP-induced platelet aggregation characteristics are provided in table II, and their corresponding aggregation curves are illustrated in figure I. Collagen-induced platelet aggregation characteristics are described in table III. Their corresponding aggregation curves are also illustrated in figure I. Figure II presents aggregation curves for ewes whose thrombocytes were nonreactive to collagen during OPT. Standard errors in table III are large due to this occurrence. In figures I and II, sharp drops appear in all curves at 2min. These are artifacts from the addition of aggregating agents.

During OPT, there was a delayed to absent collagen-induced aggregatory response, with increased lag time, and decreased percent aggregation, and primary and secondary aggregation slopes. An essentially normal ADP-induced aggregation response was noted throughout the hypertensive period, except for a decreased primary slope. After administration of thromboxane synthetase inhibitors, the aggregation slope characteristics of both collagen and ADP-induced curves returned to baseline levels. This hypoaggregatory response is in direct contrast to the hyperaggregation previously reported during ovine pregnancy-induced hypertension.[3] Instrumentation of the animals in other studies, however, would have been enough to promote the hyperaggregation that was reported.

Thrombocyte and Aggregate Ultrastructure:

Figures III, IV, and V illustrate the ultrastructural changes that occurred in unaggregated and collagen and ADP-induced aggregated platelets throughout baseline and OPT, and after thromboxane synthetase inhibition. Degranulation and swelling of the canalicular tubule system were noted in platelets which produced abnormal or no aggregation response. However, the ultrastructural abnormalities that were observed did not necessarily correspond to hypertensive periods. No gross abnormalities were seen in platelet ultrastructure after thromboxane synthetase inhibitor administration.

Discussion

The single most significant finding of this investigation was the hypoaggregatory response of platelets during hypertension, a time when plasma thromboxane B_2 levels were significantly elevated. Changes in the chemical environment of circulating blood are more commonly thought of as making platelets hypersensitive,[6] presumably due to alterations in the sensitivity of platelet eicosanoid receptors.[7] This phenomenon has been seen in thromboembolic disorders. A platelet "exhaustive" syndrome, where platelet function is decreased, has been described.[8] Normally, platelets will not aggregate in the presence of PGI_2. However, platelets have been reported to lose their sensitivity to PGI_2.[9] A situation where platelets are capable of producing thromboxane, but are unresponsive to the thromboxane that is produced has also been described.[10]

Addition of placental cytosol to PRP reduces or prevents aggregation by collagen. Reduced maximum aggregation and slope values at the inflection point, as well as increased lag times are characteristic of these aggregation curves.[11] Since preeclampsia involves the alteration of both vascular and platelet eicosanoid oxygenation pathways, it is of interest to note that there may be a differentiation between vascular and platelet receptors for thromboxane A_2.[12] Potency of thromboxane agonists is different in platelets than in vascular smooth muscle from the same species.[13]

Impaired collagen-induced platelet aggregation with normal ADP-induced aggregation has been described in human disease,[14,15] even in the face of normal total platelet counts. Lack of aggregation response to collagen but normal response to ADP, epinephrine, and ristocetin has

been reported clinically,[16] and defective platelet receptors for collagen have been observed.[17] We speculate that the ovine platelet abnormality described here is due to an abnormality of the membrane receptor specific for collagen-platelet interactions, much like that theorized for humans. As the response to collagen was abolished and later returned, it is unlikely that the abnormality was due to the collagen itself. It is possible, but less likely, that these platelets could have disturbances in intracellular calcium mobilization.

Platelet survival in preeclamptic patients appears to be reduced even in the absence of thrombocytopenia, and the platelet surface is altered in women with preeclampsia and their neonates, even in the absence of bleeding.[18] However, normal total platelet counts during human pregnancy-induced hypertension have been reported.[19] Normal platelet counts during hypertension were observed in this study as well. This finding, in noninstrumented ewes, differs from that which has been previously reported in chronically instrumented ewes.[3] The artificial surfaces of the instrumentation devices used in previous studies may have been responsible for the hyperaggregability and decreased platelet counts that were observed. Platelet counts may have decreased after thromboxane synthetase inhibitor administration in the present study due to an enhanced ability of the reticuloendothelial system to recognize functionally abnormal cells.

Defects in function may or may not be accompanied by ultrastructural abnormality.[20] Similarly, coagulation tests may be normal in the face of abnormal platelet function tests and ultrastructure.[1] When ultrastructural anomalies do occur, the most commonly occurring problem is

abnormal granulation. Disrupted and disassembled marginal microtubules, defective membranous structures, retention of the rough endoplasmic reticulum, and hypermitochondrionism are also found.[1] In this study, defects in platelet function were generally accompanied by ultrastructural changes. Degranulation was the most striking change noted, with canalicular swelling also being observed. The presence of microtubules during collagen-induced aggregation was particularly prevalent in samples taken after thromboxane synthetase inhibitor administration. No correlation can be drawn between OPT and ultrastructural changes, as the most pronounced changes did not always occur during this period.

Mild increases in the serum levels of FDP's are present in preeclamptics.[21] We have also observed FDP's in the ovine model, but without any degree of consistency.

Cerebral, hepatic, renal, and pulmonary hemorrhage and hemorrhagic necrosis are all characteristic lesions of preeclampsia-eclampsia.[1] Extensive intervillous thrombosis is a danger in preeclamptics with hematologic complication, as it may lead to placental lesions and separation. Disseminated intravascular coagulation, initiated by premature placental separation, may lead to a progressive consumptive coagulopathy. Fibrin microthrombi, together with systemic vasospasm, lead to further hemorrhage and necrosis.[22] Elevated activity of the platelet TxA_2 metabolic pathway[23] and increased rates of platelet activation[1] and consumption[24] occur in pregnancy-induced hypertension. However, the significance of pregnancy-induced coagulopathy is quite controversial, as moderate activation of platelets is present in normal

pregnancy.[19] The arising disagreements over coagulation abnormalities that occur during this syndrome stem from uncertainties as to how the disease itself might best be managed.[21]

The dangers of intravascular coagulation, especially during pregnancy, are clear. However, antithrombotic therapy with such agents as aspirin, dipyridamole, or heparin could be devastating in hypertension complicated by hemorrhage. Both hypoaggregation and hyperaggregation are causes of immediate concern. The maintenance of proper platelet function is paramount to the maintenance of homeostasis. Therapy with thromboxane synthetase inhibitors appears to restore normal function to platelets.

To more clearly understand the coagulopathy that occurs with pregnancy-induced hypertension, further investigation is necessary. The ovine model of pregnancy-induced hypertension is also an interesting model for coagulopathy in this disease. Future efforts should be concentrated on the mechanisms of thrombocyte receptors, particularly those of collagen. Quantitation of ovine platelet thromboxane release in PRP during normal pregnancy and OPT would also be helpful.

The technical assistance of Karen M. Swanson, Virginia Viers, Kathy J. Lowe, Nancy Hotter, and Meagan Bamford in electron microscopy and Julie Kutruff in blood processing was greatly appreciated. This project was funded by a grant from the Ciba Giegy Corporation, Summit, New Jersey.

References

1. Ts'ao C, Rossi EC, Lestina FC. Abnormalities in platelet function and morphology in a case of thrombocythemia. Arch Pathol Lab Med 1977;101:526-533.
2. Thatcher CD, Keith JC. Pregnancy-induced hypertension: Development of a model in the pregnant sheep. Am J Obstet Gynecol 1986;155:201-207.
3. Keith JC, Thatcher CD, Schaub RG. Beneficial effects of U63,557A, a thromboxane synthetase inhibitor, in an ovine model of Pregnancy-induced Hypertension. Am J Obstet Gynecol 1987;157:199-203.
4. Miller KW, Keith JC. Erythrocyte morphology and serum chemistry in ovine pregnancy-induced hypertension treated with thromboxane synthetase inhibitors. Am J Obstet Gynecol in press.
5. Born GVR. Aggregation of blood platelets and its reversal. Nature 1962;194:927-929.
6. White JG. The ultrastructure and regulatory mechanisms of blood platelets. In: Blood platelet function and medicinal chemistry. A Lasslo (ed) Elsevier Biomedical Publishers, New York. 1984. pp.15-59.
7. Myers A, Shio H, Nicosia S, Ramwell PW. Platelet Eicosanoid receptors. In: The Platelets: Physiology and Pharmacology. Longenecker, G.L. (ed) Academic Press, Orlando, Florida. 1985. p.201.
8. Howie PW. The haemostatic mechanisms of preeclampsia. Clin Obstet Gynaecol 1977;4:595-609.
9. Mehta P, Mehta J. Platelet function studies in coronary heart

disease. VIII. Decreased platelet sensitivity to prostacyclin in patients with myocardial ischaemia. Thrombos Res 1980;18:273-277.

10. Wu KK, LeBreton GC, Jai HH, Chen YC. Abnormal platelet response to thromboxane A_2. J Clin Invest 1981;67:1801-1804.
11. Lembele-Duchesne MJ, Lahlou AL, Thaler-Dao H, Crastes de Paulet A. Inhibition of Platelet aggregation by human placenta. Thromb Haemo 1985;54:431-437.
12. Zawilska KM, Born GVR, Begent NA. Effect of ADP-utilizing enzymes on the arterial bleeding time in rats and rabbits. Br J Haematol 1982;50:317-325.
13. Mais DE, Saussy DL, Chaikhouni A, Kochel PJ, Knapp DR, Hamanaka N, Halushka PV. Pharmacologic characterization of human and canine thromboxane A_2 prostaglandin H_2 receptors in platelets and blood vessels: evidence for different receptors. J Pharmacol Exp Ther 1985;233:418-424.
14. Hirsh J, Castelan DJ, Loder PB. Spontaneous bruising associated with a defect in the interaction of platelets and connective tissue. Lancet 1967;2:18-21.
15. Caen J, LeGrand, Y. Abnormalities in the platelet collagen reaction. Ann NY Acad Sci 1972;201:194-204.
16. Mielke CH. Qualitative platelet abnormality due to absent collagen-induced aggregation. Thromb Haemost (Stuttgert) 1976;36:283-288.
17. Tiffany ML, Penner JA. Effect of complement on collagen-induced platelet aggregation. J Lab Clin Med 1980;96:796-802.
18. Samuels P, Main EK, Tomaski A, Mennuti T, Gabbe SG, Clines DB. Abnormalities in platelet antiglobulin tests in preeclamptic

mothers and their neonates. Am J Obstet Gynecol 1987;157:109-113.

19. Rekonen F, Rosi V, Ammala M, Viinikka L, Ylikorkala O. Platelet function and coagulation in normal and preeclamptic pregnancy. Thromb Res 1986;43:553-560.

20. Tiffany ML. Technical considerations for platelet aggregation and related problems. Crit Rev Clin Lab Sci 1983;119:27-69.

21. Norok Z, Weitz J, Owen J, Auerback M, Nossel HL. Fibrinogen proteolysis and platelet alpha-granule release in preeclampsia/ eclampsia. Blood 1984;63:525-531.

22. FitzGerald GA, Brash AR, Oates JA, Pedersen AK. Endogenous prostacyclin biosynthesis and platelet function during selective inhibition of thromboxane synthetase in man. J Clin Invest 1983; 71:1336-1343.

23. Wallenburg HCS, Rotmans N. Enhanced reactivity of the platelet thromboxane pathway in normotensive and hypertensive pregnancies with insufficient fetal growth. Am J Obstet 1982;144:523-528.

24. Redman CWG, Bonner J, Beilin LJ. Early platelet consumption in pre-eclampsia. Br Med J 1978;1:467-469.

TABLE I. Total platelet counts from 9 nontreated, CGS13080, and CGS12970 treated, initially normotensive, gravid ewes in which pregnancy toxemia was induced [Mean ± SE].

	BASELINE	TOXEMIC	POST Rx
Total Count	571,828 ± 25,021 [n=29]	580,722 ± 45,627 [n=9]	525,600 ± 83,894[a] [n=5]

a=Toxemia vs Treatment; $p \leq 0.01$

TABLE II. ADP-induced aggregation curve characteristics from 9 nontreated, CGS13080, and CGS12970 treated, initially normotensive, gravid ewes in which pregnancy toxemia was induced [Mean ± SE].

	BASELINE	TOXEMIC	POST Rx
% Aggregation	65.29 ± 2.63	72.13 ± 2.47	72.40 ± 1.17
Lag Time	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Primary Slope	2.99 ± 0.14	2.52 ± 0.21[a]	3.02 ± 0.41[b]
Secondary Slope	0.98 ± 0.04	0.93 ± 0.08	1.03 ± 0.25
	[n=28]	[n=8]	[n=5]

a=Baseline vs Toxemia; p±0.01

b=Toxemia vs Treatment; p±0.05

TABLE III. Collagen-induced aggregation curve characteristics from 9 nontreated, CGS13080, and CGS12970 treated, initially normotensive, gravid ewes in which pregnancy toxemia was induced [Mean ± SE].

	BASELINE	TOXEMIC	POST Rx
% Aggregation	57.81 ± 6.87	38.63 ± 14.62[b]	63.00 ± 16.16[d]
Lag Time	166.14 ± 19.15	227.25 ± 33.36[a]	176.40 ± 39.69[d]
Primary Slope	1.54 ± 0.22	0.74 ± 0.32[a]	1.27 ± 0.35[d]
Secondary Slope	0.53 ± 0.07	0.28 ± 0.11[c]	0.53 ± 0.14[d]
	[n=28]	[n=8]	[n=5]

a=Baseline vs Toxemia; p≤0.01

b=Baseline vs Toxemia; p≤0.05

c=Baseline vs Toxemia; p≤0.005

d=Toxemia vs Treatment; p≤0.005

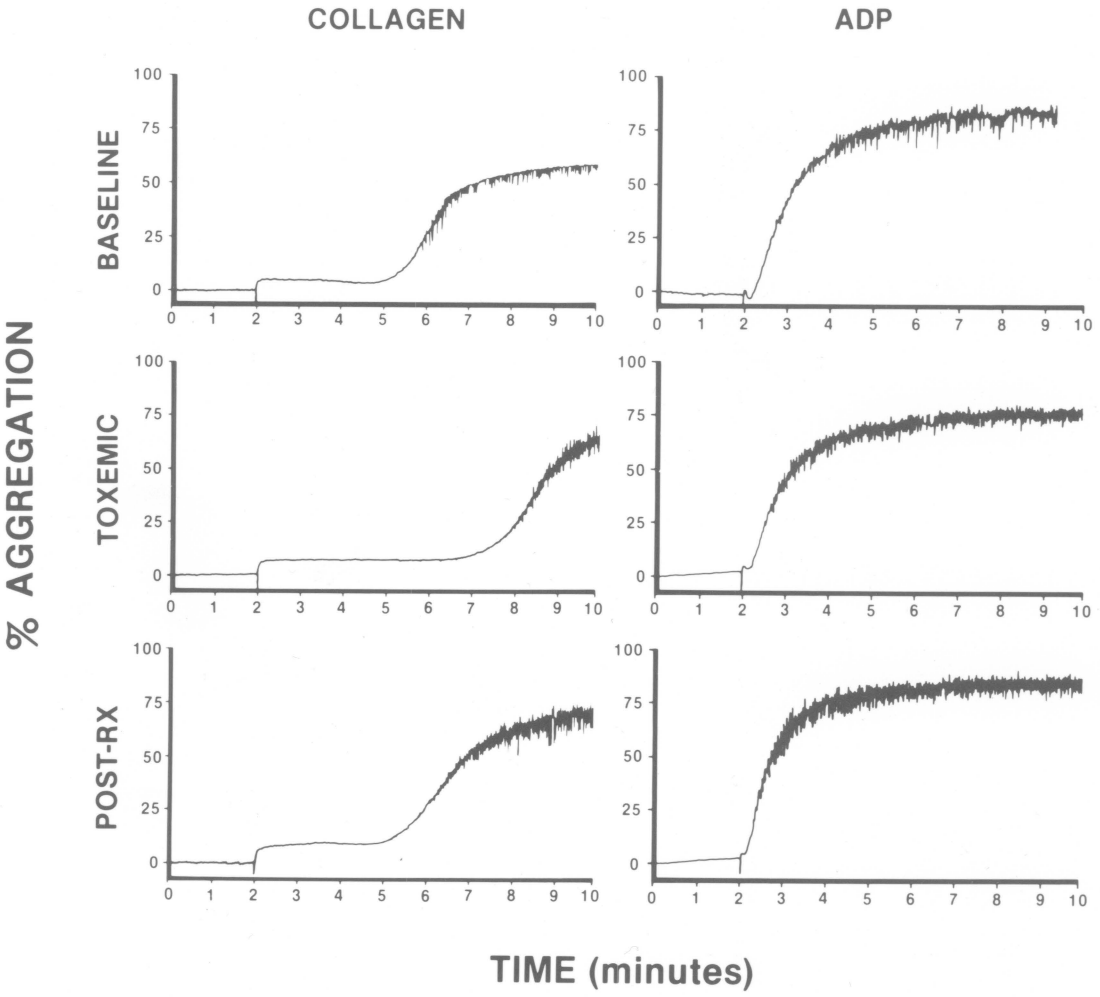


Figure 1. Representative collagen and ADP-induced aggregation curves from nontreated, CGS13080 treated, or CGS12970 treated multiparous gravid ewes.

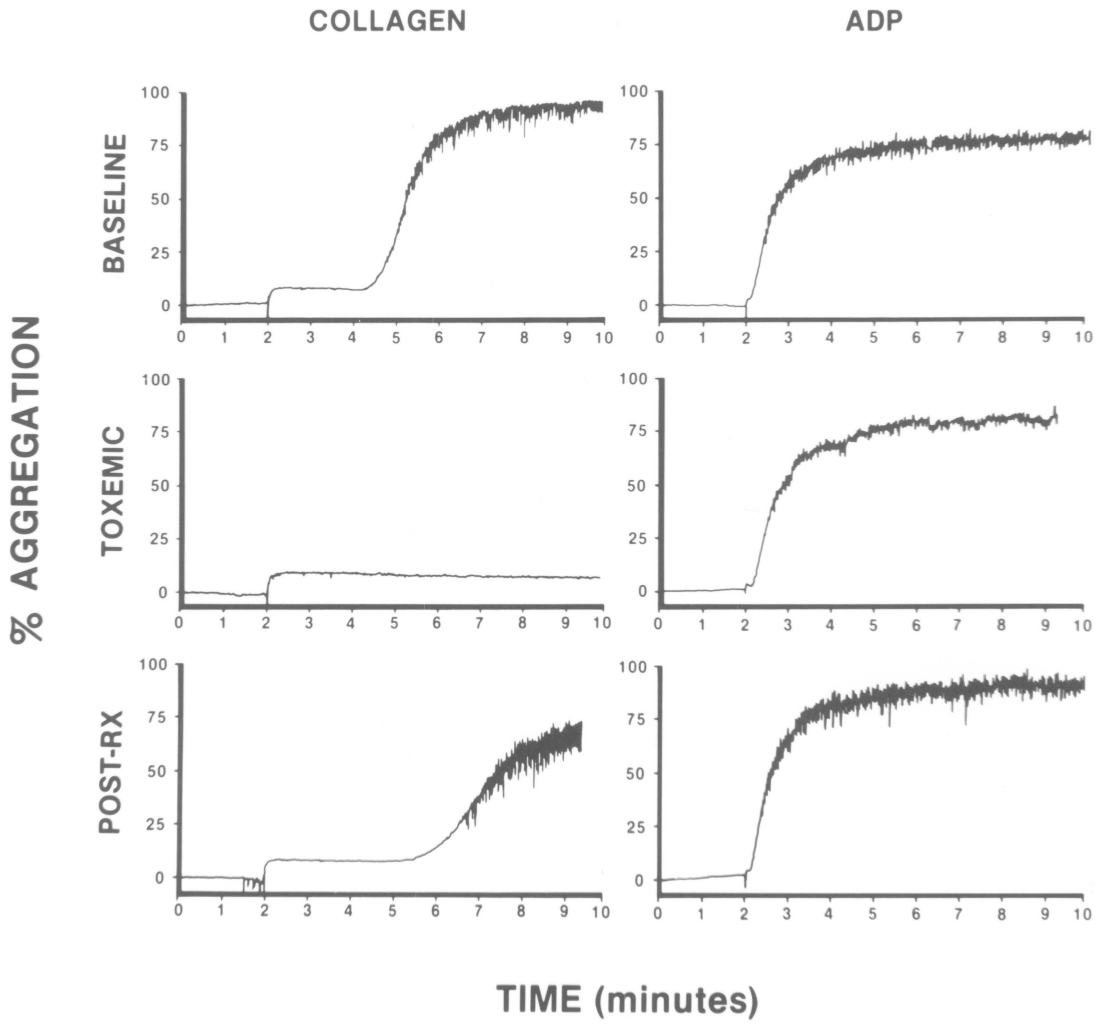


Figure 2. Representative collagen and ADP-induced aggregation curves from those nontreated, CGS13080 treated, or CGS12970 treated multiparous gravid ewes in which platelets failed to aggregate when challenged with collagen.

Figure III: Presented are representative transmission electron micrographs of thrombocytes taken from unaggregated PRP of multiparous gravid ewes.

A. Many alpha granules (ag), dense granules (dg), and microtubules (mt) are evident in this unaggregated platelet during the baseline period. Original magnification 36,000x.

B. Unaggregated platelet during OPT. No alterations in alpha granules, dense granules, or microtubules are apparent. Original magnification 36,000x.

C. Unaggregated platelets did not appear to be affected by thromboxane synthetase inhibition. Original magnification 10,000x.

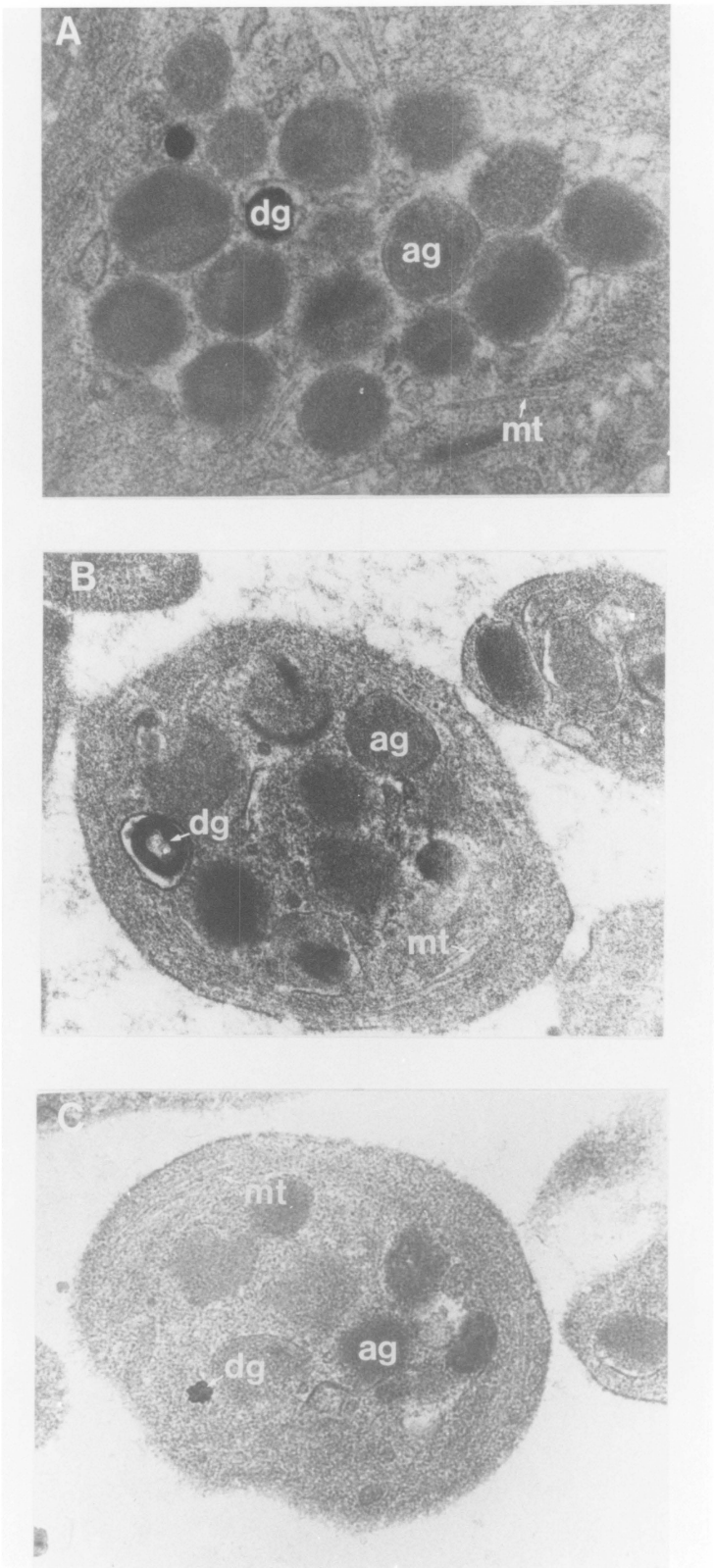

A
dg
ag
mt
B
ag
dg
mt
C
mt
dg
ag

Figure IV: Presented are representative transmission electron micrographs of thrombocytes taken from PRP of multiparous gravid ewes into which collagen was added at a final concentration of 10μg/ml.

A. Platelet aggregate during baseline. Note the centralization of microtubules (mt) and cytosolic granules (cg), as well as the swollen open canalicular system (ocs). Original magnification 14,000x.

B. Giant vacuoles (gv) were observed during OPT. Original magnification 36,000x.

C. Platelet aggregate after thromboxane synthetase inhibition. No abnormalities were detected, and microtubule formation seemed to be more pronounced than in either baseline or toxemia. Original magnification 48,000x.

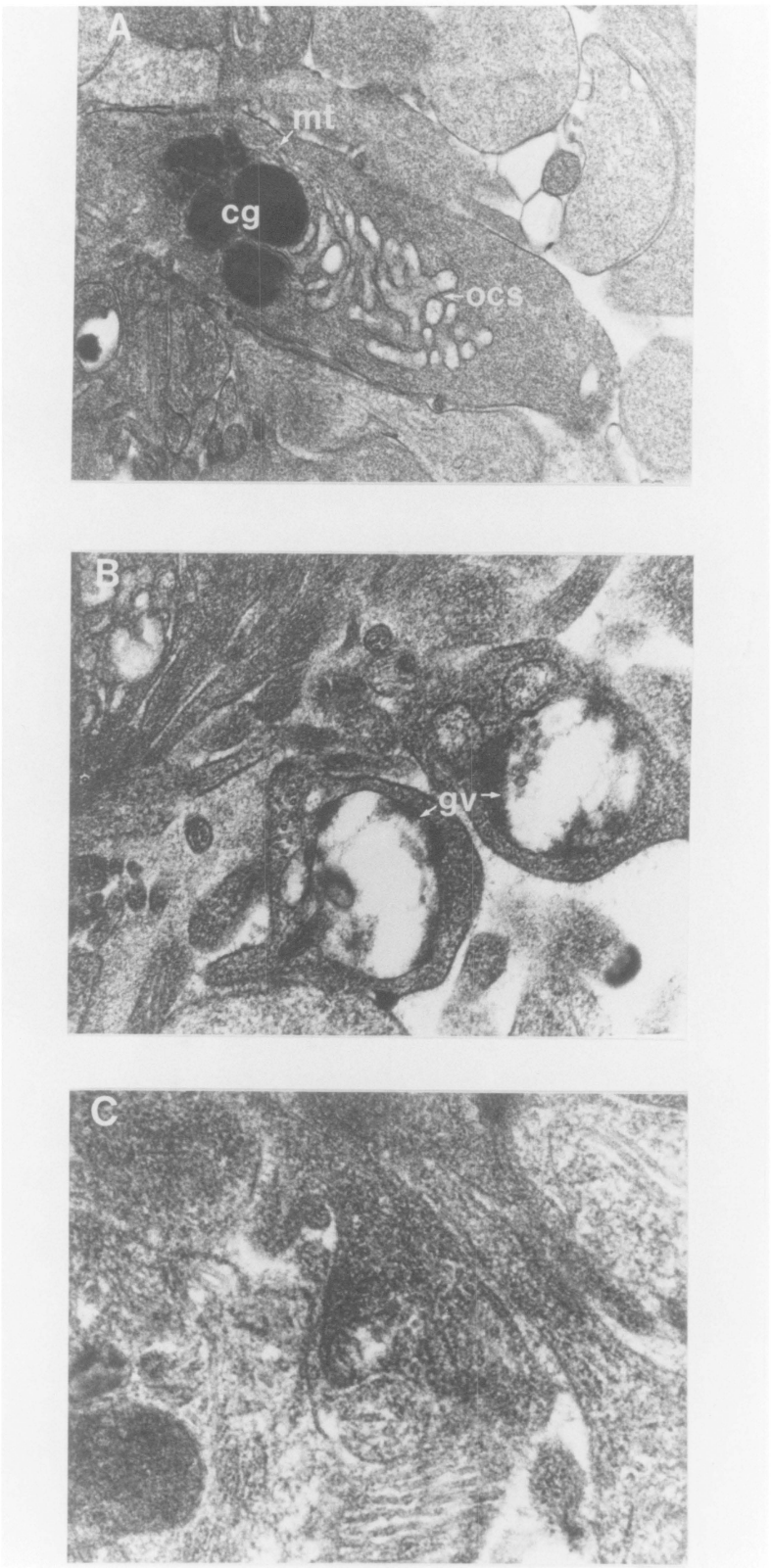
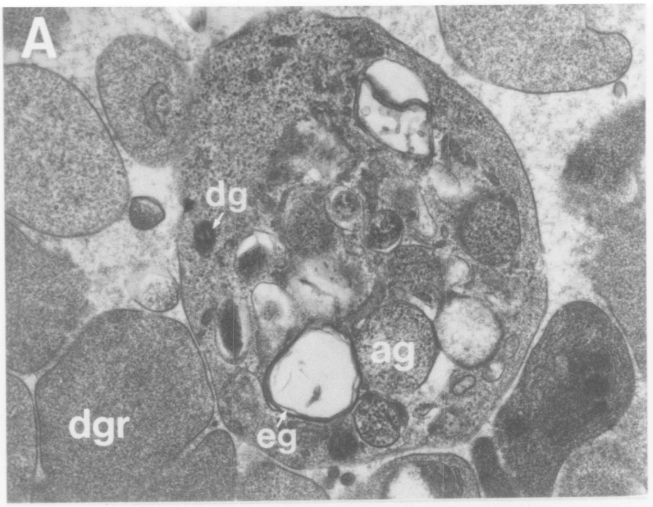

A
mt
cg
ocs
B
gv
C

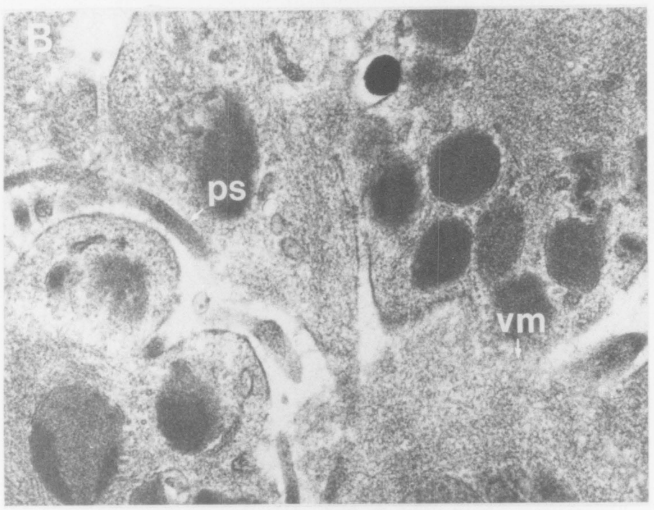
Figure V: Presented are representative transmission electron micrographs of thrombocytes taken from PRP of multiparous gravid ewes into which ADP was added at a final concentration of 8.5μg/ml.

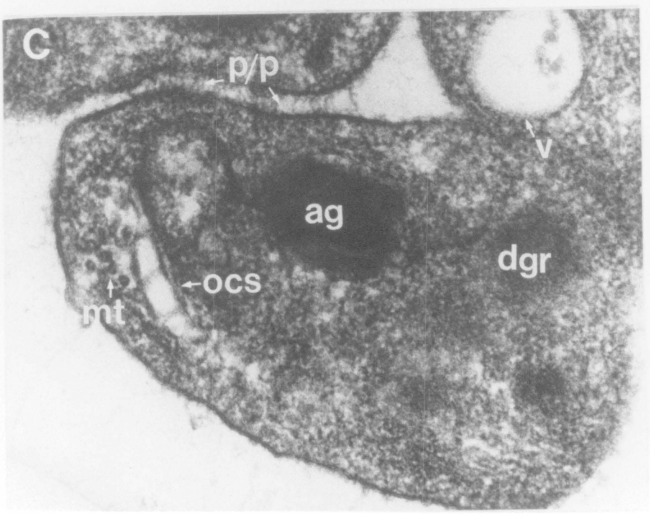
A. Platelet aggregate during baseline. Alpha (ag) and dense (dg) granules are seen in various stages of release. Empty granules (eg) and degranulated platelets (dgr) are also observed. Original magnification 28,000x.

B. Platelet aggregate during OPT, demonstrating pseudopod formation (ps) and viscous metamorphosis (vm). Original magnification 29,000x.

C. Platelet/platelet interaction (p/p) is observed in this aggregate after thromboxane synthetase inhibition. Both degranulating granules (dgr), and alpha granules (ag) are noticable. Microtubules (mt) and the open canacicullar system (ocs) are also evident. Original magnification 58,000x.


A
dg
ag
dgr
eg


B
ps
vm


C
p/p
v
ag
dgr
ocs
mt

V. MANUSCRIPT SUBMITTED TO AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY ENTITLED "ERYTHROCYTE MORPHOLOGY AND SERUM CHEMISTRY IN OVINE PREGNANCY-INDUCED HYPERTENSION TREATED WITH THROMBOXANE SYNTHETASE INHIBITORS."

Erythrocyte morphology and serum chemistry in ovine pregnancy-induced hypertension treated with thromboxane synthetase inhibitors

Kevin Wm. Miller, B.S. and James C. Keith, Jr., D.V.M., Ph.D.

Blacksburg, Virginia

From the Department of Veterinary Biosciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

This work was supported by the Ciba Giegy Corporation, Summit, New Jersey.

Reprint requests: Kevin Wm. Miller, Department of Physiology and Biophysics, 224 Basic Sciences Building, Georgetown University Medical Center, Georgetown University, Washington, D.C. 20057.

Short Title: CGS13080 and CGS12970 in Ovine Pregnancy Toxemia.

Erythrocyte morphology and serum chemistry in ovine pregnancy-induced hypertension treated with thromboxane synthetase inhibitors.

Kevin Wm. Miller, B.S. and James C. Keith, Jr., D.V.M., Ph.D.

Erythrocyte morphology and serum chemistries were studied in nine multiparous gravid ewes during experimentally induced ovine pregnancy-induced hypertension and subsequent administration of the thromboxane synthetase inhibitors CGS13080 or CGS12970. During the hypertensive period mean arterial blood pressure, plasma thromboxane B_2 levels, and serum chemistries and electrolytes were significantly altered ($p \leq 0.005$). Parameters returned to baseline values or were improved after drug administration.

Erythrocyte morphology did not change significantly with the onset of the syndrome. Echinocytosis was present during baseline measurement and persisted throughout hypertension. However, after thromboxane synthetase inhibition, percentages of discocytes increased ($p \leq 0.005$) with the same frequency that echinocyte numbers decreased ($p \leq 0.05$). Shistocytes were present throughout the study, and changes in their numbers were not detected.

Serum phosphorus, BUN, GGT, and bicarbonate rose significantly during hypertension ($p \leq 0.005$), and returned to normal levels after drug treatment. We speculate that CGS13080 or CGS12970, by decreasing thromboxane levels and blood pressure, promoted the normalization of erythrocyte membranes.

Key Words: Erythrocyte Ultrastructure, Thromboxane, Hypertension, Preeclampsia, Ovine Pregnancy Toxemia

Preeclampsia (Pregnancy-induced hypertension or PIH), a hypertensive disorder of pregnancy which usually occurs in late gestation, is a major cause of fetal and maternal mortality and morbidity.[1] Complications of PIH may arise which include hemolysis, elevated liver enzymes, and lowered platelet counts.[2] Erythrocyte shape abnormalities and hemolysis may contribute to the fetal hypoxia and maternal platelet aggregation seen in PIH. Erythrocyte morphologic changes which accompany human preeclampsia-eclampsia have been characterized.[3] No attempt, however, has been made to characterize the response of erythrocytes to preeclampsia or its treatment in an animal model of this disease. A pregnancy-induced hypertensive disorder similar to that seen in humans can be induced in gravid ewes.[4,5] The purpose of this investigation was to characterize erythrocyte morphology in an ovine model of pregnancy-induced hypertension in normal pregnancy, during hypertension, and 12hrs after the administration of thromboxane synthetase inhibitors. Blood pressure, serum chemistry, and plasma thromboxane B_2 levels were also monitored to assess the circulatory environment of these ewes during the experimental period.

Materials and Methods

Thirteen multiparous gravid ewes were obtained from a commercial breeder. Mixed hay, grain, water, and mineralized salt were offered ad lib. Ewes were taken into the laboratory in pairs and placed in metabolism crates around the 120th day of gestation. Each ewe was allowed an acclimation period of approximately 3-7 days. The laboratory environment was kept at constant temperature and photoperiod. During gestational days 127 through 134, maternal blood chemistries and electrolytes were determined, indirect measurements of maternal blood pressure were obtained, and plasma thromboxane B_2 levels were measured to establish baseline values for each ewe. Erythrocyte samples were gathered for ultrastructural examination.

On the 134th day of gestation, grain and hay were removed to induce ovine pregnancy-induced hypertension (ovine pregnancy toxemia or OPT). Water and salt were provided ad lib throughout the fast. Fasting periods varied for each ewe from 12 to 90hrs (the majority fasted for 72hrs, days 134-137).

Of thirteen animals fasted, nine developed OPT. An animal was considered to have OPT in this study when: 1.) a minimum rise in mean arterial pressure of 11mmHg was demonstrated; 2.) plasma chemistries indicated hypoglycemia and renal dysfunction (proteinuria and ketonuria were also noted); and 3.) neurologic disturbances (ie: depression or muscle tremors) were observed.

At the onset of the disease, the ewes were divided into three sets of three animals each. Each group was randomly assigned to one of three treatment blocks: control - no treatment, CGS13080 treatment,

or CGS12970 treatment (thromboxane synthetase inhibitors were obtained from Ciba Geigy Corporation, Summit, N.J.). CGS13080 [N (1-carboxy-heptyl)imidazole, imidazo 1,5-a pyridine-5-hexanoic acid] was administered at the rate of 0.1mg/kg/hr IV infusion for 6hr. CGS12970 [3-methyl-2-(3-pyridyl)-1-idolectanoic acid] was administered once at a rate of 1mg/kg IV bolus. Post treatment sampling was conducted 12hrs after drug administration. All ewes were observed for up to 48hrs post drug administration.

Maternal Blood Pressure. While the ewes stood quietly in the metabolism crates, thirty minute recordings of systolic, mean, and diastolic maternal blood pressures were obtained at 12hr intervals throughout the experiment (gestational day 127 until approximately day 140). A dinamap pressure monitoring device (Criticon) with trend recorder 950A printer (Applied Medical Research) was used to record pressures at 4min intervals during each session. Sphygmomanometer blood pressure cuffs (Criticon) were placed on the left thoracic limb, over the anterior cephalic artery. Cuff size was determined by the formula: limb circumference X (0.4).[6]

Hematologic Chemistry and Electrolyte Analysis. 10ml whole blood without anticoagulant was obtained once a day by clean jugular venipuncture. After the sample had clotted, the blood was centrifuged at 1,500rpm for 10min at room temperature. Serum was submitted to the Clinical Pathology Laboratory at the Virginia-Maryland Regional College of Veterinary Medicine for chemical and electrolyte analysis.

Thromboxane B_2 Determination. 9ml whole blood was obtained once a day by clean jugular venipuncture and mixed thoroughly with 1ml of 3.8%

sodium citrate solution containing indomethacin (50μg/ml). The blood was immediately centrifuged at 1,500rpm for 10min at room temperature. Plasma was decanted from the cellular fraction, and immediately frozen at -70°C until analyzed. Radioimmunoassays were performed on 300μl aliquots of the unextracted plasma for thromboxane B_2, the stable metabolite of thromboxane A_2, by commercially available (^3H) RIA kits (Amersham) as has been previously reported.[5]

Erythrocyte Ultrastructure. 1ml of blood anticoagulated with 3.2% sodium citrate (9 parts blood : 1 part citrate) was centrifuged at 1,500rpm for 10min at room temperature. Plasma was decanted, and the erythrocytes were fixed in modified McDowell-Trumps fixative for at least 2hr. The cells were then washed 2x in Tyrode's buffer (pH 7.4). The cells were then post fixed in 1% osmium tetroxide for 1hr, and again washed 2x in Tyrode's buffer. Dehydration in a graded ethanol series followed. When in absolute ethanol, the cells were placed on a millipore filter (VMR) and critical point dried in a Ladd critical point dryer. The filters were mounted on stubs and coated with gold in a sputter coater (SPI).

For each sample, 100 randomly chosen erythrocytes from vicinal fields were examined with a Jeol Model 35C scanning electron microscope. Cells were categorized as discocytes, echinocytes, or shistocytes according to procedures described for human cells.

Statistical Analysis. Each ewe served as its own control and statistical analysis was performed by paired t test comparisons. Probability of ≤ 0.05 was considered to be significant. All results are reported as Mean ± SE.

Results

Maternal Blood Pressure. Baseline oscillometric arterial pressures from the nine animals were: 131 ± 1.11mmHg (systolic), 90 ± 1.07mmHg (mean arterial), and 66 ± 1.03mmHg (diastolic) [n=356]. Blood pressure rose significantly ($p \leq 0.005$) during the toxemic period to 150 ± 1.24mmHg (systolic), 106 ± 1.37mmHg (mean arterial), and 80 ± 1.48mmHg (diastolic) [n=216]. 12hr after the administration of CGS13080 or CGS12970, systolic (144 ± 2.70mmHg), mean arterial (97 ± 3.15mmHg), and diastolic (74 ± 3.12mmHg) blood pressures fell to levels that were significantly different from those of the hypertensive period ($p \leq 0.005$)[n=60], but not of baseline.

Hematologic Chemistry and Electrolyte Analysis. Hematologic values are given in table I. The marked rise in plasma phosphorus levels during hypertension may lend evidence to the occurrence of microangiopathic hemolysis or renal dysfunction. Low albumin and high BUN levels, indicators of hepatic and renal dysfunction, accompanied this rise, as did a nonsignificant rise in bilirubin. Elevated liver enzymes and hemoglobinuria, however, were not demonstrated. Changes in serum anion gap were consistent with elevations in the urinary ketone levels during the progression of OPT. Despite many statistically significant changes in biochemical values during hypertension and after treatment, only glucose and phosphorus levels deviated from the normal range reported for sheep.

Thromboxane B_2 Determination. Plasma levels of thromboxane B_2 rose significantly from 57.69 ± 3.1pg/ml [n=21] during baseline to 99.87 ± 8.28pg/ml during hypertension [n=15] ($P \leq 0.005$). After CGS13080 or

CGS12970 administration, thromboxane B_2 levels returned to 42.57 ± 5.21 pg/ml, a level significantly lower than either toxemic or baseline values ($P \leq 0.005$). The least detectable concentration of thromboxane B_2 was 5pg/tube. Interassay variation was ≤ 10%, and intraassay variation was ≤ 5%. Fifty percent binding of the standard curves for thromboxane B_2 was 27pg. Recovery rates for added thromboxane B_2 ranged from 91-134%.

Erythrocyte Ultrastructure. Percentages of normal to abnormal erythrocytes are given in Table II. Figure I provides high power scanning electron microscopic views of representative cell types: discocytes, echinocytes, and shistocytes. Figure II includes scanning electron micrographs of representative fields during baseline pregnancy, OPT, and after thromboxane synthetase administration. Both CGS13080 and CGS12970 affected the percentages of abnormal to normal cells by the same amount. Thus, one drug was not observed to have more of a therapeutic effect than the other.

Comment

Sharp and sustained rises in blood pressure and plasma thromboxane B_2 levels during OPT are central to the findings of this study. The results of these changes add additional stress and distorting forces on the plasma membranes of circulating erythrocytes. There was a 15% reduction in the echinocyte population and a corresponding 15% elevation in the discocyte population after administration of the thromboxane synthetase inhibitors CGS13080 and CGS12970. This may be a combined result of echinocyte breakdown during the later stages of hypertension and membrane stabilization after drug administration.

Echinocyte membranes are rigid,[7-9] and are characterized by projections distributed over their surface.[10] Echinocytes demonstrate significantly impaired flow patterns *in vitro*.[8] Rigid sickle cells stay in the microvasculature much longer than cells with normal deformability and reduce blood flow to a greater extent than would be expected based on their percentage to the total RBC population.[11] If the circulatory behavior of echinocytes and shistocytes is similar to that of sickle cells, they could add significantly to the increased whole blood viscosity and decreased blood flow that is characteristic of preeclampsia. Vasospastic episodes in isolated resistance vessels of preeclamptic patients[12] promote microangiopathic hemolysis.

The degree of hypertension and thromboxane B_2 excess demonstrated in this study created a harsh environment for the circulating erythrocytes which could have subjected them to shear stress. Erythrocytes subjected to such stress release ADP which aggregates platelets in citrated PRP *in vitro*.[13] Although the significance of erythrocyte ADP

release is still debated, elevated activity of the platelet TxA_2 metabolic pathway[14] and increased rates of platelet activation[1] and consumption[15] occur in human pregnancy-induced hypertension.

Extensive intervillous thrombosis is a danger in preeclamptics with hematologic complication, as it may lead to placental lesions and separation. Disseminated intravascular coagulation, initiated by premature placental separation, may lead to a progressive consumptive coagulopathy. Fibrin microthrombi, together with systemic vasospasm, lead to further hemorrhage and necrosis.[16]

Changes in erythrocyte populations may also be due to the enhanced ability of the reticuloendothelial cells to clear abnormal erythrocytes from the circulation after drug treatment. Normalized ratios of circulating prostanoids after thromboxane synthetase inhibition may also help to stabilize erythrocyte plasma membranes so that echinocyte formation does not occur.

The majority of phosphorus found in ovine blood is contained within the erythrocyte, and hemolysis would increase serum phosphorus levels. The hyperphosphatemia demonstrated in this investigation, however, is more likely due to factors other than hemolysis.

Parathyroid hormone increases during hypocalcemia and results in a decrease in plasma phosphorus levels. In this study, however, hypocalcemia was not observed. There is also evidence that a hormone independent relationship exists between calcium and phosphorus. It is currently debated whether calcium concentrations affect phosphorus reabsorption by the kidney. This relationship may be especially important in preeclampsia, where hyperphosphotemia can occur with decreased glomerular

filtration rate (GFR). The hyperphosphotemia that occurred here most likely resulted from decreased GFR associated with azotemia. BUN levels rose significantly with the onset of OPT and fell significantly after thromboxane synthetase inhibitor administration. Metabolic acidosis further complicates phosphorus metabolism. GGT and bilirubin levels did not rise significantly during hypertension, however, so we assume that liver function was maintained.

Echinocytosis found during hypertension in this ovine study is consistent with that found in human literature.[3] Thromboxane synthetase inhibition with CGS13080 and CGS12970 clearly normalized both the erythrocyte and its circulating environment. Further study into the fatty acid composition of both the cell membranes and the blood plasma during pregnancy-induced hypertension in this ovine model is warranted. As echinocytosis was also seen during baseline measurements, more study is also needed of pregnant ewes in a pastoral setting to see what stress, if any, laboratory conditions alone contribute to these morphologic changes.

Acknowledgements. The technical assistance of

in electron microscopy, and in blood processing was greatly appreciated.

References

1. Ramon de Alvarez, R.: Current Obstetric and Gynecological Diagnostics and Treatment. Lange Medical Publications, Los Altos, California. 1984. p.763.
2. Weinstein, L.: Syndrome of hemolysis, elevated liver enzymes, and low platelet count: a severe consequence of hypertension in pregnancy. Am. J. Obstet. Gynecol. 1982;142:159-167.
3. Cunningham, F.G., Lowe, T., Guss, S., and Mason, R.: Erythrocyte morphology in women with severe preeclampsia and eclampsia. Am. J. Obstet. Gynecol. 1985;153:358-363.
4. Thatcher, C.D., and Keith, J.C.: Pregnancy-induced hypertension: Development of a model in the pregnant sheep. Am. J. Obstet. Gynecol. 1986;155:201-207.
5. Keith, J.C., Thatcher, C.D., and Schaub, R.G.: Beneficial effects of U-63,557A, a thromboxane synthetase inhibitor, in an ovine model of Pregnancy-induced Hypertension. Am. J. Obstet. Gynecol. 1987;157:199-203.
6. Hassler, C.R., Lutz, G.A., Linebaugh, R., and Cummings, K.D.: Identification and evaluation of non-invasive blood-pressure measuring techniques. Toxicol. Appl. Pharmacol. 1979;47:193-201.
7. Reinhart, W.H., and Chien, S.: Red cell rheology in stomatocyte-echinocyte transformation: Roles of cell geometry and cell shape. Blood. 1986;67:1110-1118.
8. Weed, R.I., LaCelle, P.I., and Merrill, E.W.: Metabolic dependence of red cell deformability. J. Clin. Invest. 1969;48:795-809.

9. Bessis, M.: Red Cell Shapes. An illustrated classification and its rationale. In: Red Cell Shape. Bessis, M., Weed, R.I., and Leblond, P.F. (eds) Springer-Verlag, New York. 1973. pp.1-26.

10. Chien, S.: Rheology of sickle cells and the microcirculation. N. Engl. J. Med. 1984;311:1567-1569.

11. Bauer, T.W., Moore, G.W., and Hutchins, G.M.: Morphologic evidence for coronary artery spasm in eclampsia. Circulation 1982;65:255-259.

12. Born, G.V.R.: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962;194:927-929.

13. Aursnes, I., Gjesdal, K., and Abildgaard, U.: Platelet aggregation induced by ADP from unsheared erythrocytes at physiological Ca++ concentrations. Brit. J. Haematol. 1981;47:149-152.

14. Gorman, R.G.: Modulation of human platelet function by prostacyclin and thromboxane A2. Fed. Proc. 1979;38:83-88.

15. Neufeld, E.J., and Majerus, P.W.: Arachidonate release and phosphatidic acid turnover in stimulated human platelets. J. Biol. Chem. 1983;258:2461-2467.

16. FitzGerald, G.A., Brash, A.R., Oates, J.A., and Pedersen, A.K.: Endogenous prostacyclin biosynthesis and platelet function during selective inhibition of thromboxane synthetase in man. J. Clin. Invest. 1983;71:1336-1343.

TABLE I. Blood serum chemistry and electrolyte values from 9 non-treated, CGS13080, or CGS12970 treated, initially normotensive, gravid ewes in which pregnancy toxemia was induced [Mean ± SE].

	BASELINE	TOXEMIC	POST Rx
Albumin	3.55 ± 0.05	3.42 ± 0.07[a]	3.45 ± 0.23
GGT	38.21 ± 1.32	33.22 ± 1.66[a]	35.50 ± 3.60[d]
Bilirubin	0.39 ± 0.04	0.47 ± 0.08	0.41 ± 0.12
Creatinine	1.58 ± 0.06	1.67 ± 0.11	1.36 ± 0.17[de]
Glucose	45.10 ± 3.18	27.46 ± 2.28[a]	38.52 ± 4.73[de]
Total Protein	6.87 ± 0.09	6.99 ± 0.15	6.93 ± 0.23
BUN	17.75 ± 0.89	22.53 ± 3.18[a]	17.90 ± 1.84[cf]
Calcium	9.00 ± 0.11	8.62 ± 0.12	8.88 ± 0.47
Magnesium	2.24 ± 0.06	2.52 ± 0.68	1.83 ± 0.10[bd]
Phosphorus	5.80 ± 0.28	10.06 ± 1.34[a]	6.60 ± 0.45[de]
Bicarbonate	22.35 ± 0.64	17.69 ± 0.67[a]	18.50 ± 1.25[d]
Chloride	108.72 ± 0.82	108.80 ± 1.44	108.52 ± 1.74
Potassium	4.35 ± 0.08	3.80 ± 0.10	4.15 ± 0.18[b]
Sodium	148.14 ± 1.02	147.78 ± 1.24	144.38 ± 2.73[ce]
Anion Gap	21.83 ± 0.53	25.39 ± 0.73[a]	21.45 ± 1.69[d]
	[n = 30]	[n = 9]	[n=6]

a=Baseline vs Toxemia; p≤0.005 d=Toxemia vs Treatment; p≤0.005

b=Toxemia vs Treatment; p≤0.05 e=Baseline vs Treatment; p≤0.05

c=Toxemia vs Treatment; p≤0.01 f=Baseline vs Treatment; p≤0.01

TABLE II. Erythrocyte morphology from 9 nontreated, CGS13080, and CGS12970 treated, initially normotensive, gravid ewes in which pregnancy toxemia was induced [Mean ± SE].

	BASELINE	TOXEMIC	POST Rx
% Discocytes	38 ± 2	35 ± 5	50 ± 5[a]
% Echinocytes	35 ± 3	36 ± 4	21 ± 1[b]
% Shistocytes	27 ± 1	29 ± 4	29 ± 6
	[n = 1900]	[n = 600]	[n = 600]

a=Toxemia vs Treatment; $p \leq 0.005$

b=Toxemia vs Treatment; $p \leq 0.05$

Figure I: Presented are representative scanning electron micrographs of erythrocytes from multiparous gravid ewes treated with CGS13080 or CGS12970. These cells demonstrate the cell types observed in this investigation.

A. Discocyte during the baseline period, original magnification 3,600x.

B. Shistocyte after treatment with a thromboxane synthetase inhibitor, original magnification 3,000x.

C. Echinocyte during hypertension, original magnification 6,600x.

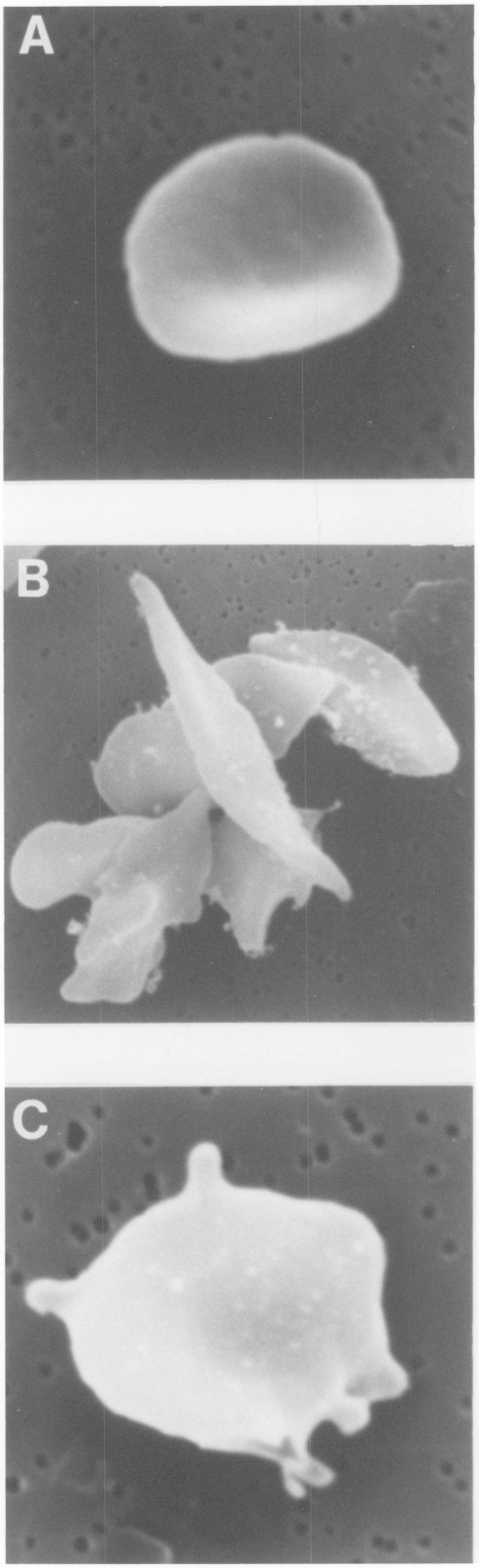
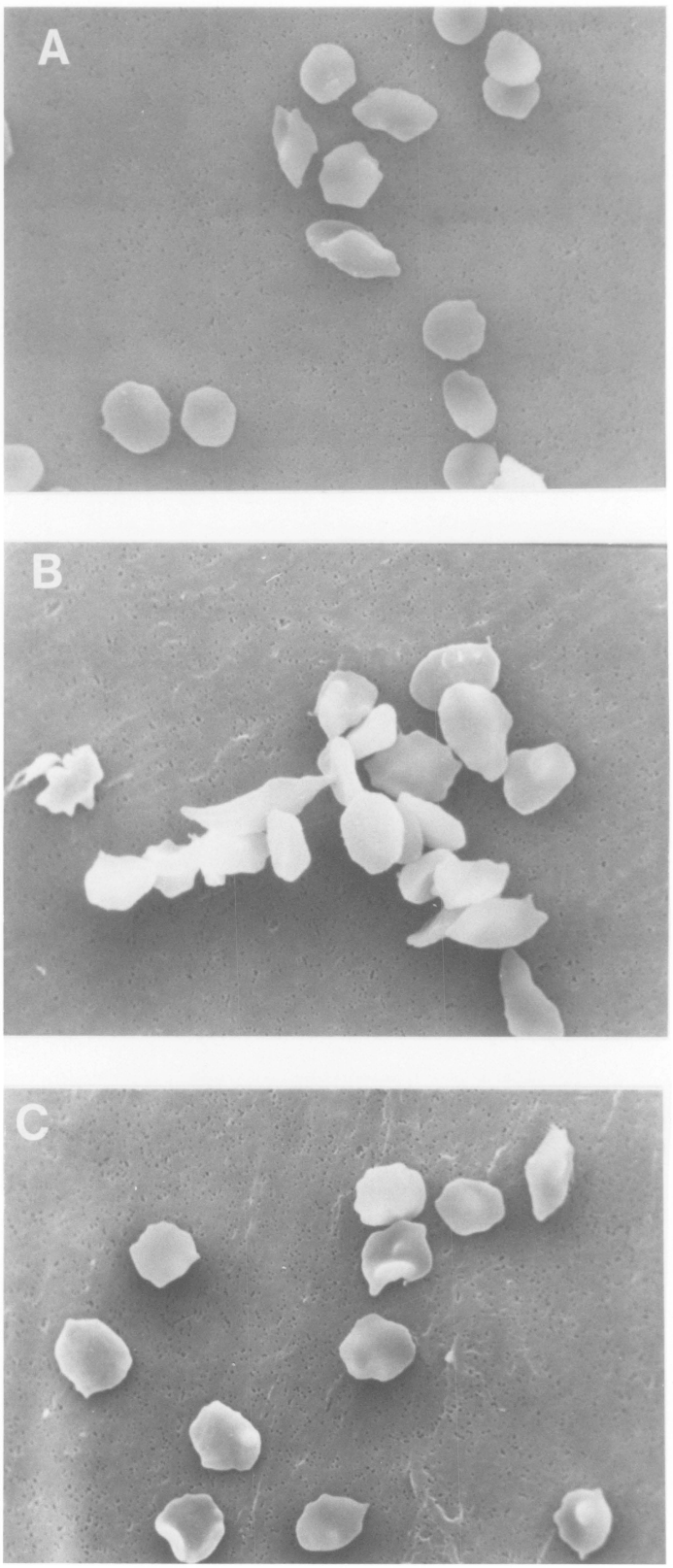

A
B
C

Figure II: Presented are representative scanning electron micrographs of erythrocyte fields from multiparous gravid ewes treated with CGS13080 or CGS12970.

A. During the baseline period, original magnification 2,600x.

B. During hypertension, original magnification 3,000x.

C. 24hrs after treatment with a thromboxane synthetase inhibitor, original magnification 2,600x.


A
B
C

VI. THESIS SUMMARY

This thesis attempted to address the potential role of abnormal prostanoid metabolism on erythrocytes and thrombocytes in an ovine model of pregnancy-induced hypertension. This was accomplished by the study of erythrocyte morphology and thrombocyte function and morphology during normal pregnancy, ovine pregnancy-induced hypertension, and thromboxane synthetase inhibitor administration. Measurements of arterial blood pressure, plasma thromboxane B_2, serum chemistries and electrolytes, total platelet count, and fibrin/fibrinogen degratory products were obtained in order to elucidate the extent of change in the circulating environment of these cells.

This work supports the general hypothesis that thromboxane synthetase inhibitors are therapeutic in the treatment of ovine pregnancy-induced hypertension. This is evidenced by clear and significant drops in systolic, mean arterial, and diastolic blood pressures, and in plasma thromboxane B_2 levels, as well as the normalization of serum chemistry and electrolyte values after CGS13080 or CGS12970 therapy.

Significant findings support the specific hypotheses of this study as well. Ultrastructural changes in ovine thrombocytes are consistent with those found in humans during pregnancy-induced hypertension. These ultrastructural changes, although subjectively noted, corresponded to objectively quantitated abnormal in vitro collagen and ADP-induced aggregation responses.

These data support the presently held theory that platelets, when exposed to an excessive stimulus such as the excessive plasma levels of thromboxane B_2 as noted in pregnancy-induced hypertension, become refractory to the stimulus. This exhaustive effect may be of particular concern when preeclampsia is complicated by hemorrhage. Treatment may best by managed by thromboxane synthetase inhibition particularly when these complications arise, as traditional antithrombotic therapy would destroy platelet function and severely compromise the patient's condition.

Alterations in the circulating environment during pregnancy did not appear to change erythrocyte membrane characteristics. Thromboxane synthetase inhibition, however, appeared to promote normal membrane morphology.

Peripheral findings such as the hyperphosphatemia demonstrated during hypertension are relevant and interesting. Additional investigation of electrolyte changes during pregnancy-induced hypertension is warranted. Further study of pregnant ewes in a pastoral setting also needs to be conducted to compare any differences in morphologic changes that may occur due to the stress caused by changes in environmental setting.

It is clear that both normal and abnormal metabolism of the oxygenated eicosanoids affect blood cellular function and morphology. In the future, work needs to be concentrated on characterizing exact mechanisms of prostanoid metabolism in blood cells. The current ovine model of pregnancy-induced hypertension can add much to comparative medicine and its hope of a treatment for human pregnancy-induced hypertension.

VII. REFERENCES

1. Ramon de Alvarez, R. Current Obstetric and Gynecologic Diagnostics and Treatment. Lange Medical Publications, Los Altos, California, 1984. p.763.

2. Lindheimer, M.D., Katz, A.I.: Pathophysiology of Preeclampsia. Ann. Rev. Med. 1981;32:273-289.

3. Mackenzie, I.Z., Machean, D.A., and Mitchell, M.D.: Prostaglandins in the human fetal circulation in mid-trimester and term pregnancy. Prostaglandins 1980;20:649-654.

4. Dadak, C., Kefalides, A., Sinzinger, H., and Weber,G.: Reduced umbilical artery prostacyclin formation in complicated pregnancies. Am. J. Obstet. Gynecol. 1982; 144:792-795.

5. Yamaguchi, M., and Mori, N.: 6-Keto Prostaglandin F1 alpha, thromboxane B_2, and 13,14-dihydro-15-keto prostaglandin F concentrations of normotensive and preeclamptic patients during pregnancy, delivery, and the post partum period. Am. J. Obstet. Gynecol. 1985;151:121-127.

6. Makila, U., Vunikka, L., and Ylikorkala, O.: Evidence that prostacyclin deficiency is a specific feature in preeclampsia. Am. J. Obstet. Gynecol. 1984;148:772-774.

7. Walsh, S.W.: Preeclampsia: An imbalance in placental prostacyclin and thromboxane production. Am. J. Obstet. Gynecol. 1985;152:335-340.

8. Makila, U.M., Vunikka, L., and Ylikorkala, O.: Increased thromboxane A_2 production but normal prostacyclin by the placenta in hypertensive pregnancies. Prostaglandins 1984;27:87-95.

9. Naden, R.P., and Rosenfeld, C.R.: Systemic and uterine responsiveness to angiotension II and norepinephrine in estrogen treated nonpregnant sheep. Am. J. Obstet. Gynecol. 1985;153:417-425.

10. Ueda, S., Fortune, V., Bull, B.S., Valenzuela, G.J., and Longo, L.D.: Estrogen effects on plasma volume, arterial blood pressure, interstitial space, plasma proteins, and blood viscosity in sheep. Am. J. Obstet. Gynecol. 1986;155:195-201.

11. Rosenfeld, C.R., and Jackson, G.M.: Estrogen-induced refractoriness to the pressor effects of infused angiotensin II. Am. J. Obstet. Gynecol. 1984;148:429-435.

12. Hou, S.: Pregnancy in women with chronic renal disease. N. Engl. J. Med. 1985;312:836-839.

13. Newsom, I.E.: Newsom's Sheep Diseases. March, H (ed) Williams and Wilkins, Baltimore. 1965. p.294.

14. Kronfeld, D.S.: Ketosis in pregnant sheep and lactating cows: A review. Aus. Vet. J. 1972;48:680-687.

15. Schulz, L.M., and Riese, R.L.: Pregnancy toxemia in the ewe. Iowa State Vet 19;45:11-13.

16. Kaufman, C.F., and Bergman, E.N.: Renal function studies in normal and toxemic pregnant sheep. Cornell Vet 1978;68:124-137.

17. Morriss, F.H., Rosenfeld, C.R., Crandell, S.S., and Adcock, E.W.: Effects of fasting on uterine blood flow and substrate uptake in sheep. J. Nutr. 1980;110:2433-2443.

18. Ferris, T.F., Herdson, P.B., Dunnill, M.S., and Lee, M.R.: Toxemia of Pregnancy in Sheep: A clinical physiological and pathological study J. Clin. Invest. 1969;48:1643-1655.

19. Keith, J.C., Thatcher, C.D., and Schaub, R.G.: Beneficial effects of U-63,557A, a thromboxane synthetase inhibitor, in an ovine model of Pregnancy-induced Hypertension. Am. J. Obstet. Gynecol. 1987;157:199-203.

20. Thatcher, C.D., and Keith, J.C.: Pregnancy-induced hypertension: Development of a model in the pregnant sheep. Am. J. Obstet. Gynecol. 1986;155:201-207.

21. Goodnight, S.H., Inkeles, S.B., Kovach, N.L., and Connor, W.E.: Reduced prostacyclin survival after fasting-induced elevation of plasma free fatty acids. Thromb. Haemos. 1985;54:418-421.

22. Bassett, J.M.: Endocrine factors in the control of nutrient utilization: Ruminants Proc. Nutr. Soc. 1978;37:273-280.

23. Lindsay, D.B.: Carbohydrate metabolism in ruminants. In: Physiology of Digestion and Metabolism in the ruminant. Philipson, A.T. (ed) Oriel Press: Newcastle upon-Tyne. 1970. pp.438-451.

24. Wastney, M.E., Arcus, A.C., Bickerstaffe, R., and Wolff, J.E.: Glucose tolerance in ewes and susceptibility to pregnancy toxaemia. Aust. J. Biol. Sci. 1982;35:381-392.

25. Cunningham, F.G., Lowe, T., Guss, S., and Mason, R.: Erythrocyte morphology in women with severe preeclampsia and elampsia. Am. J. Obstet. Gynecol. 1985;153:358-363.

26. Colli, S., Maderna, P., Tremuli, E., Baraldi, A., Ravati, G.E., Gianfranceschi, G., and Nicosla, S.: Prostacyclin-lipoprotein interactions: studies on human platelet aggregation and adenylate cyclase. Biochem. Pharmacol. 1985;34:2451-2457.

27. Kernoff, P.B.A., Willis, A.L., Stone, K.J., Davies, J.A., and McNicol, G.P.: Antithrombotic potential of dihomo-gamma linolenic acid in

man. Br. Med. J. 1977;1441-1444.

28. Gerrard, J.M.: The Red Cell. In: Prostaglandins and Leukotrienes: Blood and vascular cell function. Marcel Dekker, Inc. N.Y., N.Y. 1985. pp.107-116.

29. Smith, W.L., and Borgeat, P.: The Eicosanoids: Prostaglandins, thromboxanes, leukotrienes, and hydroxyeicosaenoic acids. In: Biochemistry of Lipids and Membranes. Vance, D.E., and Vance, J.E. (eds) The Benjamin/Cummings Publishing Company, Menlo Park, California. 1985. p.331.

30. Sibai, B.M., Taslimi, M., Abdella, T.N., Brooks, T.F., Spinnator, J.A., and Anderson, G.D.: Maternal and perinatal outcome of conservative management of severe preeclampsia in mid-trimester. Am. J. Obstet. Gynecol. 1985;152:32-37.

31. Hamberg, M., Svensson, J., and Samuelsson, B.: Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxidases. Proc. Nat. Acad. Sci. U.S.A. 1975;72:2994-3000.

32. Gorman, R.G.: Modulation of human platelet function by prostacyclin and thromboxane A_2. Fed. Proc. 1979;38:83-88.

33. Neufeld, E.J., and Majerus, P.W.: Arachidonate release and phosphatidic acid turnover in stimulated human platelets. J. Biol. Chem.

1983;258:2461-2467.

34. Ritterhouse-Simmons, S., and Deykin, D.: Platelets in biology and pathology. Gordon, J.L. (ed) Amsterdam: Elsevier/ North Holland. 1981. pp.349-371.

35. Hamberg, M., and Samuelsson, B.:Prostaglandin endoperoxidases. Novel transformation of arachidonic acid in human platelets. Proc. Nat. Acad. Sci. U.S.A. 1974;71:3400-3404.

36. Moncada, S., and Vane, J.R.: The role of prostacyclin in vascular tissue. Fed. Proc. 1979;38:66-71.

37. Mehta, J., and Mehta, P. (eds): Platelets and Prostaglandins in Cardiovascular Disease. Mount Kisco, Futura Publishing Co., 1981. pp.1-21.

38. Dodds, W.J.: Platelet function in animals: species specificities In: Platelets: A Multidisciplinary Approach. De Daetano, G., Garattini, S. (eds) New York, Raven Press. 1978. pp. 45-59.

39. Rand, M.L., Greenberg, J.P., Packham, M.A., and Mustard, J.F.: Density subpopulations of rabbit platelets: Size, protein, and sialic acid content, and specific radioactivity changes following labeling with ^{35}S-sulfate in vivo. Blood 1981;57:741-746.

40. Tanaka, Y., and Goodman, J.R.: Megakaryocytes and Platelets. In: Elecron Microscopy of Human Blood Cells. Harper and Rowe, New York. 1972. p.196.

41. Caen, J.P., Cronberg, S., and Gubisz, P.: Platelets: Physiology and Pathology. Stratton Intercontinental Medial Book Corp, New York. 1977. pp.3-22.

42. Farrell, R.J.: Haemorrheology, platelets, and atherogenesis. Thromb. Res. 1980;18:747-748.

43. Hope, W., Martin, T.J., Chesterman, C.N., and Morgan, F.J.: Human beta-thromboglobulin inhibits PGI_2 production and binds to a specific site in bovine aortic endothelial cells. Nature 1979;282:210-212.

44. White, J.G., and Clawson, C.C.: The surface-connected canalicular system of blood platelets - a fenestrated membrane system. Am. J. Pathol. 1980;101:353-364.

45. Gimbrone, M.A., Aster, R.H., Cotran, R.S., Corkery, J., Jandl, J.H., and Folkman, J.: Preservation of vascular integrity on organs perfused in vitro with a platelet-rich medium. Nature 1969; 1222:33-36.

46. Gentry, P.A., Schneider, M.D., and Miller, J.K.: Plasma clot promoting

effect of collagen in relation to collagen-platelet interaction. Am. J. Vet. Res. 1981;42:708-715.

47. Castagna, M., Brasseur, R., Pavoine, C., Bredoux, R., Mely, B., Ruysschaert, J.M., and Levy-Toledano, S.: Protein kinase C and granule release in human platelets. In: Mechanisms of stimulus-responde coupling in platelets. Westwick, J., Scully, M.F., MacIntyre, D.E., and Kakkar, V.V. (eds) Plenum Press, New York. 1985. pp.249-263.

48. Zawilska, K.M., Born, G.V.R., Begent, N.A.: Effect of ADP utilizing enzymes on the arterial bleeding time in rats and rabbits. Br. J. Haematol. 1982;50:317-325.

49. Colman, R.W.: The role of platelets in the genesis of ischemia. Ann. N.Y. Acad. Sci. 1982;382:190-203.

50. Holmsen, H.: Biochemical and pharmacological aspects of the platelet aggregation. Energetics of some platelet functions. In: Platelet Aggregation and Drugs. Caprino, L. and Rossi, E.C. (eds) Acedemic Press, New York. 1974. p.91.

51. Meyers, K.M., Seachord, C.L., Holmsen, H., Smith, J.B., and Prieur, D.J.: A dominant role of thromboxane formation in secondary aggregation of platelets. Nature 1979;282:331-333.

52. Lagers, B.: In vitro platelet responses: Dense granule secretion. In: Platelet Responses and Metabolism. Holmsen, H. (ed) CRC Press, Boca Raton, Florida. 1986. pp. 115-143.

53. Luscher, E.F., Massini, P., and Kaser-Glanzmann, R.: The Role of Calcium Ions in the Regulation of Platelet Function and Their Pharmaceutical Control. In: Advances in Pharmacology and Therapeutics: Proceedings of the 7th International Congress on Pharmacology. Vargaftig, B.(ed) Pergamon Press, New York. 1978. pp. 87-95.

54. Condie, R.G.: A serial study of coagulation factors XII, XI, and X in plasma in normal pregnancy and in pregnancy complicated by pre-eclampsia. Br. J. Obstet Gynecol. 1976;83:636-639.

55. Vaziri, N.D., Toohey, J., Powers, D., Keegan, K., Gupta, A., Alikhani, S., Mashood, M., and Barbari, A.: Activation of intrinsic coagulation pathway in pre-eclampsia. Am. J. Med. 1986;80:103-107.

56. White, J.G.: The ultrastructure and regulatory mechanisms of blood platelets. In: Blood platelet function and medicainal chemistry. Lasslo, A. (ed) Elsevier Biomedical Publishing, New York. 1984. pp.15-59.

57. Myers, A., Shio, H., Nicosia, S., and Ramwell, P.W.: Platelet Eicosanoid receptors. In: The Platelets: Physiology and Pharmacology Longenecker, G.L. (ed) Acedemic Press, Orlando, Florida

1985. p.201.

58. Mielke, C.H.: Qualitative platelet abnormality due to absent collagen-induced aggregation. Thromb. Haemost. (Stuttgert) 1976;36:283-288.

59. Tiffany, M.L., and Penner, J.A.: Effect of complement on collagen-induced platelet aggregation. J. Lab. Clin. Med. 1980;96:796-802.

60. Tiffany, M.L.: Technical considerations for platelet aggregation and related problems. Crit. Rev. Clin. Lab. Sci. 1983;19:27-69.

61. Lands, W.E.M., Culp, B.R., Hirai, A., and Gorman, R.: Relationship of thromboxane generation to the aggregation of platelets from humans: effects of eicosapentaenoic acid. Prostaglandins 1985;30:819-825.

62. Samuels, P., Main, E.K., Tomaski, A., Mennuti, T., Gabbe, S.G., and Cines, D.B.: Abnormalities in platelet antiglobulin tests in preeclamptic mothers and their neonates. Am. J. Obstet. Gynecol. 1987;157:109-113.

63. Greer, I.A., Walker, J.J., Forbes, C.D., and Calder, A.A.: Platelet Function in Pregnancy-induced hypertension following treatment with labetalol and low dose aspirin. Thrombos. Res. 1987; 46:607-612.

64. Howie, P.W.: The haemostatic mechanisms of preeclampsia. Clinics Obstet. Gynaecol. 1977;4:595-609.

65. Mehta, P., and Mehta, J.: Platelet function studies in coronary heart disease. VIII. Decreased platelet sensitivity to prostacyclin in patients with myocardial ischaemia. Thrombos. Res. 1980; 18:273-277.

66. Lembele-Duchesne, M.J., Lahlou, A.L., Thaler-Dao, H., and Crastes de Paulet, A.: Inhibition of Platelet aggregation by human placenta. Thromb. Haemost. 1985;54:431-437.

67. Preston, F.E., Whipps, S., Jackson, C.A., French, A.J., Wyld, P.J., and Stoddard, C.J.: Inhibition of Prostacyclin and Platelet Thromboxane A_2 after low dose aspirin. N. Engl. J. Med. 1981; 304:76-79.

68. Schalm, O.W.: Schalm's Veterinary Heamatology. Jain, N.C. (ed) W.B. Saunders Co., Philadelphia. 1974. pp.527-562.

69. Leblond, P.F.: The discocyte-echinocyte transformation of the human red cell: Deformability characteristics. In: Red Cell Shape. Bessis M., Weed, R.I., and Leblond, P.F. (eds). Springer Verlag, New York. 1973. pp.95-104.

70. Reinhart, W.H., and Chien, S.: Red cell rheology in stomatocyte-

echinocyte transformation: Roles of cell geometry and cell shape. Blood 1986;67:1110-1118.

71. Weed, R.I., LaCelle, P.I., and Merrill, E.W.: Metabolic dependence of red cell deformability. J. Clin. Invest. 1969;48:795-809.

72. Bessis, M.: Red Cell Shapes. An illustrated classification and its rationale. In: Red Cell Shape. Bessis M., Weed, R.I., and Leblond, P.F. (eds) Springer-Verlag, New York. 1973. pp.1-26.

73. Chien, S.: Rheology of sickle cells and the microcirculation. N. Engl. J. Med. 1984;311:1567-1569.

74. Bauer, T.W., Moore, G.W., and Hutchins, G.M.: Morphologic evidence for coronary artery spasm in eclampsia. Circulation. 1982; 65:255-259.

75. Born,G.V.R.: Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962;194:927-929.

76. Aursnes, I., Gjesdal, K., and Abildgaard, U.: Platelet aggregation induced by ADP from unsheared erythrocytes at physiological Ca^{++} concentrations. Brit. J. Haematol. 1981;47:149-152.

77. Wallenburg, H.C.S., and Rotmans, N.: Enhanced reactivity of the platelet thromboxane pathway in normotensive and hypertensive

pregnancies with insufficient fetal growth. Am. J. Obstet. 1982;144:523.

78. Redman, C.W.G., Bonnar, J., and Beilin, L.J.: Early platelet comsumption in pre-eclampsia. Br. Med. J. 1978;1:467-469.

79. Weinstein, L.: Syndrome of hemolysis, elevated liver enzymes, and low platelet count: a severe consequence of hypertension in pregnancy. Am. J. Obstet. Gynecol. 1982;142:159-167.

80. Didisheim, P.: Pathophysiology of platelet-vessel interactions. Biblthca Haemat. 1978;44:174-180.

81. Suonio, S., Saarikoski, S., Tahvanainen, K., Paakkonen, A., and Olkkonen, H.: Acute effects of dihydralazine mesylate, furosemide, and metroprolol on maternal hemodynamics in pregnancy induced hypertension. Am. J. Obstet. Gynecol. 1985;155:122-125.

82. Cotton, D.B., Longmire, S., Jones, M.M., Dorman, K.F., Tessmen, J., and Joyce, T.H.: Cardiovascular alterations in severe pregnancy-induced hypertension: Effects of intravenous nitroglycerin coupled with blood volume expansion. Am. J. Obstet. Gynecol. 1986;154:1053-1059.

83. Hardisty, R.M.: Antiplatelet drugs: Rationale for use. Biblthca Haemat. 1978;44:181-187.

84. Wallenburg, H.C.S., Makovitz, J.W., Dekker, G.A., and Rotmans, P.: Low-dose aspirin prevents pregnancy-induced hypertension and pre-eclampsia in angiotensin-sensitive primigravidae. Lancet 1986;8471: 1-20.

85. Pedersen, A.K., and FitzGerald, G.A.: Dose related kinetics of aspirin, presystemic acetylation of platelet cyclooxygenase. N. Engl. J. Med. 1984;311:1206-1211.

86. Kelton, J.G.:Controversial aspects of the use of acetylsalicylic acid as an antithrombotic agent. In: Acetylsalicylic Acid-New Uses for an Old Drug. Barnett, H.J.M., Hirsh, J., and Mustard, J.F. (eds) Raven Press: New York. 1982. pp.95-107.

87. Baenziger, N.L., Dillender, M.J., and Majerus, P.W.: Cultured Human skin fibroblasts and arterial cells produce a labile platelet-inhibitory prostaglandin. Biochem. Biophys. Res. Commun. 1977;78:294-301.

88. Greer, I.A., Walker, J.J., McLaren, M., Calder, A.A., and Forbes, C.D.: A comprehensive study of the effects of adrenoceptor antagonists on platelet aggregation and thromboxane generation. Thromb. Haemost. 1985;54:480-484.

89. Jouppila, P., Kirkinen, P., Koivula, A., and Ylikorkala, O.:

Labetalol does not alter the placental and fetal blood flow or maternal prostanoids in pre-eclampsia. Br. J. Obstet. Gynecol. 1986;93:543-547.

90. Frolich, J., Hollifield, J., and Dormois, J.: Supression of plasma renin activity by indomethacin in man. Circ. Res. 1976; 39:447-452.

91. Everett, R.B., Worley, R.J., MacDonald, P.C., and Gant, N.F.: Prostagland in synthase inhibitors on pressor response to angiotensin II in human pregnancy. J. Clin. Endocrinol. Metab. 1978;46:1007-1010.

92. Wierda, A., Verhoeff, J., vanDijk, S., Dorresteijn, J., Wensing, T.: Effect of trenbolone acetate and propylene glycol on pregnancy toxaemia in ewes. Vet. Rec. 1985;116:284-287.

93. Ranaweera, A., Ford, E.J.H., and Evans, J.: Gluconeogenesis from glycerol by ketotic sheep pregnant with twins. Res. Vet. Sci. 1981;30:303-308.

94. Heitzman, R.J., Herriman, I.D., and Austin, A.R.: The response of sheep with pregnancy toxemia to trenbolone acetone. Vet. Rec. 1977;100:317-318.

95. Silver, M.J., Hoch, W., Koisis, J.J., Ingerman, C.M., and Smith, J.B.: Arachidonic acid causes sudden death in rabbits. Science (Washington, D.C.) 1974;183:1085-1087.

96. Myers, A., Rabbani, F., Penhos, J.C., Ramey, E., and Ramwell, P.W.: Protective effects of lidocaine cyproteroneactetate and a thromboxane synthetase inhibitor against arachidonate induced mortality. Fed. Proc., Ped. Am. Soc. Exp. Biol. 1981;40:662-668.

97. Mais, D.E., Saussy, D.L., Chaikhouni, A., Kochel, P.J., Knapp, D.R., Hamanaka, N., and Halushka, P.V.: Pharmologic characterization of human and canine thromboxane A_2 prostaglandin H_2 receptors in platelets and blood vessels: evidence for different receptors. J. Pharmacol. Exp. Ther. 1985;233:418-424.

98. Needleman, P., Wyche, A., and Raz, A.: Platelet and blood vessel arachidonate metabolism. J. Clin. Invest. 1979;63:345-349.

99. Bertele, V., Stemerman, M., Schafer, A., Adelman, B., Smith, M., Fuhro, R., and Salzman, E.: Refractoriness of platelets to prostaglandins after infusion in rabbits. J. Lab. Clin. Med. 1985;106:551-561.

100. Reilly, I.A.G., Doran, J.B., Smith, B., FitzGerald, G.A.: Increased thromboxane biosynthesis in a human preparation of platelet activation: biochemical and functional consequences of selective inhibition of thromboxane synthetase. Circulation 1986;73:1300-1309.

101. Barrett, P.A., Butler, K.D., Shand, R.A., and Wallis, R.B.:

Intrathoracic platelet accumulation in the guinea-pig induced by intravenous administration of arachidonic acid-effect of cyclo-oxygenase and thromboxane synthetase inhibitors. Thromb Haemost 1986;56:311-317.

102. Fischer, S., Struppler, M., Bohlig, B., Bernutz, C., Wober, W., and Weber, P.C.: The influence of selective thromboxane synthetase inhibition with a novel imidazole derivative, UK-38,485, on prostanoid formation in man. Circulation 1983;68:821-826.

103. Baenziger, N.L., Becherer, P.A., and Majerus, P.W.: PGI_2 production in cultured human arterial smooth muscle cells, venous endothelial cells and skin fibroblasts. Cell 1979;16:967-974.

104. Roth, G.J., and Majerus, P.W.: The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particulate fraction protein. J. Clin. Invest. 1975; 56:624-632.

105. FitzGerald, G.A., Brash, A.R., Oates, J.A., and Pedersen, A.K.: Endogenous prostacyclin biosynthesis and platelet function during selective inhibition of thromboxane synthetase in man. J. Clin. Invest. 1983;71:1336-1343.

106. FitzGerald, G.A., and Oates, J.A.: Selective and nonselective inhibition of thromboxane formation. Clin. Pharmacol. Ther. 1984;35:633-640.

107. Naden, R.P., Iliya, C.A., Arant, B.S., Gant, N.F., and Rosenfeld, C.R.: Hemodynamic effects of indomethacin in chronically instrumented pregnant sheep. Am. J. Obstet. Gynecol. 1985;151: 484-493.

108. Rosenfeld, C.R., and Gant, N.F.: The chronically instrumented ewe-a model for studying vascular reactivity to angiotensin II in pregnancy. J. Clin. Invest. 1981;67:486-492.

109. Rankin, J.H.G., Pernetton, T.M., Anderson, D.F., and Berssenburgge, A.D.: Effect of prostaglandin I_2 on ovine placental vasculature. J. Dev. Physiol. 1979;1:151-160.

The vita has been removed from the scanned document