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THE EFFECT OF ANTI-HYPERTENSIVE AGENTS ON PLATELETS,
PROSTACYCLIN AND THROMBOXANE AND OBSERVATIONS ON PROSTACYCLIN
AND THROMBOXANE IN NORMAL AND HYPERTENSIVE PREGNANCY

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DECLARATION

The studies presented in this thesis were performed in the University Departments of Medicine and Obstetrics and Gynaecology at Glasgow Royal Infirmary and Glasgow Royal Maternity Hospital, between August 1982 and July 1985. All the in vitro studies were conceived, designed and performed by the author in person, with the exception of the radioimmunoassays used to measure prostacyclin metabolites and thromboxane B₂ which were developed and performed by Mrs. Margaret McLaren of the University Department of Medicine and to whom I am deeply indebted.

The clinical studies on prostacyclin and thromboxane in pregnancy described in Chapters 10, 11 and 12 of this thesis were conceived, designed and performed in association with Dr. James J. Walker of the University Department of Obstetrics and Gynaecology, and we were assisted in the collection of clinical material by Dr. Myriam Bonduelle and Dr. Alan D. Cameron also of the University Department of Obstetrics and Gynaecology.

The analysis of the data, the interpretation of the results and the opinions expressed in this thesis are, however, those of the author.

Most of the studies in this thesis have already been published or are in press in learned journals. Copies of these papers are to be found at the end of this thesis. A list of these papers published or in press follows:-

- Greer, I.A., Walker, J.J., Calder, A.A. and Forbes, C.D. (1985)
Aspirin with an adrenergic or a calcium channel blocking agent
as new combination therapy for arterial thrombosis.
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Immunoreactive prostacyclin and thromboxane metabolites in
normal pregnancy and the puerperium.
British Journal of Obstetrics and Gynaecology, 92, 581 - 585.
- Greer, I.A., Walker, J.J., McLaren, M., Calder, A.A. and Forbes,
C.D. (1985)
Inhibition of thromboxane and prostacyclin production in whole
blood by adrenoceptor antagonists.
Prostaglandins, Leukotrienes and Medicine, 19, 209 - 217.
- Greer, I.A., Walker, J.J., McLaren, M., Calder, A.A. and Forbes,
C.D. (1985)
A comparative study of the effects of adrenoceptor antagonists
on platelet aggregation and thromboxane generation.
Thrombosis and Haemostasis, 54, 480 - 484.
- Greer, I.A., Walker, J.J., Cameron, A.D., McLaren, M., Calder,
A.A. and Forbes, C.D.
A prospective longitudinal study of immunoreactive prostacyclin
and thromboxane metabolites in normal and hypertensive
pregnancy.
Clinical and Experimental Hypertension - Part B - Hypertension
in Pregnancy (In Press).
- Greer, I.A., Walker, J.J., Calder, A.A. and Forbes, C.D.
Inhibition of platelet aggregation in whole blood by
adrenoceptor antagonists.
Thrombosis Research (In Press).
- Greer, I.A., Walker, J.J., McLaren, M., Calder, A.A. and
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Inhibition of whole blood platelet aggregation by nicardipine
and synergism with prostacyclin in vitro.
Thrombosis Research (In Press).

The following communications based on work in this thesis have
been presented personally by the author:-

1. Prostacyclin and thromboxane production in normal and
pathological pregnancy.
British Congress of Obstetrics and Gynaecology, Birmingham,
July 1983.
2. Thromboxane in pregnancy induced hypertension.
European Society for Clinical Investigation, Milan, April
1984.

3. Thromboxane in pregnancy induced hypertension: Influence of therapy with labetalol in vivo and in vitro.
Fourth World Congress of the International Society for the Study of Hypertension in Pregnancy, Amsterdam, June 1984.
4. Effect of labetalol on platelet consumption and thromboxane in pregnancy induced hypertension.
Scottish Society for Experimental Medicine, Aberdeen, June 1984.
5. A comparative study of the effects of adrenoceptor antagonists on platelet aggregation and thromboxane generation.
British Society for Haemostasis and Thrombosis, London, September 1984.
6. Prostacyclin in Pregnancy.
Medical Research Council Reproductive Biology Unit, and Department of Obstetrics and Gynaecology, University of Edinburgh, combined seminar, Edinburgh, January 1985.
7. Aspirin with an adrenergic or a calcium channel blocking agent: A new combination therapy for arterial thrombosis
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8. A prospective longitudinal study of prostacyclin and thromboxane in normal and hypertensive pregnancy.
The Second International Congress on Essential Fatty Acids, Prostaglandins and Leukotrienes, London, March 1985.
9. Effect of adrenergic blocking agents on thromboxane A₂ and prostacyclin production from whole blood.
The Second International Congress on Essential Fatty Acids, Prostaglandins and Leukotrienes, London, March 1985.
10. A comparative study of the effects of adrenergic blocking agents on platelet aggregation and thromboxane generation.
The Second International Congress on Essential Fatty Acids, Prostaglandins and Leukotrienes, London, March 1985.
11. Influence of treatment on platelet consumption and thromboxane production in pregnancy induced hypertension.
Registrars Research Meeting, Royal College of Physicians and Surgeons of Glasgow, Glasgow, March 1985.
12. Inhibition of platelet aggregation and thromboxane generation in whole blood by adrenoceptor antagonists in vitro.
British Meeting of the International Society for the Study of Hypertension in Pregnancy, Newcastle, July 1985.
13. Effect of nicardipine on platelet aggregation and prostacyclin and thromboxane production from whole blood, and synergism with low dose aspirin - a possible antihypertensive and platelet protective strategy for pregnancy induced hypertension.
British Meeting of the International Society for the Study of Hypertension in Pregnancy, Newcastle, July 1985.

14. Prostacyclin metabolites in normal and hypertensive pregnancy: a prospective longitudinal study.
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15. A comparative study on the effects of adrenergic blocking agents on whole blood platelet aggregation and synergism with prostacyclin and aspirin.
Xth International Congress on Thrombosis and Hemostasis, San Diego, July 1985.
16. Inhibition of whole blood platelet aggregation by nicardipine, and synergism with low dose aspirin: A new combination therapy for arterial thrombosis.
Xth International Congress on Thrombosis and Hemostasis, San Diego, July 1985.
17. Effect of adrenergic blocking agents on thromboxane A₂ and prostacyclin production from whole blood.
Xth International Congress on Thrombosis and Hemostasis, San Diego, July 1985.
18. Influence of treatment on platelet consumption and thromboxane in pregnancy induced hypertension.
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20. Inhibition of whole blood platelet aggregation by nicardipine, and synergism with prostacyclin and aspirin in vitro.
British Society for Haemostasis and Thrombosis, London, September 1985.

SUMMARY

Prostacyclin (PGI₂) is a potent vasodilator and anti-platelet prostaglandin which is the major product of arachidonic acid metabolism in vascular tissue. It is thought to function as a local vasoprotective agent, protecting blood vessels from platelet deposition and subsequent damage. Thromboxane A₂(TxA₂) is the major product of arachidonic acid metabolism in platelets, and has actions directly opposed to those of prostacyclin as it is a potent vasodilator and platelet aggregating agent, which is synthesised and released when platelet aggregation occurs. It has been proposed that a balance exists between these two substances to maintain vascular integrity. Disturbance of this balance may be important in diseases associated with platelet consumption and vasoconstriction such as pregnancy induced hypertension (PIH) and prostacyclin deficiency and excess TxA₂ production have both been implicated in the pathophysiology of this condition. Recently, adrenoceptor antagonists and calcium channel blocking agents have been used in the treatment of PIH. The aims of this thesis were firstly to study the effects of these drugs on platelet function and PGI₂ and TxA₂ production in vitro, and secondly, to study plasma levels of PGI₂ and TxA₂ metabolites in normal pregnancy and PIH, and assess the effect of treatment with an adrenoceptor antagonist (labetalol) on levels of these substances and platelet consumption in PIH.

The effects of seven adrenoceptor antagonists on platelet aggregation and TxA₂ production in platelet rich plasma were studied in vitro. Labetalol, pindolol and propranolol inhibited

platelet aggregation and TxA_2 generation in a dose dependent manner, probably by preventing release of arachidonic acid from membrane phospholipids. This effect was independent of any adrenoceptor blocking property, as several other adrenoceptor antagonists - atenolol, metoprolol, timolol and prazosin were without effect. This platelet inhibitory effect may be related to membrane stabilising activity or lipid solubility as these properties were common to all three effective agents.

The effects of labetalol, pindolol and propranolol on vascular PGI_2 production was studied using umbilical artery, however, these agents had little effect, causing only slight inhibition of PGI_2 production at high drug concentrations. These 3 agents acted synergistically with PGI_2 - either exogenous or derived from vessel wall - to inhibit platelet aggregation in platelet rich plasma.

It has recently become possible to measure platelet aggregation in whole blood. This may be more physiological than traditional turbidometric techniques using platelet rich plasma as it leaves platelet in their natural milieu surrounded by red and white cells which can themselves influence platelet aggregation. The effects of labetalol, pindolol, propranolol and atenolol on platelet aggregation in whole blood was studied. Labetalol, pindolol and propranolol inhibited aggregation in a dose dependent manner and acted synergistically with exogenous PGI_2 to inhibit platelet aggregation. They also inhibited PGI_2 and TxA_2 production from whole blood. Atenolol had no effect on either aggregation or TxA_2 or PGI_2 production. The calcium channel

blocking agent nicardipine was similarly assessed and found to inhibit aggregation but it had no effect on TxA₂ or PGI₂ production. Labetalol and nicardipine were also found to act synergistically with low concentrations of aspirin to inhibit platelet aggregation in whole blood.

Cross-sectional and longitudinal studies of plasma levels of PGI₂ and TxA₂ metabolites throughout normal pregnancy were performed. Prostacyclin metabolites were found to be increased in the first trimester compared to the non-pregnant state, while TxA₂ metabolite levels were slightly reduced in the second and third trimesters. Cross-sectional and longitudinal studies of these substances in PIH were also performed and plasma PGI₂ metabolites were found to be reduced, providing further evidence that PIH is associated with PGI₂ deficiency. No change in TxA₂ metabolites was noted in PIH. The effects of treatment with labetalol on plasma levels of PGI₂ and TxA₂ metabolites, and on platelet consumption were studied in PIH. Labetalol therapy did not affect levels of TxA₂ metabolites but seemed to increase PGI₂ metabolite levels if these were initially low, although no consistent effect on plasma PGI₂ metabolite levels was noted if these were initially within the normal range. Following therapy with labetalol platelet consumption was significantly reduced especially in severe PIH. This reduction in platelet consumption suggests that the anti-platelet properties of labetalol studied in vitro may well exist in vivo, and such platelet protective therapy may be of value in the treatment of this disease.

The anti-platelet properties of adrenoceptor antagonists, used either alone or in combination with prostacyclin or aspirin, which have been described in this thesis, require further clinical evaluation, but offer new therapeutic possibilities not only in PIH but also in other vascular disorders associated with platelet activation.

CHAPTER 1

PROSTACYCLIN AND THROMBOXANE

1.1 DISCOVERY OF PROSTAGLANDINS PROSTACYCLIN AND THROMBOXANE

The first observations on the biological effects of prostaglandins were made by Raphael Kurzok, a New York gynaecologist with an interest in artificial insemination. He noted that when seminal fluid was injected into the uterus it caused uterine contraction and immediate expulsion of the fluid (Kurzok and Lieb, 1930).

The findings of Kurzok and Lieb were quickly extended by other investigators. In 1935 Goldblatt discovered that this biologically active substance in seminal plasma was soluble in organic solvents and therefore appeared to be a lipid or lipid-like substance. von Euler (1936) reported that similar substances were found in sheep seminal vesicles and proposed that the active substance had the properties of a fatty acid. It was Von Euler who named these substances prostaglandins as he initially thought that they originated in the prostate gland. The active principle was later found to be in the fraction of seminal fluid containing unsaturated hydroxy acids (Bergström, 1967).

The methodological advances of gas-liquid chromatography and mass spectrometry in the 1960's were to allow further characterisation of the prostaglandins. It became apparent that prostaglandins contained 20 carbon atoms and a 5 membered ring. The hypothesis that they might be derived from a C₂₀ polyunsaturated fatty acid was tested by van Dorp et al., (1964) and Bergström, Danielsson and Samuelsson (1964) using tritium labelled arachidonic acid.

This was incubated with sheep seminal vesicles and prostaglandin E₂ was identified as the end product. Thus, it became known that essential fatty acids were the precursors of prostaglandins.

A discovery of major significance was the demonstration that aspirin inhibited prostaglandin synthesis (Vane, 1971; Smith and Willis, 1971). This allowed much information to be gained on the role of prostaglandins in physiology, and stimulated interest in the regulation of prostaglandin production. Since it was already known that aspirin inhibited platelet aggregation (Weiss, Aledort and Kochwa, 1968), prostaglandins produced by platelets became implicated in haemostasis.

In experiments to study the effects of anaphylaxis on guinea pig lung, Piper and Vane (1969) noted that an unstable substance was produced which had the ability to stimulate contraction of rabbit aorta. It became clear that this was not a classical prostaglandin, although aspirin had the ability to inhibit its production. Vargaftig and Zirinus (1973) also reported that addition of arachidonic acid to platelets induced an aggregatory response and resulted in formation of this same rabbit aorta contracting substance.

This substance was later discovered to be thromboxane A₂, a derivative of prostaglandin endoperoxides (Hamberg, Svensson and Samuelsson, 1975), and the major product of arachidonic acid metabolism in platelets, which itself can stimulate vasoconstriction and platelet aggregation (Hamberg et al., 1975; Hamberg et al., 1974).

Reasoning that platelets and blood vessels had functional similarities, Moncada, Vane and colleagues started to study blood vessel homogenates, to ascertain whether they, like platelets, produced thromboxane A_2 . A feature which might explain the vasoconstriction which immediately follows cutting of a small vessel. Unexpectedly they discovered that blood vessel homogenates produced a hitherto unknown prostaglandin, which they called prostaglandin X. This substance had platelet inhibitory and vasodilatory properties (Moncada et al., 1976; Bunting et al., 1976); properties directly opposed to those of thromboxane A_2 . At about the same time, Kulkarni et al., (1976) independently found that intact blood vessel preparations converted arachidonic acid into an unstable vasodilatory substance.

The chemical structure of prostaglandin X was soon defined as 9-deoxy-6,9 α -epoxy-delta-prostaglandin $F_{1\alpha}$ (Johnson et al., 1976) and rapidly renamed prostacyclin. Prostacyclin was found to be the main product of arachidonic acid metabolism in vascular tissue (Salmon et al., 1978). The main hydrolytic product of prostacyclin was found to be 6-keto-prostaglandin $F_{1\alpha}$, a substance which had been described several years previously by Pace-Asciak and Wolfe (1971) as a product of arachidonic acid metabolism in rat stomach.

Thus, from a chance observation over 50 years ago, our knowledge of prostaglandins has developed through numerous contributions from various disciplines, into what appears to be an important

regulatory system both in physiology and pathology. The rapid proliferation of knowledge on prostacyclin and thromboxane, which has occurred in the last 10 years, has also highlighted the importance of these two substances both in the physiology and pathology of haemostasis, thrombosis and vascular disease.

1.2 NOMENCLATURE, STRUCTURE, SYNTHESIS AND METABOLISM OF PROSTAGLANDINS, AND IN PARTICULAR PROSTACYCLIN AND THROMBOXANE

1.2.1 Nomenclature and Structure

Prostaglandins and thromboxanes are derived from essential fatty acids. Essential fatty acids cannot be synthesised by the body and therefore must be derived in sufficient quantities from the diet (Crawford, 1983). Prostaglandins contain 20 carbon atoms made up into a cyclopentane ring with 2 side chains. Prostacyclin differs from this as it has a bicyclic structure (figure 1.1) and thromboxanes contain a completely different ring structure which is essentially an oxane. Due to the absence of the pentane ring thromboxanes are not considered to be prostaglandins.

Prostaglandins and thromboxanes can be synthesised from 3 different essential fatty acid precursors; dihomo-gamma-linolenic acid which contains 3 double bonds, arachidonic acid, which contains 4 double bonds and eicosapentaenoic acid which contains 5 double bonds (Crawford, 1983). Prostaglandins and thromboxanes derived from these essential fatty acids are given the subscripts 1, 2 and 3, respectively (e.g., PGE₁, PGI₂), indicating their fatty acid precursor. Within each series the compounds are identified by letters (e.g., PGE, PGF, PGI). In the 1960's

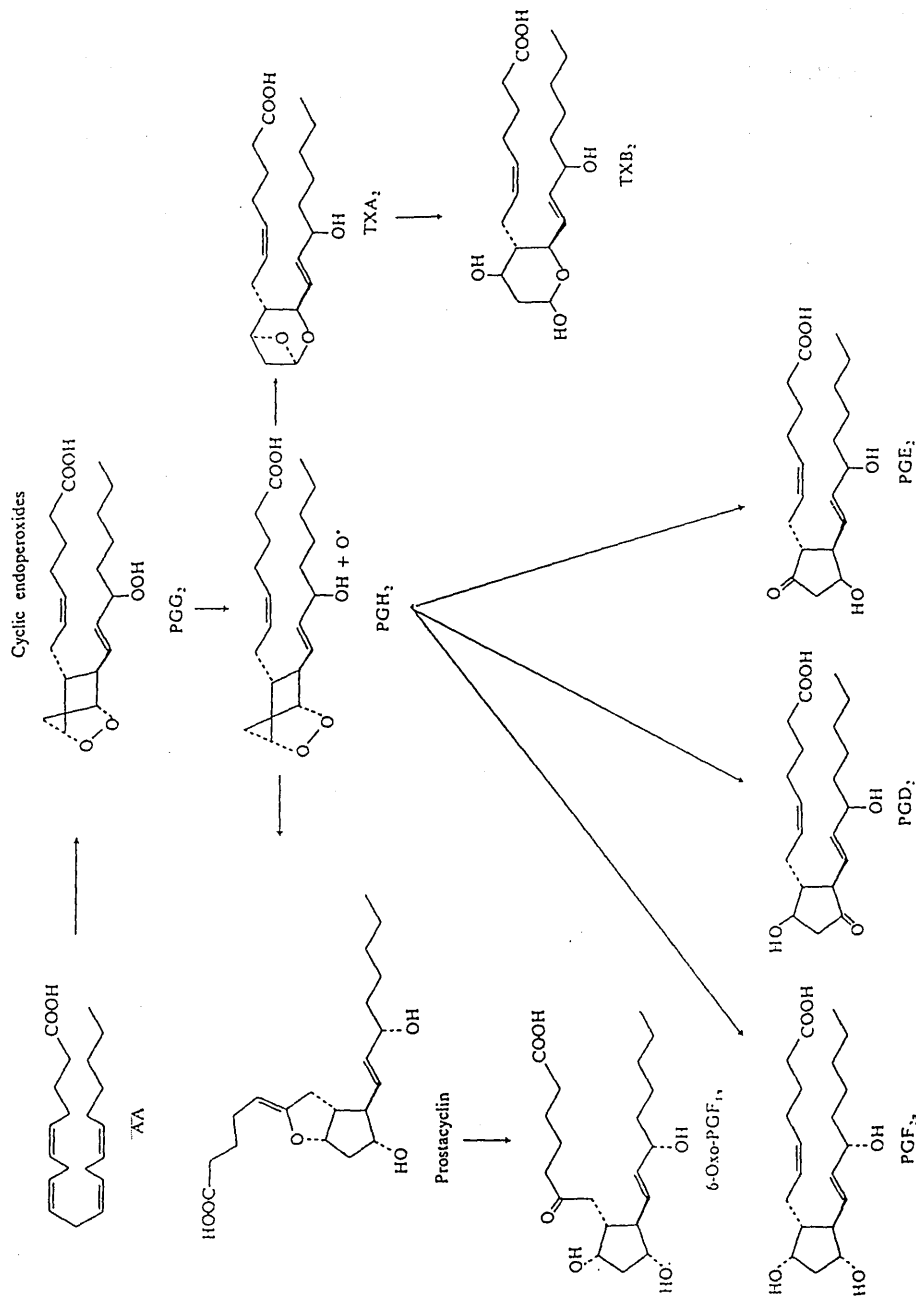


FIGURE 1.1

Metabolism of Arachidonic Acid via the
Cyclooxygenase System

Swedish investigators successfully isolated two pure prostaglandins from seminal fluid. The first they termed PGE (as it was soluble in ether), and the second PGF (as it was soluble in phosphate buffer phosphate being spelled with an "F" in Swedish). Since then a large number of prostaglandins have been identified and for clarity have been given letters starting at the beginning of the alphabet. Thus, the most recent addition, prostacyclin is termed PGI₂. An additional subscript α or β is given to "F" series prostaglandins to indicate the stereoisomeric position of the hydroxyl group at the carbon position number 9, for example PGF_{1 α} .

The prostaglandins and thromboxanes of the "two" series are currently considered to be the most important in man. Arachidonic acid can be obtained from two sources, either directly from the diet or by formation from its precursor linoleic acid. Linoleic acid is first converted to γ -linolenic acid, then by elongation of the carbon chain to dihomo- γ -linolenic acid (the precursor of the "one" series prostaglandins) and then by further desaturation to arachidonic acid (Ramwell *et al.*, 1980). The bulk of arachidonic acid does not remain in the circulation, but is incorporated into cell membrane phospholipids (Ramwell *et al.*, 1980). Since prostaglandin formation requires free arachidonic acid it must first be liberated before serving as substrate.

1.2.2 Release of Arachidonic Acid

Prostaglandins are not stored but are released as soon as arachidonic acid is freed from its esterified form in the cell membrane and this is the rate limiting step in prostaglandin production (Ramwell et al., 1980). The breakdown of membrane phospholipids to produce free arachidonic acid is brought about by the action of various phospholipases, which are triggered as soon as the integrity of the cell membrane phospholipid bilayer is disturbed. The main enzyme involved is thought to be phospholipase A₂ but the combined action of phospholipase C and diglyceride lipase can also release arachidonic acid. Phospholipase A₂ requires the presence of calcium ions (Ca²⁺) for optimal activity (Blackwell and Flower, 1983), and unlike phospholipase C, phospholipase A₂ is present within the cell membrane and can be activated when certain receptors on the cell surface are occupied (Blackwell and Flower, 1983). Once arachidonic acid is available it serves as substrate for either the lipoxygenase pathway or the cyclo-oxygenase pathway. The lipoxygenase enzyme system forms leucotrienes which are involved in the inflammatory response and are the constituents of the so called slow reacting substance of anaphylaxis (Piper, 1983). The cyclo-oxygenase enzyme system forms prostaglandins and thromboxanes.

1.2.3 The Cyclo-oxygenase Pathway

Cyclo-oxygenase is present in all tissues except red blood cells. It is a substrate activated enzyme which transforms arachidonic acid into PGG₂ by oxygenation. It also has a peroxidase component which converts PGG₂ to PGH₂ (Bakhle, 1983). Since the

endoperoxides PGG₂ and PGH₂ are synthesised in all prostaglandin and thromboxane producing cells, there is no apparent heterogeneity in the cyclo-oxygenase enzyme, regardless of cell type.

The endoperoxides are unstable in aqueous solution and isomerise spontaneously or enzymatically to the stable prostaglandins, namely PGD₂, PGE₂ and PGF_{2α} (figure 1.1). For prostacyclin and thromboxane A₂ production there are two well recognised enzymes, prostacyclin synthetase and thromboxane synthetase (Bakhle, 1983).

The substances formed via the cyclo-oxygenase pathway have widely differing biological properties. The actual product formed shows marked specificity for cell, tissue and species. The clearest example of this is the ability of vascular endothelium to form prostacyclin, and of platelets to form thromboxane A₂. These two substances have diametrically opposing biological properties yet are produced from the same substrate.

Prostaglandins and thromboxanes are not stored, but are synthesised and released on demand, production being triggered by release of arachidonic acid. An exception to this is primate seminal vesicles where prostaglandins are "stored" in seminal fluid.

1.2.4 Catabolism of Prostacyclin and Thromboxane A₂

Prostacyclin is an unstable molecule with a half life of 3 minutes at 37°C and physiological pH (Dusting, Moncada and Vane, 1978). It is rapidly hydrolysed to the inactive derivative 6-keto-PGF_{1α}. Stability of prostacyclin can be maintained by storing it at alkaline pH (>9) and in a cold environment.

Both prostacyclin and 6-keto-PGF_{1α} may be enzymatically degraded by prostaglandin dehydrogenase and subsequently by delta-13-reductase. The metabolites so formed are then subjected to beta-oxidation (removal of 2 carbon units from the C₁ position) and omega-oxidation (removal of 2 carbon units from the C₂₀ position) (Ramwell et al., 1980). The rate limiting step is that of prostaglandin dehydrogenase (Bakhle, 1983).

Most tissues possess these cytoplasmic enzymes, however, their intracellular situation means that the extracellular substrate has to pass through the cell membrane before inactivation can proceed. Unlike other prostaglandins, such as PGE₁ and PGF_{2α}, prostacyclin is not cleared by the lungs (Bakhle, 1983) as prostacyclin is not a good substrate for the cell membrane uptake process. However, this selectivity of prostaglandin inactivation is not shared by the liver where all prostaglandins are inactivated. The main clearance of prostacyclin is by liver and kidney.

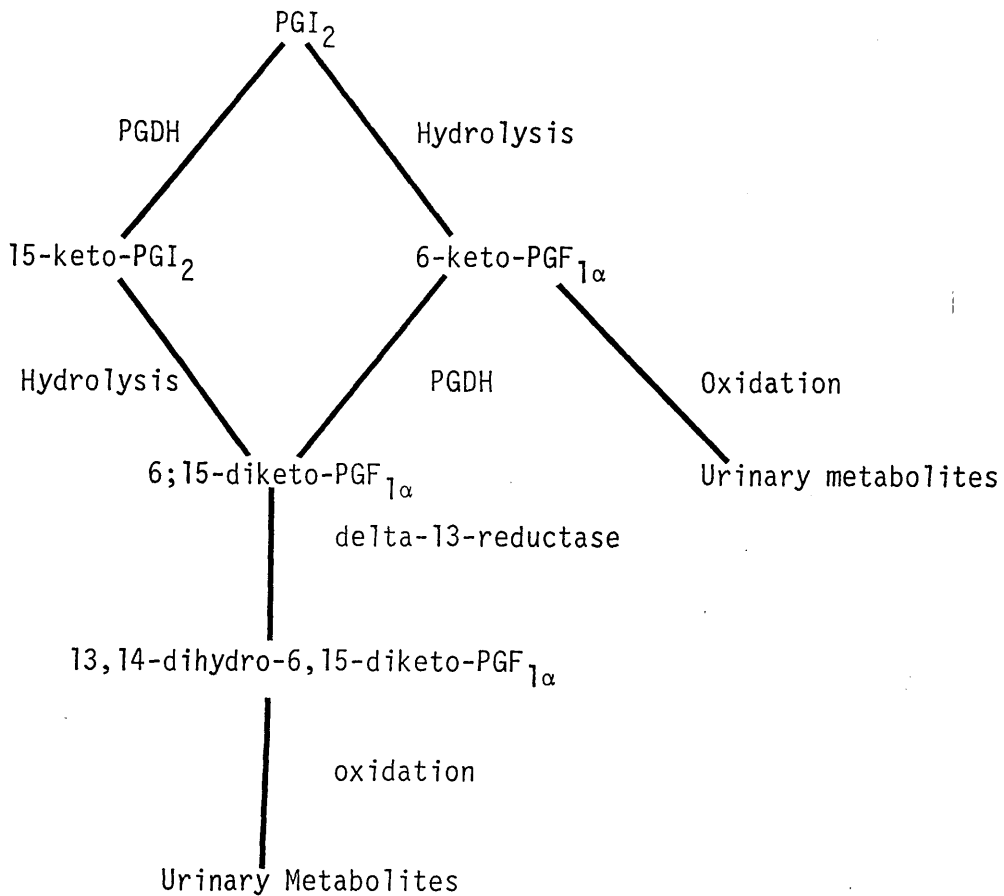


FIGURE 1.2

Metabolism of Prostacyclin (PGI₂)

PGI₂ is rapidly hydrolysed to 6-keto-PGF_{1α} or enzymatically converted to 15-keto-PGI₂ by prostaglandin dehydrogenase (PGDH). 6-keto-PGF_{1α} can be directly oxidised and excreted in the urine, or further metabolised prior to excretion.

There are many urinary metabolites of prostacyclin, the most important being 2,3,dinor-6-keto-PGF_{1α} and 6,15,diketo-14-15,dihydro,2-3,dinor PGF_{1α} (Rosenkranz et al., 1980). The catabolism of prostacyclin is shown in figure 1.2.

Thromboxane A₂ (TxA₂) is also an unstable molecule with a half life of 30 seconds under physiological conditions (Hamberg et al., 1975). It is rapidly hydrolysed to its inactive derivative thromboxane B₂ (TxB₂), which can subsequently only undergo one enzymatic step, that of beta-oxidation, to form 2,3,-dinor TxB₂ the major urinary product of thromboxane A₂ (Roberts et al., 1977).

1.3 TISSUE DISTRIBUTION OF PROSTACYCLIN AND THROMBOXANE PRODUCTION

Prostacyclin is the major product of arachidonic acid metabolism in vascular tissue (Salmon et al., 1978). Initial studies on prostacyclin synthesis were performed either with arterial microsomal preparations (Bunting et al., 1976) or with rings or strips of arterial or venous tissue. In 1977 Moncada et al., examined the differential formation of prostacyclin by layers of rabbit aorta, and found that prostacyclin production was highest, in the intimal layer and progressively decreased to the adventitial surface. Weksler, Marcus and Jaffe (1977) demonstrated that human endothelial cells produced prostacyclin and McIntyre, Pearson and Gordon (1978) showed that porcine endothelial and smooth muscle cells produce prostacyclin. It has

also been shown that following removal of the endothelium from rabbit aorta, production of prostacyclin at the luminal surface ceased (Eldor et al., 1981).

Fetal tissue appears to be more active than adult tissue in producing prostacyclin (Terragno, McGiff and Smigel, 1978). The human placenta also shows a high capacity for synthesising prostacyclin (Myatt and Elder, 1977). Arterial tissue seems to produce greater amounts of prostacyclin than venous tissue on a weight for weight basis (Johnson et al., 1980).

Non-vascular cells such as fibroblasts (Baenziger, Becherer and Majerus, 1980), gastric mucosa cells (Whittle, Moncada and Vane, 1978) and white cells (Deckmyn, Proesmans and Vermylen, 1983; Blackwell et al., 1978) produce prostacyclin, however, platelets do not.

Thromboxane A₂ is produced from the endoperoxides PGG₂ and PGH₂ by the action of thromboxane synthetase. It is the major product of arachidonic acid metabolism in platelets and is synthesised and released when platelets aggregate (Hamberg et al., 1975).

Other cells are capable of synthesising thromboxane A₂ including polymorphnuclear leukocytes, and human lung fibroblasts. Some vascular tissues have been shown to be capable of producing thromboxane A₂, including human umbilical artery and rabbit pulmonary artery (Bunting, Moncada and Vane, 1983), although this is unlikely to be physiologically relevant. However, it has been shown that kidneys rendered hydronephrotic by ligation of the

ureters will begin to synthesise thromboxane A₂ (Morrison et al., 1981) suggesting that tissue damage may reorientate arachidonic acid metabolism and this may be important in the pathological situation.

1.4 BIOLOGICAL EFFECTS OF PROSTACYCLIN AND THROMBOXANE

Prostacyclin and thromboxane A₂ have opposing biological actions. Prostacyclin is a potent anti-platelet agent, vasodilator, bronchodilator and cyto-protective agent, while thromboxane A₂ is a potent platelet aggregating agent, vasoconstrictor and cyto-destructive agent. The majority of these properties are mediated through alterations in intracellular cyclic adenosine monophosphate (cAMP) levels. Cyclic AMP in turn controls the free Ca²⁺ concentration which is responsible for cellular response. Cyclic AMP is formed from adenosine triphosphate (ATP) by the action of adenylate cyclase, and is broken down to 5' adenosine monophosphate by the action of phosphodiesterase. The activity of these two enzymes therefore controls intracellular cAMP levels. Prostacyclin and thromboxane A₂ oppose each other through regulation of adenylate cyclase activity (Tateson, Moncada and Vane, 1977).

Prostacyclin is the most potent endogenous stimulus to cAMP production in man, acting by directly stimulating adenylate cyclase (Gorman, Bunting and Miller, 1977). There are specific receptors for prostacyclin on the cell membranes of platelets, vascular cells and other mammalian tissues and these receptors are coupled to adenylate cyclase (Shepherd et al., 1983; MacDermot et al., 1981; Siegl et al., 1979).

Stimulation of cAMP production by prostacyclin has been shown to occur in several types of cell including platelets (Gorman et al., 1977), and endothelial cells (Hopkins and Gorman, 1981). A direct stimulatory effect on cAMP levels in intact arterial tissue has also been documented and the increase in cAMP was shown to precede vascular smooth muscle relaxation (Weksler, 1982). This increase in cAMP and smooth muscle relaxation can be potentiated by phosphodiesterase inhibitors (Moncada and Korb, 1978). However, prostacyclin has no inhibitory effect on phosphodiesterase (Hopkins and Gorman, 1981) and its biological effects can be prevented by inhibitors of adenylate cyclase (Weksler, 1982), confirming its direct stimulatory effect on adenylate cyclase.

The mechanism by which the prostacyclin-stimulated elevation in cAMP affects cellular response is perhaps best documented in platelets where mobilisation of Ca^{2+} is required for shape change (Hathaway, Eaton and Adelstein, 1980), phospholipase activity and subsequent thromboxane A_2 production (Blackwell and Flower, 1983) and the release reaction (Feinman and Ditwiler, 1974). When levels of cAMP are high Ca^{2+} is sequestered in the platelet dense tubular system thereby lowering Ca^{2+} availability, and inhibiting platelet function. When levels of cAMP are low there is increased Ca^{2+} availability and platelet activation is free to occur. Since prostacyclin is a potent stimulator of adenylate cyclase, cAMP levels will rise and Ca^{2+} will be sequestered in intra-cellular storage sites, thereby inhibiting all stages of platelet activation.

In contrast, thromboxane A₂ inhibits adenylate cyclase and therefore reduces cAMP levels, but this only occurs when cAMP has already been increased by a stimulator of adenylate cyclase such as prostacyclin (Whittle and Moncada, 1983). Thromboxane A₂ does not lower basal levels of cAMP, so that although this is the site of an important interaction between prostacyclin and thromboxane A₂, it does not account for all the effects of thromboxane A₂ on platelets and vascular tissue. This can be supported by evidence showing that an adenylate cyclase inhibitor which lowers platelet cAMP levels does not on its own induce platelet aggregation (Salzman et al., 1978).

Prostacyclin affects many aspects of platelet function, and is the most potent endogenous inhibitor of platelet activation in man. It inhibits shape change and platelet procoagulant activity (Ehrman and Jaffe, 1980), availability of fibrinogen receptors which are important for platelet aggregation (Hawiger, Parkinson and Timmons, 1980), and platelet aggregation (Higgs et al., 1978; Ubatuba, Moncada and Vane, 1979). In addition, systemic prostacyclin infusion can inhibit electrically induced thrombus formation in the carotid artery of rabbits (Ubatuba et al., 1979). Prostacyclin may also cause disaggregation of platelet aggregates. Inhibition of platelet adhesion, however, only occurs at very high concentrations (Higgs et al., 1978) which are unlikely to be achieved in vivo. Prostacyclin inhibits thromboxane A₂ production from platelets by reducing Ca²⁺ availability, which inhibits the action of phospholipase and prevents the release of substrate for thromboxane A₂ production.

Thromboxane A₂ is a potent stimulator of platelet aggregation, acting not only by inhibiting adenylate cyclase, but also by directly promoting Ca²⁺ release from intra-cellular storage sites (Gerrard, Butler and White, 1977). It also promotes the platelet release reaction which in turn promotes further aggregation, amplifying the aggregatory response. It is clear therefore that the balance between prostacyclin and thromboxane may be important in the control of platelet function, haemostasis and thrombosis.

Prostacyclin is a potent vasodilator, relaxing vascular smooth muscle, by reducing Ca²⁺ availability secondary to increased levels of cAMP. It affects both large and small vessels, and also acts on both the systemic and pulmonary circulations (Weksler, 1982). As it is not cleared by the lungs, prostacyclin is equally effective following either intra-venous or intra-arterial administration (Armstrong *et al.*, 1978). It is also active in the microcirculation and may be involved in the modulation of local blood flow (Whittle and Moncada, 1983). The contribution of prostacyclin to maintenance of normal vascular tone, however, is unknown but circulating levels are lower than the concentrations needed to affect vascular tone. While prostacyclin cannot function as a circulating vasodilator it is still likely to be important in the local control of vascular tone, and maintenance of vascular integrity in the presence of platelet activation (Whittle and Moncada, 1983). The presence of specific prostacyclin receptors on vascular tissue also supports a role for prostacyclin in control of vascular tone and local blood flow (MacDermot *et al.*, 1981).

Thromboxane A₂ is a potent vasoconstrictor acting on all vascular beds including umbilical artery, coronary artery, cerebral artery and mesenteric artery (Whittle and Moncada, 1983). Although it is unlikely to be involved in the physiological regulation of vascular tone and blood flow, it may be an important mediator of vasospasm in conditions associated with platelet activation and vasoconstriction.

Prostacyclin and thromboxane have opposing actions on renal vessels, however, prostacyclin also promotes renin release independently of beta-adrenergic stimulation or haemodynamic changes and this may reflect a local role for prostacyclin in regulating juxta-glomerular function (Patrono et al., 1982).

In the bronchopulmonary system, prostacyclin and thromboxane A₂ again have opposing actions. Prostacyclin causes pulmonary vasodilation and bronchodilation while thromboxane A₂ causes pulmonary vasoconstriction and bronchoconstriction (Whittle and Moncada, 1983).

In the stomach prostacyclin reduces acid production and increases blood flow. It also seems to have a gastric cytoprotective action which is separate from its effects on acid output and blood flow. Thromboxane A₂ on the other hand promotes gastric ulceration and reduces mucosal blood flow (Whittle and Moncada, 1983).

The cyto-destructive properties of thromboxane A_2 can usually be explained by its vasoconstrictor and platelet aggregatory functions, which cause tissue ischaemia. It may also, however, have a direct cytotoxic effect, possibly due to a lytic effect on cell membranes leading to release of tissue destructive lysosomal enzymes. These enzymes enhance the tissue damage caused by local ischaemia (Whittle and Moncada, 1983). In contrast, prostacyclin has been shown to have some cytoprotective effects as it can reduce infarct size, oxygen demand and enzyme release in experimental models of myocardial infarction independently of its inhibitory effect on platelet aggregation and coronary vasodilation (Ogletree *et al.*, 1979). It has also been shown to have cytoprotective effects in other situations such as endotoxic shock and hypoxic liver damage (Whittle and Moncada, 1983). These cytoprotective qualities may also occur in the clinical situation as a symptomatic improvement in peripheral vascular disease and Raynaud's phenomenon has been documented up to several months after termination of prostacyclin infusions (Belch *et al.*, 1983a; Belch *et al.*, 1983b). Clearly this benefit cannot be attributed to the short lived vasodilator and anti-platelet effects of prostacyclin infusion, which disappear a few minutes after stopping the infusion. This cytoprotective effect of prostacyclin therefore does seem to occur both in the experimental and clinical situations but as yet is poorly understood.

Prostacyclin was initially thought to function as a circulating hormone in man (Moncada *et al.*, 1978). This concept was supported by reported levels for its stable hydration product

6-keto-PGF_{1α} of 70-100 pg/ml, measured both by radioimmunoassay (Mitchell, 1978) and by gas-chromatography mass-spectrometry (Hensby et al., 1979). In 1982, however, it was reported that the absolute value for 6-keto-PGF_{1α} in peripheral venous plasma was below 5 pg/ml using the technique of negative ion chemical ionisation gas chromatography - mass spectrometry (Blair et al., 1982). This was compatible with the results of other workers using gas chromatography with electron capture detection (Christ-Hazelhof and Nugteren, 1981) and radioimmunoassay (Siess and Dray, 1982) who reported similarly low levels. These levels are probably not compatible with the concept that prostacyclin is a circulating hormone in man. However, this does not preclude a role for prostacyclin functioning as a local hormone in the regulation of platelet - vessel wall interaction and in local control of vascular tone and blood flow (Patrono, Preston and Vermlyen, 1984). There is also considerable evidence to support a functional role for prostacyclin in vivo. There are specific prostacyclin receptors coupled to adenylate cyclase in platelets, vascular cells and other mammalian tissues (Shepherd et al., 1983; MacDermot et al., 1981; Siegl et al., 1979). Furthermore, it has been shown that mild stimuli can stimulate prostacyclin production from human blood vessels (Ritter et al., 1983). It is thought that following vascular injury the extent of thrombus formation is limited and vascular patency maintained by prostacyclin. Failure of this mechanism might therefore result in excessive platelet consumption and intravascular thrombosis. Prostacyclin deficiency has been documented in diseases such as pregnancy induced hypertension (Goodman et al., 1982; Downing, Shepherd and Lewis, 1980; Remuzzi et al., 1980) and haemolytic

uraemic syndrome (Remuzzi et al., 1981) which are characterised by platelet consumption and intravascular thrombosis in the microcirculation, and this would support the concept of prostacyclin acting as a vasoprotective hormone.

1.5 THE REGULATION OF PROSTACYCLIN SYNTHESIS AND THE EFFECT OF DRUGS ON PROSTACYCLIN PRODUCTION

Prostacyclin synthesis is modulated by a wide variety of naturally occurring biological substances and by drugs. Thrombin induces prostacyclin production in human endothelial cells and this may be a physiological mechanism to prevent the unlimited growth of thrombi at sites of vascular injury (Weksler, Ley and Jaffe, 1978). Trypsin, bradykinin, histamine and angiotensin II can also stimulate prostacyclin production (Weksler, 1982).

High density lipoproteins can increase prostacyclin production while low density lipoproteins can inhibit it (Weksler, 1982) and this may be important in the association between low density lipoproteins and atherosclerosis.

Lipid peroxidation induced by free radical formation may also be important in the regulation of prostacyclin production. Lipid peroxides inhibit prostacyclin production by inactivating prostacyclin synthetase and also cyclo-oxygenase (Warso and Lands, 1983). Atherosclerotic plaques have long been known to have increased levels of lipid peroxides which may predispose to further vascular damage and thrombus formation by inhibiting prostacyclin production (Warso and Lands, 1983).

Serum is known to possess prostacyclin stimulating activity and the factor responsible may be platelet derived growth factor, which has been shown to stimulate prostacyclin production independently of its mitogenic effect on smooth muscle cells (Bunting, Moncada and Vane, 1983).

Control of prostacyclin production therefore is possibly due to a variety of substances, however, the precise mechanisms are yet unknown.

Aspirin is the most studied inhibitor of prostacyclin production, it irreversibly acetylates cyclo-oxygenase and therefore permanently inactivates the enzyme. It was initially thought that vascular cyclo-oxygenase was less sensitive than platelet cyclo-oxygenase, suggesting that low dose aspirin might function as a selective inhibitor of thromboxane A₂ (Burch et al., 1978). It was subsequently shown that vascular cyclo-oxygenase was equally sensitive to aspirin (Jaffe and Weksler, 1979). However, unlike platelets, endothelial cells have the capacity to regenerate cyclo-oxygenase and will recover significantly within 4 - 6 hours of a single dose of aspirin and recover completely within 24 - 36 hours (Jaffe and Weksler, 1979).

More recent studies have shown that daily ingestion of aspirin at doses as low as 20 or 40 mg will result in cumulative inhibition of both prostacyclin and thromboxane A₂ production (Preston, Greaves and Jackson, 1981; Fitzgerald et al., 1983), although there may be a relative sparing of prostacyclin production (Weksler et al., 1983). The use of infrequently administered low

dose aspirin may therefore permit a discrimination to be made between inhibition of prostacyclin and thromboxane production. In support of this hypothesis, a recent study has shown that a daily dose of 9 mg of aspirin can significantly reduce thromboxane A₂ while sparing prostacyclin production (Toivanen, Ylikorkala and Viinikka, 1984).

Non-steroidal anti-inflammatory agents also inhibit cyclo-oxygenase but do so reversibly and are much less potent than aspirin (Weksler, 1982). Corticosteroids inhibit prostacyclin production by inhibiting phospholipase A₂ and thereby reducing substrate availability (Blackwell and Flower, 1983).

Selective inhibition of thromboxane synthetase by drugs such as dazoxiben, also represents a possible method of enhancing prostacyclin production. Vascular endothelium has been shown to have the ability to use platelet derived endoperoxides to form prostacyclin (Marcus et al., 1980). Inhibition of thromboxane synthetase may therefore divert endoperoxides from platelets to vascular tissue and thereby increase prostacyclin production. Dazoxiben has been shown to effectively block thromboxane synthetase in human platelets, although it is not clear whether vascular prostacyclin production is enhanced (Tyler, Saxton and Parry, 1981; Randall et al., 1981).

Nitroglycerin has been shown to increase prostacyclin production from human endothelial cells (Levin et al., 1981), and dipyridamole, a phosphodiesterase inhibitor, may enhance the effect of prostacyclin by reducing breakdown of cAMP.

Dipyridamole was initially thought also to stimulate prostacyclin production (Vermylen, Chamone and Verstraete, 1979), but this has since been shown not to occur (Deckmyn et al., 1984). Dipyridamole does, however, delay exhaustion of prostacyclin production from irritated vessels (Deckmyn et al., 1984) by reducing peroxides which would otherwise inactivate vascular cyclo-oxygenase (Deckmyn et al., 1984).

It is clear that there are several possibilities to protect or enhance prostacyclin production, or to re-orientate the prostacyclin - thromboxane balance, however, the most effective technique in the clinical situation remains to be established.

Regulation of thromboxane A₂ production and platelet function are discussed in chapter 2.

1.6 PROSTACYCLIN AND THROMBOXANE IN DISEASE

Several conditions associated with endothelial damage and platelet activation have been associated with a disturbance of prostacyclin and thromboxane metabolism, although no direct causal relationship has yet been documented.

Atherosclerotic tissue is known to have a decreased capacity to produce prostacyclin (Sinzinger, Feigl and Silberbauer, 1979). This may be due to increased levels of lipid peroxides, which can inactivate the enzymes responsible for prostacyclin production (Warso and Lands, 1983). Low density lipoproteins can reduce prostacyclin production possibly again through an increase in lipid peroxide formation (Weksler, 1982) and this may be involved

in the association between hyperlipidaemia and arteriosclerosis. Diabetes mellitus is well known to be associated with premature vascular disease and increased thromboxane A₂ production enhanced platelet aggregation and reduced vascular prostacyclin production have all been documented in this condition (Halushka, Dollery and MacDermot, 1983).

Thromboxane A₂ has also been implicated in the pathogenesis of myocardial infarction (Coker *et al.*, 1981), arrhythmias (Coker and Parratt, 1983), vasospastic angina (Lewy *et al.*, 1979; Robertson *et al.*, 1981) and angina in patients with ischaemic heart disease (Hirsh *et al.*, 1981). Haemolytic - uraemic syndrome, thrombotic thrombocytopenic purpura and pregnancy induced hypertension, are also diseases associated with reduced prostacyclin production (Remuzzi *et al.*, 1981; Goodman *et al.*, 1982; Bussolino *et al.*, 1980; Downing, Shepherd and Lewis, 1980). In conclusion, alterations in the synthesis of both thromboxane and prostacyclin appear to be involved in the pathophysiology of several cardiovascular diseases, however, their precise roles in the aetiology of these conditions remains to be defined.

1.7 THERAPEUTIC APPLICATIONS OF PROSTACYCLIN

In view of prostacyclin's potent anti-platelet and vasodilatory properties, infusion of prostacyclin may be of benefit in situations associated with platelet consumption and vasoconstriction. As it is not metabolised by the lungs it can be given effectively by intra-venous infusion. It initially found a role in extracorporeal circulations, such as charcoal haemoperfusion, haemodialysis and cardiopulmonary bypass, where

it reduced both platelet loss and activation (Weksler, 1982). However, because of its therapeutic potential its use was rapidly explored in other situations. It has been used successfully in both severe peripheral vascular disease and Raynaud's phenomenon (Belch et al., 1983a; Belch et al., 1983b) and its beneficial effects in these situations were still apparent up to several months after termination of the infusion, despite the short half-life of prostacyclin. This prolonged benefit may be due to the poorly understood cyto-protective effect which prostacyclin seems to possess. Its use is also being explored in ischaemic heart disease where it may limit infarct size and reduce the frequency of anginal attacks (Henriksson, Edhag and Wennmalm, 1985; Szczeklik et al., 1985).

Prostacyclin infusion has also been used in haemolytic uraemic syndrome (Beattie et al., 1981) where it may help reduce platelet consumption. It has more recently been used in pregnancy induced hypertension where it was of some value both as an hypotensive and anti-platelet agent (Belch et al., 1985).

However, prostacyclin is a difficult drug to use. It is unstable, requires to be given by continuous intravenous infusion and the dose related side effects of headache, flushing, nausea, and hypotension limit the dose range and mean that patient supervision is required. Despite these limitations the potential benefits of prostacyclin infusion are enormous and further studies are required to establish which diseases might benefit from its use. The application of prostacyclin therapy to

clinical situations may be facilitated by the development of more stable and orally active analogues, which would overcome some of the difficulties associated with the use of this powerful drug.

CHAPTER 2

PLATELETS

2.1 PLATELET STRUCTURE

The circulating platelet is an anucleate cytoplasmic fragment, originating from megakaryocytes. They are biconvex discs with a mean diameter of 3.6 ± 0.7 (SD) μm , 0.9 ± 0.3 μm thick and 7.06 ± 4.85 fl in volume (Frojmovic and Panjwani, 1976) and are the smallest formed elements of the blood. When studied by scanning electron microscopy their surface appears fuzzy and contains many indentations, which are the openings of an elaborate tubular system which extends throughout the interior of the platelet, forming the so called open canalicular system (White and Gerrard, 1976). Transmission electron microscopy has allowed more detailed study of the platelet anatomy and the recognition of several distinct regions (White, Clawson and Gerrard, 1981), a peripheral zone, a sol-gel zone, and an organelle zone (figure 2.1).

2.1.1 The Peripheral Zone

The peripheral zone forms the platelet wall with the many invaginations of the open canalicular system connecting the cell surface with the interior. It plays a major role in platelet function, maintaining cell integrity, providing receptor sites for stimulating or inhibiting agents, mediates platelet-platelet and platelet-surface interactions and provides a phospholipid surface to accelerate blood coagulation. The peripheral zone is made up of 3 morphological parts, the external coat or glycocalyx, the unit membrane and the submembrane region.

ULTRA STRUCTURES OF DISCOID PLATELET IN CROSS SECTION (EQUATORIAL PLANE)

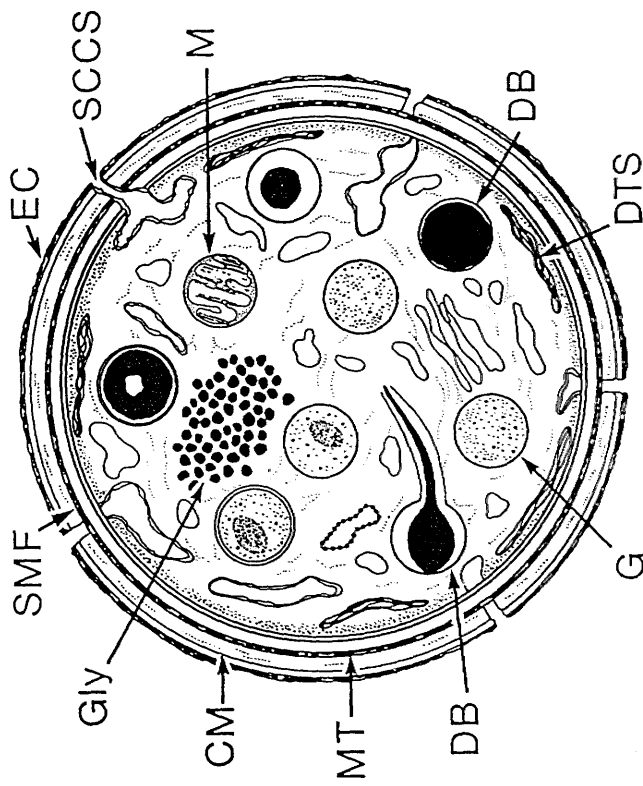


FIGURE 2.1

Diagrammatic representation of platelets as they appear in equatorial cross section on transmission electron microscopy. The peripheral zone includes the exterior coat (EC), the unit membrane (CM), and the submembrane filament area (SMF) which also lines the channels of the open or surface connected canalicular system (SCCS). The sol-gel zone is the matrix of the platelet containing actin, myosin, microtubules (MT) and glycogen (Gly). Within the sol-gel zone lie the organelles including mitochondria (M), granules (G), dense bodies (DB) and dense tubular system (DTS) collectively termed the organelle zone (After White and Gerrard, 1976).

The external coat is rich in glycoproteins, some of which are unique to platelets, and several platelet membrane glycoproteins have been characterised (GP I, II, III, etc.) (Coller, 1984). The role of these substances in platelet adhesion and aggregation has been elucidated by studies on individuals with congenital disorders of platelet function, in whom there is a deficiency of certain of these glycoproteins. GP I for example is necessary for normal platelet adhesion while GP II and III are required for platelet aggregation (Phillips, 1980). The surface glycoproteins have terminal sialic acid carboxyl groups which contribute to a negative surface charge on the platelet, and it is likely that this electrostatic repulsion plays an important role in preventing spontaneous aggregation, since reducing the charge has been shown to increase aggregation (Greenberg et al., 1975).

The unit membrane is a phospholipid bilayer which plays an important role in accelerating coagulation, and contains enzymes involved in membrane transport and cyclic AMP metabolism. In addition it is rich in arachidonic acid, the lipid precursor of prostaglandins and thromboxane (Marcus, 1976).

The submembrane region is the space between the unit membrane and the circumferential band of microtubules. It contains a system of filaments which are involved in maintaining platelet shape and in pseudopod formation and clot retraction.

2.1.2 The Sol-Gel Zone

The sol-gel zone is a viscous matrix inside the platelet composed of the contractile proteins, actin and myosin, and the non-contractile protein, tubulin (Coller, 1984), which make up the platelet cytoskeleton. The cytoskeleton is responsible for the platelets normal disc-like shape and also for the rapid shape change which can occur. When platelets are activated, they transform into small irregular spheres with filopodia and pseudopodia (figure 2.2). This is a contractile response of the cytoskeleton, due to Ca^{2+} dependent phosphorylation of myosin by the enzyme myosin kinase which is triggered by an increase in free cytoplasmic Ca^{2+} acting in concert with calmodulin (Nachmias, Kavalier and Jacobowitz, 1985; Hathaway, Eaton and Adelstein, 1980). The phosphorylated myosin in turn interacts with actin and contraction ensues. A further control mechanism also exists as the kinase can be inhibited by increased levels of intracellular cyclic AMP.

2.1.3 Organelle Zone

Within the platelet are a large number of granules which have been subdivided into α -granules, dense bodies (due to their electronmicroscopic appearance) and lysosomes. They have a random distribution but become centralised when platelet activation occurs. They act as stores for a large number of pharmacologically active substances (table 2.1). The α -granules are the most numerous of these structures and contain proteins such as β -thromboglobulin, platelet factor 4 and platelet basic protein, which have pro-coagulant, chemotactic and heparin neutralising actions (Coller, 1984).

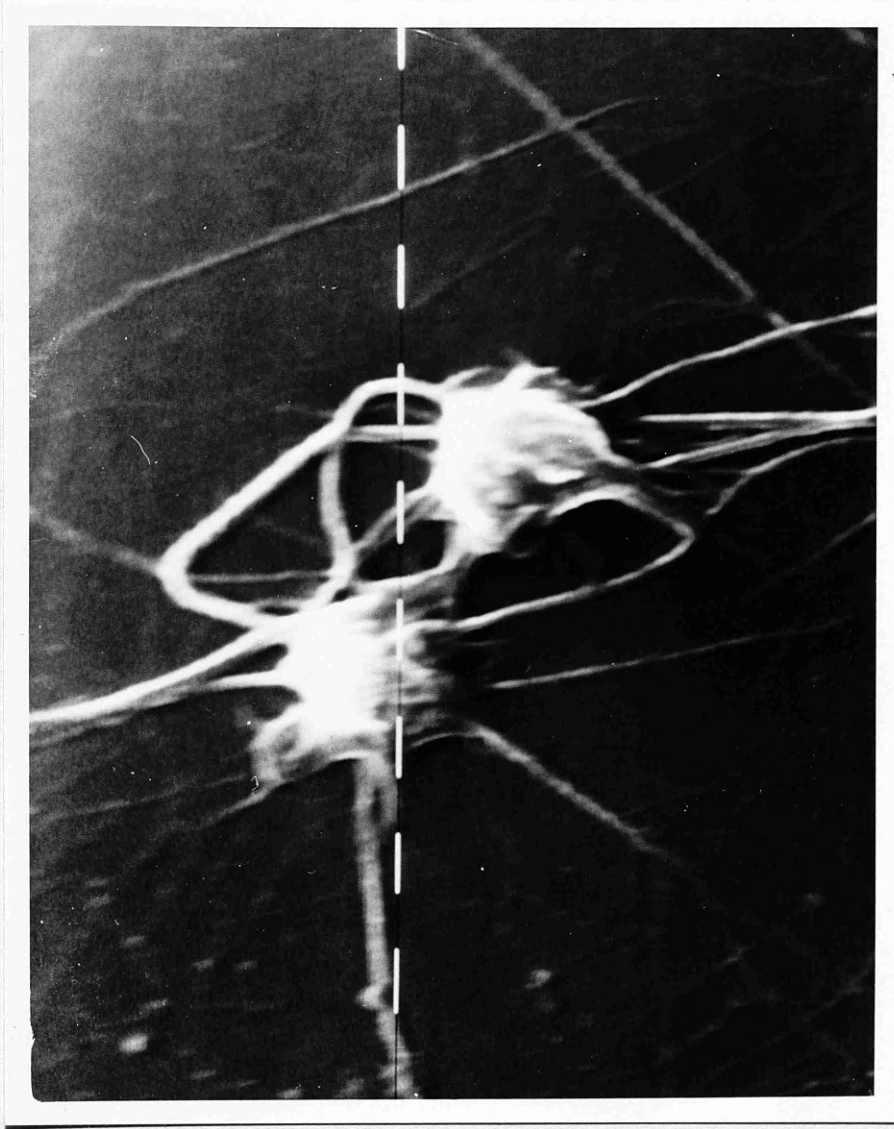


FIGURE 2.2

Scanning electron micrograph of platelet stimulated with thrombin showing shape change and pseudopodia formation (magnification x 5,000). Courtesy of A.R. Saniabadi

TABLE 2.1

Dense Granules	α -granules	Lysosomes
	Platelet basic Protein	
Adenosine diphosphate (ADP)	Platelet Factor 4	β -Galactosidase
	β -Thromboglobulin	β -N-Acetyl-glucosamidase
Adenosine Triphosphate (ATP)	Platelet Derived Growth Factor	β -glycerophosphate
	Fibrinogen	Aryl sulphatase
Ca^{2+}	Fibronectin	
Serotonin	von Willebrand's Factor	
Pyrophosphate	Thrombospondin	
	Factor V	

Platelet Granule Contents

In addition, α -granules also contain platelet derived growth factors which have mitogenic activity for several cell lines including smooth muscle cells and fibroblasts (Niewiarowski, 1981). These substances are released when platelets adhere to the subendothelium of damaged blood vessels, and while these factors may be part of the normal repair process, they have been implicated in the initiation of atherosclerosis by stimulating proliferation of smooth muscle cells within the media of blood vessels (Ross and Glomset, 1976). The α -granules also contain several glyco-proteins such as fibrinogen, von Willebrand factor and fibronectin. Lysosomes are membrane bound organelles containing hydrolytic enzymes which may be involved in the inflammatory response by altering vascular permeability.

Dense bodies contain high concentrations of ADP, ATP, calcium and serotonin. Since ADP and calcium are important in platelet aggregation, release of the contents of dense bodies acts as a positive feedback mechanism for promoting aggregation. Serotonin on its own has only weak platelet aggregating activity, however it enhances the aggregatory response to other inducers and causes vasoconstriction.

Other platelet organelles include the dense tubular system (White et al., 1981) a membranous system which sequesters Ca^{2+} analogous to the sarcoplasmic reticulum of skeletal muscle, several mitochondria (White et al., 1981), and the occasional Golgi apparatus (White and Gerrard, 1976).

2.2 PLATELET FUNCTION

2.2.1 Platelet adhesion

Platelets normally interact only with red cells, white cells and vascular endothelium. All other surfaces are foreign to them and they will adhere to any such foreign surface which they encounter. Much of the available information on platelet adhesion comes from the work of Baumgartner (1973). Baumgartner exposed de-endothelialised aortic strips to flowing blood, then examined the aortic strips microscopically following fixation with glutaraldehyde. Using this technique, many of the factors influencing platelet adhesion were studied. Both platelet adhesion and mural thrombus formation are influenced by the rate of blood flow over the subendothelial surface. The higher the flow rate the greater the number of platelets deposited (Baumgartner, 1973; Turitto and Baumgartner, 1979). The number of platelets in the blood and the haemataocrit are also important, as red cells physically enhance platelet deposition by increasing the number of platelet-vessel wall interactions which occur (Turitto and Baumgartner, 1975).

Further information on adhesion came from studies of patients with von Willebrand's disease. In von Willebrand's disease factor VIII-von Willebrand factor is reduced or abnormal and it has long been known that platelet adhesion is impaired in this condition (Salzman, 1963), and can be corrected by adding factor VIII-von Willebrand's factor from normal plasma (Weiss et al ., 1978). Factor VIII-von Willebrand's factor seems to be especially important at high flow rates (Baumgartner, Tschopp and Meyer, 1980).

In Bernard-Soulier syndrome platelet count is low, the platelets are very large and biochemically abnormal, as they lack glycoprotein Ib on the cell surface (Nurden and Caen, 1979), and they have reduced adhesion to subendothelium. Further evidence for the role of glycoprotein Ib in adhesion came from the work of Tobelem et al., (1976) who showed that an antibody to glycoprotein Ib inhibited the adhesion of normal platelets to damaged vascular tissue.

Platelets also adhere more readily to structures lying deep within the vessel wall, such as collagen, than the structures immediately below the vascular endothelium (Crowley and Pierce, 1981).

2.2.2 Platelet Aggregation

The understanding of platelet reactivity owes much to the development of the photometric aggregometer (Born, 1962; Born and Cross, 1963; O'Brien, 1962) which has proved invaluable in the study of platelet aggregation. Platelet aggregation is a series of morphological and functional changes, starting with shape change and going on to aggregation (figure 2.3) and release of a variety of substances such as Ca^{2+} , ADP, thromboxane A_2 and serotonin, which amplify the aggregation response.

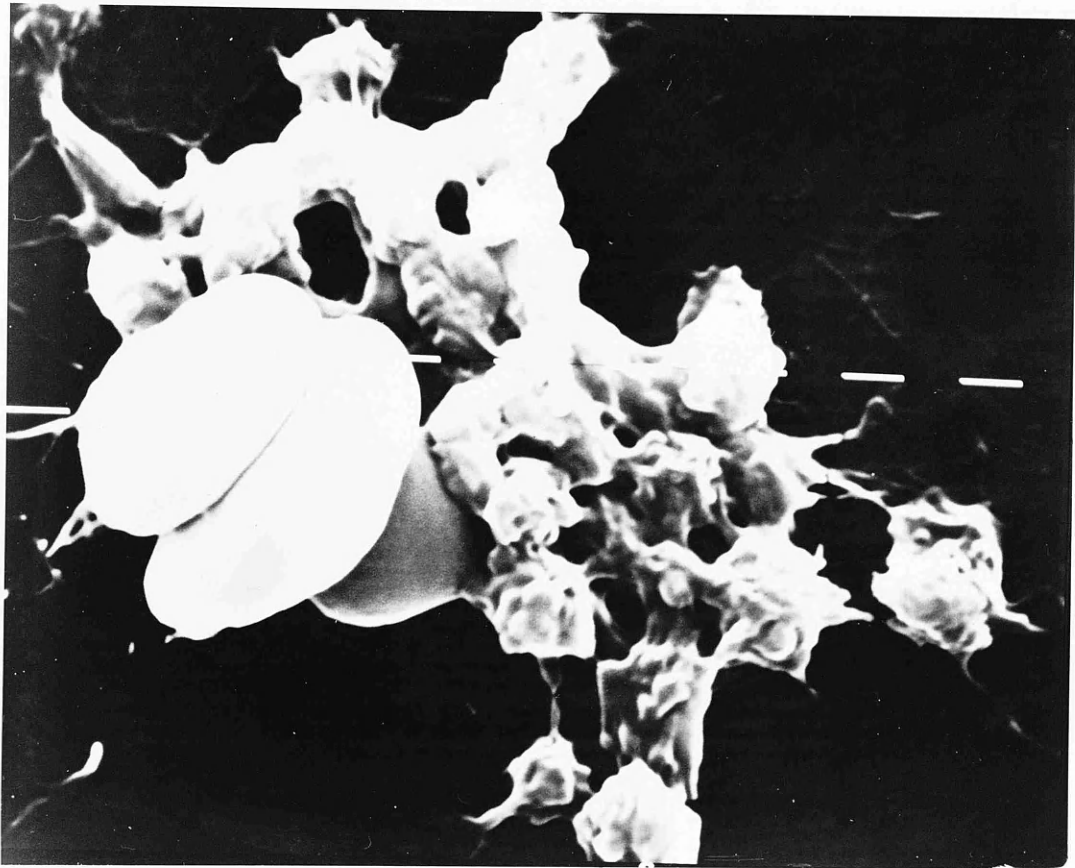


FIGURE 2.3

Scanning electron micrograph showing a platelet aggregate, following stimulation by thrombin in whole blood, note the red cells on the left of the micrograph (magnification x 5,000) Courtesy of A.R. Saniabadi.

Aggregation can be induced by a variety of agonists such as ADP, collagen, thrombin, adrenalin, ristocetin, arachidonic acid and platelet activating factor (PAF). The primary stimulus involves interaction of the agonist with a receptor site, which is often specific, on the platelet membrane.

While shape change usually precedes aggregation, this is not an absolute prerequisite as adrenalin induced aggregation is not preceded by shape change (Coller, 1984). The physiological role of shape change is not entirely clear but is thought to expose the open canalicular system where specific fibrinogen and factor VIII-von Willebrand factor receptors may be located. It also allows a greater surface area for contact with other platelets or foreign surfaces.

There are three pathways of aggregation which will be described (Vargaftig, Chignard and Benveniste, 1981).

The First Pathway of Aggregation

Adenosine diphosphate is a specific platelet stimulating agent, which causes a typical biphasic aggregation response which is dependent on both fibrinogen (Cross, 1964) and divalent cations (Han and Ardlie, 1974).

The primary or reversible phase is produced by an agent such as ADP which exposes fibrinogen receptors on the platelet membrane allowing aggregation to occur (Bennett and Vilaire, 1979), by means of fibrinogen molecules occupying their receptors and forming cell bridges with other platelets.

The second or irreversible phase is mediated by generation of prostaglandins, thromboxane A_2 , and the subsequent release of substances such as ADP, serotonin, and fibrinogen. Inhibitors of cyclo-oxygenase such as aspirin will inhibit the second phase of ADP induced aggregation (Vargaftig et al., 1981) by preventing prostaglandin and thromboxane A_2 formation. Whether the second phase of aggregation will occur depends on the dose of ADP. If insufficient ADP is present to induce secondary aggregation then disaggregation will occur and the platelets will resume their non-adhesive disc-like shape.

The Second Pathway of Aggregation

The second pathway of aggregation is arachidonic acid dependent, and is triggered by agonists such as collagen and thrombin (Vargaftig et al., 1981). The agonist binds to a receptor and stimulates phospholipase A_2 , possibly by increasing Ca^{2+} as this enzyme is Ca^{2+} dependent. Phospholipase A_2 breaks down phosphatidyl-ethanolamine, a membrane phospholipid, to produce free arachidonic acid, which is converted by cyclo-oxygenase to endoperoxides, and then by thromboxane synthetase to thromboxane A_2 . Thromboxane A_2 stimulates aggregation both directly, as it is a potent inducer of aggregation (Hamberg et al., 1975), and indirectly by release of the contents of the platelet granules (Hamberg et al., 1974; Hamberg et al., 1975) which will cause further aggregation. This forms a positive feedback loop.

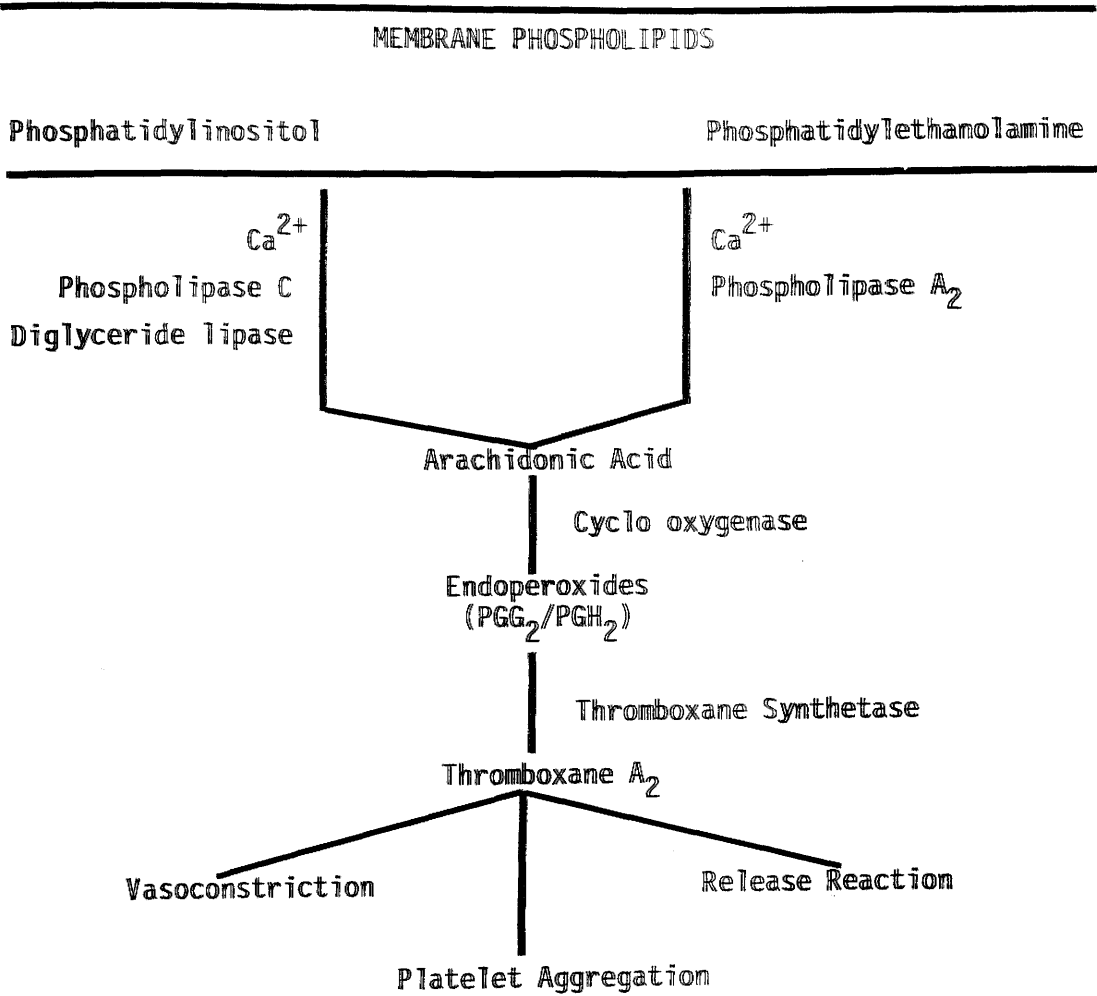


FIGURE 2.4

The second or arachidonic acid dependent pathway of aggregation. Collagen and thrombin will stimulate this pathway by activating the phospholipases. Exogenous arachidonic acid will bypass the phospholipase step, and induces aggregation by generation of thromboxane A₂.

Arachidonic acid can also be liberated by the action of phospholipase C and diglycerol lipase, which are also Ca^{2+} dependent, on phosphatidylinositol in the cell membrane.

Exogenous arachidonic acid is not a direct stimulator of aggregation but acts through production of thromboxane A_2 . This pathway is illustrated in figure 2.4.

The Third Pathway of Aggregation

The existence of a possible third pathway of aggregation came to light when it was discovered that platelet aggregation induced by Ca^{2+} ionophore or high concentrations of thrombin or collagen, was not abolished by blocking both ADP and arachidonic acid pathways simultaneously, and PAF, a phospholipid, may be responsible for this (Vargaftig et al., 1981). PAF induces aggregation and release of both α -granules and dense bodies and is independent of arachidonic acid and ADP. The mode of release of PAF is unknown but Ca^{2+} , and high dose collagen and thrombin will cause release of sufficient PAF from the platelets to cause aggregation (Vargaftig et al., 1981).

2.3 PLATELET PRODUCTION, KINETICS AND SIZE

Platelets are produced from the fragmentation of the cytoplasm of megakaryocytes. Megakaryocytes are derived from multipotential cells in the bone marrow and are unique as they can reproduce their deoxyribonucleic acid (DNA) without undergoing cell division, a process termed endomitosis. They start as diploid stem cells and their DNA content doubles several times until they are polyploid with somewhere between 2 and 32 times the DNA

content of a single cell. The cytoplasm then enlarges and granules are formed as the cell matures. The whole maturation process takes between 4 and 5 days. The cytoplasm then fragments and platelets are released into the circulation (Coller, 1984).

Recently it has been suggested that the whole megakaryocyte is released from the marrow and fragments after being trapped in the pulmonary circulation (Trowbridge, Martin and Slater, 1982).

Platelet production is thought to be under the control of a thrombopoietic hormone (Coller, 1984; Firkin, 1984) which increases when platelet count falls and vice-versa.

The normal circulating platelet count in man is $150 - 350 \times 10^9$ /litre in whole blood (Sloan, 1951), and the platelet lifespan in normal humans is around 8 - 10 days as determined by radioisotope (Gardner, 1972) and aspirin labelling (Rakoczi et al., 1979; Wallenburg and van Kessel, 1978) techniques.

Platelets are heterogeneous in terms of size and density. Large heavy platelets are thought to be young cells, and progress to smaller lighter platelets with age. The large young platelets are thought to be metabolically and functionally more active than the small older ones (Karparkin and Charmatz, 1969; Karparkin, 1969; Karparkin, 1978).

2.4 PLATELETS AND COAGULATION

Platelets play a central role in haemostasis. Following damage to the vessel wall a platelet plug forms to arrest bleeding. This is dependent on platelet adhesion to collagen in the damaged vessel wall, and subsequent platelet aggregation. The importance of this is seen in von Willebrand's disease where adhesion is defective (Salzman, 1963) and as a result the bleeding time is prolonged.

The platelet plug is insufficient on its own to provide long-term haemostasis and activated platelets are involved in the activation of the coagulation cascade. This culminates in the formation of a fibrin mesh at the site of bleeding which maintains haemostasis and promotes wound healing.

The coagulation cascade is a chain of enzymatic reactions in which proenzymes are converted to active enzymes which in turn activate further enzymes (Davie and Ratnoff, 1964). This finally results in thrombin formation which in turn cleaves fibrin from fibrinogen. The interaction of these clotting factors is accelerated by their being absorbed and concentrated onto a suitable phospholipid surface thereby amplifying the response. Platelets provide such a surface, and in addition activate factors XI and XII directly (Walsh, 1972a; Walsh, 1972b).

Activation of factor X by activated factor IX, and of prothrombin by activated factor X, occur on a negatively charged lipid surface. Platelets have an assymmetric distribution of phospholipids in the two layers of the cell membrane, with the

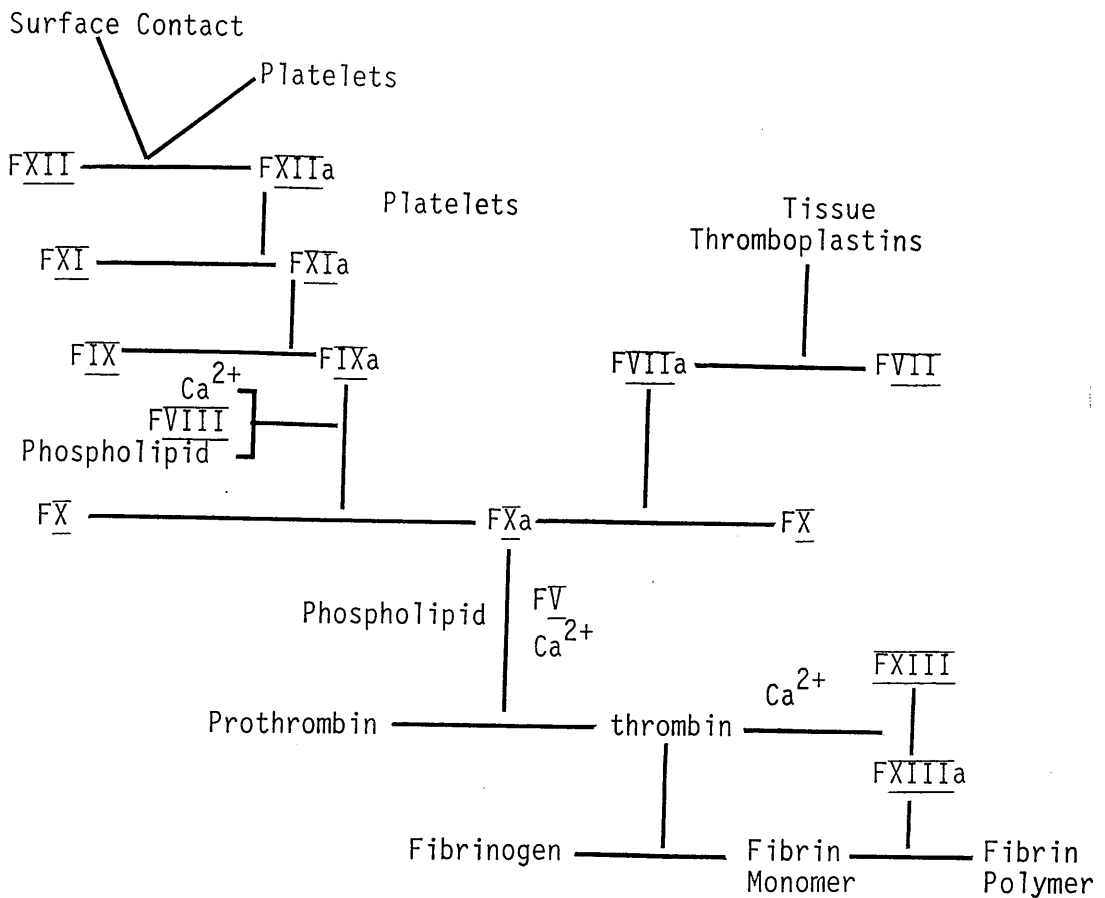


FIGURE 2.5

The Coagulation Cascade

Coagulation is initiated by either the intrinsic pathway, activated by surface contact, or the extrinsic pathway, activated by tissue thromboplastins. In the intrinsic pathway, phospholipid is provided by platelets and in the extrinsic pathway by the phospholipid component of tissue thromboplastins. After activation of factor X both pathways converge. This diagram is simplified as inhibitors of the steps and interactions between the two pathways have been omitted.

(FXII = factor XII; FXIIa = Activated FXII etc.)

most negatively charged phospholipids on the inside. Following activation by thrombin or collagen a transmembrane "flip-flop" of phospholipids occurs and the most negatively charged phospholipids move to the outside, thus providing a catalytic surface for factor X and prothrombin activation (Bangham, 1961; Schick, 1979). This capacity of activated platelets to enhance prothrombin activation has been termed platelet factor 3 (Coller, 1984).

Platelets also release factor V from α granules and bind factor V. When activated, factor V acts as an additional receptor for activated factor X on the platelet membrane promoting thrombin formation (Majerus, Miletich and Kane, 1980). The factor VIII related antigen bound by the platelet may also act as a binding site for the factor VIII coagulant moiety, thereby increasing the concentration of factor VIII coagulant on the platelet surface where it may act as a regulatory protein enhancing activation of factor X (Saito, 1984). Finally, the fibrin becomes intimately involved in the platelet mass (Coller, 1984). A simplified scheme of the coagulation cascade is given in figure 2.5.

2.5 PLATELETS AND THROMBOSIS AND ARTERIOSCLEROSIS

From the foregoing description of platelet function and platelets in coagulation, it is apparent that platelets may play a role in the pathogenesis of thrombotic vascular occlusion and arteriosclerosis.

Platelets probably contribute to arteriosclerosis in two ways. Firstly, platelets form thrombi at the site of endothelial injury and these become organised and incorporated into the vessel wall causing arteriosclerotic narrowing (Jorgensen et al., 1972; Fuster, 1981). Secondly, when platelets are activated following adhesion to the subendothelium, they release a variety of vasoactive substances including thromboxane A₂ which increases vascular permeability and causes vasoconstriction. A mitogenic factor, platelet derived growth factor, is also released and this causes proliferative changes in the vessel wall which have been implicated in the pathogenesis of arteriosclerosis (Ross and Glomset, 1976). Additional evidence for the role of platelets in arteriosclerosis comes from animal models. Animals rendered thrombocytopaenic, are protected from arteriosclerosis induced by repeated endothelial injuries (Friedman et al., 1977) while animals rendered thrombocythaemic develop increased arteriosclerotic lesions if they are also fed on a high cholesterol diet (Cohen and McCombs, 1967).

In the clinical situation patients with vascular disease have been shown to have increased platelet turnover rates (Zahavi and Zahavi, 1985; Murphy and Mustard, 1962; Steele et al., 1973), and abnormal platelet function has been reported in patients with a wide variety of vascular disease (Zahavi and Zahavi, 1985; Steele et al., 1973; Mehta, Mehta and Pepine, 1978; Walsh, Pareti and Corbett, 1977). In addition decreased sensitivity of platelets from patients with ischaemic heart disease to prostacyclin has been reported (Mehta and Mehta, 1980). Thromboxane A₂, the major arachidonic acid metabolite in platelets (Hamberg et al., 1975),

which is a potent platelet aggregating agent and vasoconstrictor, has been implicated in thrombotic and arteriosclerotic situations such as ischaemic heart disease (Hirsh et al., 1981; Mehta, 1983) and peripheral vascular disease (Zahavi and Zahavi, 1985).

2.6 CONTROL OF PLATELET FUNCTION

Control of platelet function centres on the concentration of free intracellular Ca^{2+} , which is the intermediary linking receptor occupancy with cellular response. Stimuli triggering platelet activation such as collagen, thrombin or ADP act by promoting Ca^{2+} release from its storage sites in the dense tubular system.

Increasing Ca^{2+} release promotes platelet shape change by activating the Ca^{2+} dependent phosphorylation of myosin, and the subsequent myosin-actin interaction responsible for contraction (Hathaway et al., 1980).

Free intracellular Ca^{2+} also promotes the release reaction (Feinman and Ditwiler, 1974) by causing the platelet granules to fuse with the cell membrane thereby discharging their contents.

In addition Ca^{2+} stimulates production of endoperoxides and thromboxane A_2 , by activating Ca^{2+} dependent phospholipases which act on membrane phospholipids to release arachidonic acid. Arachidonic acid is then converted via cyclo-oxygenase and thromboxane synthetase to thromboxane A_2 .

The free intraplatelet Ca^{2+} concentration is controlled by cyclic adenosine monophosphate (cAMP) concentrations. When levels of cAMP are high Ca^{2+} is taken up by the dense tubular system. When cAMP levels are low Ca^{2+} concentrations can increase and platelet activation occurs (Kaser-Glanzmann, Gerber and Lüscher, 1979).

Cyclic AMP is regulated by 2 enzymes, adenylate cyclase which forms cAMP from adenosine triphosphate (ATP), and phosphodiesterase which breaks down cAMP to 5' adenosine monophosphate (5' AMP). The activity of these enzymes is also regulated by Ca^{2+} concentration. Adenylate cyclase is inhibited by Ca^{2+} while phosphodiesterase is stimulated. Consequently when free intraplatelet Ca^{2+} is high it inhibits formation, and promotes breakdown of cAMP, thereby maintaining high levels of Ca^{2+} and promoting platelet activation.

Thromboxane A_2 and prostacyclin oppose each other through regulation of adenylate cyclase (Tateson, Moncada and Vane, 1977). The proaggregatory substances thromboxane A_2 and prostaglandins G_2 and H_2 inhibit adenylate cyclase allowing free intraplatelet Ca^{2+} to rise. They also have a direct effect, promoting Ca^{2+} release from intracellular storage sites (Gerrard, Butler and White, 1977).

On the other hand, platelet inhibitory prostaglandins such as PGI_2 stimulate adenylate cyclase, increasing cAMP and reducing Ca^{2+} (Tateson et al., 1977).

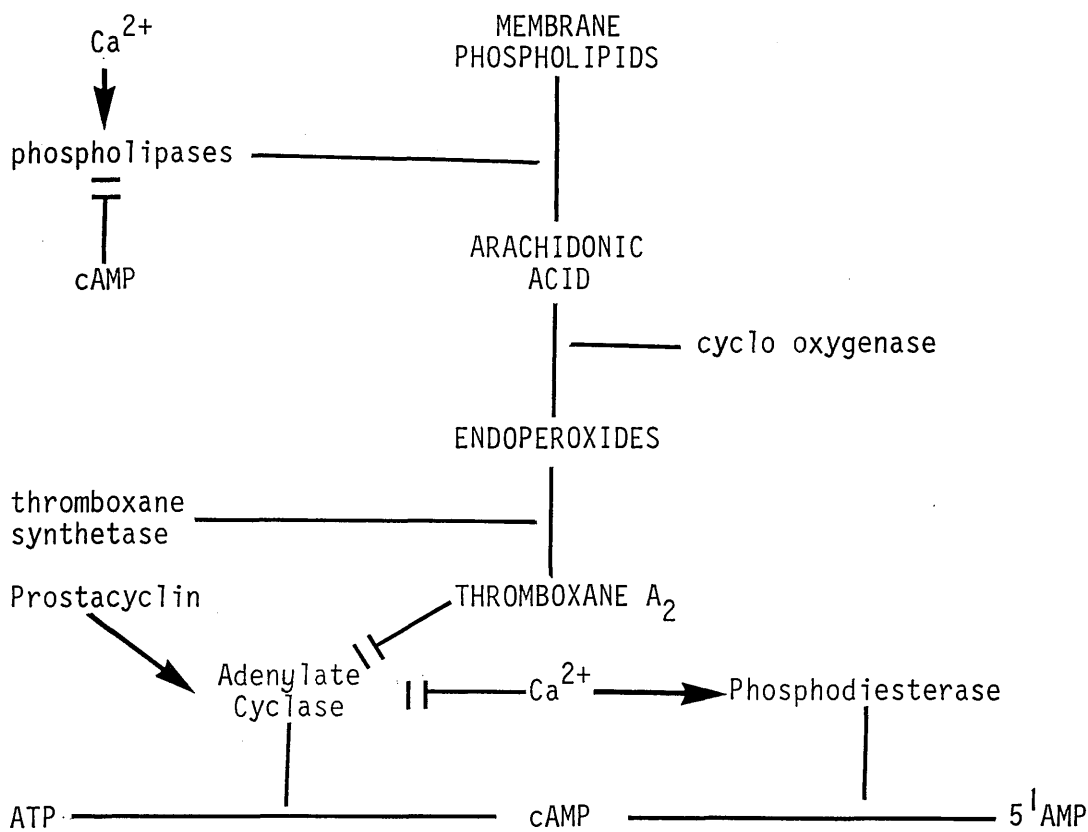


FIGURE 2.6

Effect of Ca^{2+} and cAMP on platelet arachidonic acid metabolism.

(\longrightarrow = stimulatory effect: ---|| = inhibitory effect)

cAMP exerts its effect by lowering Ca^{2+} concentration. Thromboxane A_2 also directly stimulates Ca^{2+} release.

The effects of Ca^{2+} and cAMP on platelet arachidonic acid metabolism are illustrated in figure 2.6.

2.7 PROSTACYCLIN AND THROMBOXANE IN THE PLATELET-ENDOTHELIUM INTERACTION

Platelets do not normally adhere to vascular endothelium (figure 2.7). The reasons for this are unclear, however, there are several possibilities. Firstly, both platelets and endothelium have a negative surface charge, making them naturally repel each other. This, however, cannot be the sole explanation, as platelets adhere readily to the sub-endothelium which has a similar negative charge.

A second possibility is a continuous release of prostacyclin by the endothelium itself. Prostacyclin is the major product of arachidonic acid metabolism in vascular tissue. The ability of the vascular wall to generate prostacyclin is greatest on the endothelial surface, and decreases progressively to the adventitial surface (Moncada et al., 1977).

Prostacyclin is a potent inhibitor of platelet activation, acting by stimulating platelet adenylate cyclase to increase cAMP, which in turn reduces Ca^{2+} availability and maintains platelet stability. Maintenance of the normal disc like shape is also physically unsuitable for cell-cell contact. Prostacyclin also reduces fibrinogen receptor exposure on the platelet and this may also help maintain platelet stability (Hawiger, Parkinson and Timmons, 1980). It is therefore thought that prostacyclin plays a major role in preventing platelets from adhering to endothelium. However, prostacyclin is not present in sufficient

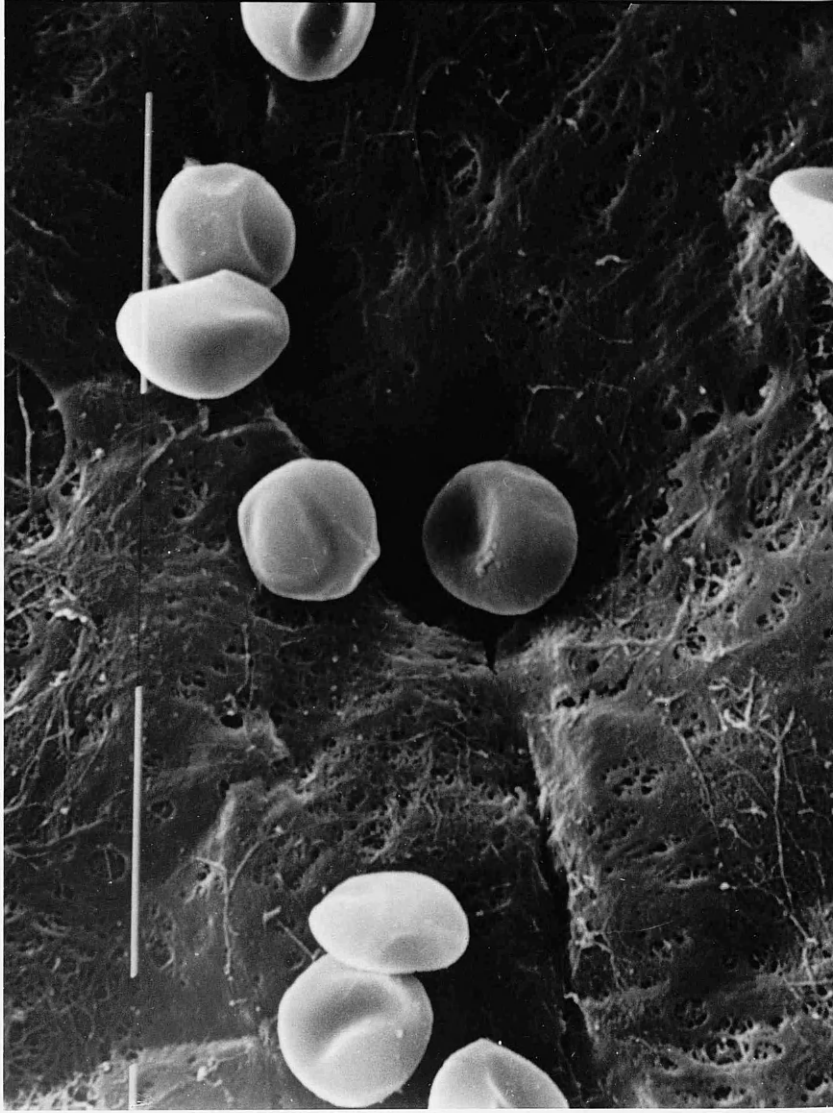


FIGURE 2.7

Scanning electron micrograph of healthy umbilical artery perfused with oxygenated Ringer's lactate buffer for 2 hours then fresh citrated whole blood from a healthy volunteer. Note lack of platelet deposition on the vessel wall, and healthy endothelium, in contrast to figure 2.8. (magnification x 2,500)

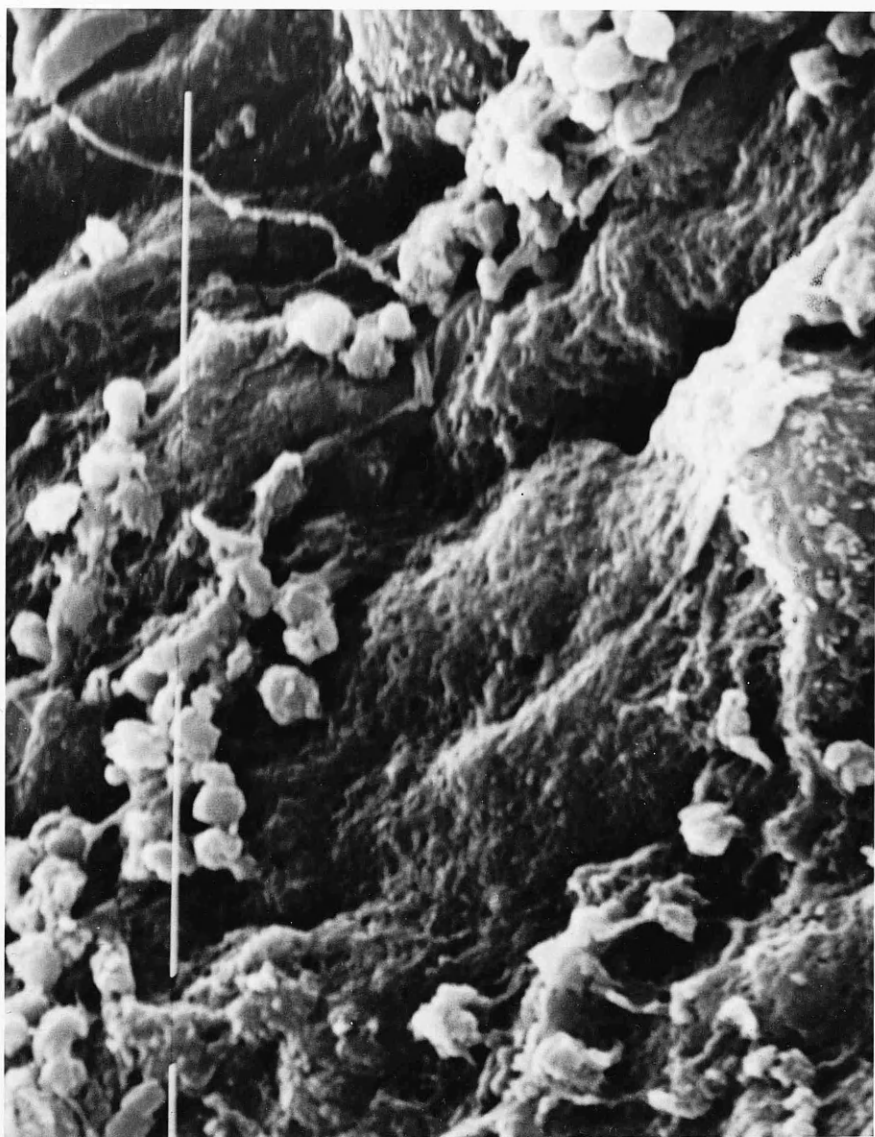


FIGURE 2.8

Scanning electron micrograph of a section of the same umbilical artery as in figure 2.7. This segment was perfused for 18 hours with unoxygenated Ringer's lactate buffer to exhaust it of prostacyclin production (confirmed by radioimmunoassay of prostacyclin metabolite), and induce hypoxic endothelial damage. Then perfused with fresh citrated whole blood from the same donor as in figure 2.7. Note damaged endothelial surface and platelet adhesion and aggregation. (magnification x 2,500)

quantity to function as a circulating anti-platelet agent (Blair et al., 1982), although it is still thought to be important in the local platelet-vessel wall interaction (Patrono, Preston and Vermynen, 1984). In addition, the concentration of prostacyclin required to inhibit adhesion is much greater than that required to inhibit aggregation (Higgs et al., 1978), and is unlikely to be achieved even on the endothelial surface. Consequently, prostacyclin cannot alone be responsible for inhibiting platelet adhesion to the vessel wall. It may, however, be important in limiting platelet aggregation and thrombus formation in damaged vessels. Following damage to the endothelium, platelets quickly adhere to the subendothelium (figure 2.8) and the surrounding traumatised vascular tissue releases relatively large amounts of prostacyclin which limit subsequent platelet aggregation and thrombus formation. In addition, the activated platelets release endoperoxides (Hamberg et al., 1974) which can be utilised by the surrounding vessel wall to produce prostacyclin (Bunting et al., 1976; Marcus, Weksler and Jaffe, 1978; Marcus et al., 1980). This in turn may limit further platelet aggregation. These phenomena may be part of a negative feedback loop to prevent excessive platelet activation and thrombus formation at the site of endothelial injury. However, the ability of platelets to provide endoperoxides for vascular conversion to prostacyclin has not been confirmed by other workers (Hornstra, Haddeman and Don, 1979), thus bringing the existence of this possible mechanism into question. A further possible mechanism limiting platelet aggregation and thrombus formation is that thrombin, which is generated by activation of the coagulation system, can stimulate

prostacyclin production (Weksler, Ley and Jaffe, 1978) which could maintain vascular patency by limiting platelet aggregation and producing local vasodilation.

Other possible mechanisms for reducing platelet adhesion, aggregation and thrombus formation include the presence of ADPase and activators of fibrinolysis (Ashford, 1969; Heyns et al., 1974) in the endothelium. However, it is likely that many factors contribute to the "thromboresistance" of vascular endothelium.

2.8 EFFECT OF DRUGS ON PLATELET FUNCTION

From the foregoing discussions it is clear that platelets play a major role in the pathophysiology of vascular disease and a great deal of work has centred on drugs which inhibit platelet activation. There have been innumerable studies on anti-platelet agents, which affect platelet function in a variety of ways. The most widely studied groups are drugs which affect arachidonic acid metabolism, cAMP and Ca^{2+} availability.

2.8.1 Drugs Affecting Arachidonic Acid Metabolism

Inhibitors of Phospholipase

Phospholipases break down platelet membrane phospholipids to produce free arachidonic acid, the substrate for thromboxane A_2 .

Corticosteroids inhibit the action of phospholipases (Hong and Levine, 1976), and also block vascular prostacyclin production, and may thus contribute to the thrombotic problems occasionally seen with steroid administration (Blajchman et al., 1979).

The antimalarial drug, mepacrine, also blocks arachidonic acid release (Winocour, Kinlough-Rathbone and Mustard, 1979). Local anaesthetics reduce phospholipase activity possibly by acting on calmodulin (Wong and Cheung, 1979), an intracellular Ca^{2+} binding protein involved in intra-cellular Ca^{2+} transport. None of these drugs, however, have been tested in clinical situations.

Adrenoceptor antagonists inhibit platelet aggregation and thromboxane A_2 production (Weksler, Gillick and Pink, 1977; Campbell *et al.*, 1981), possibly by reducing phospholipase activity (Vanderhoek and Feinstein, 1979). Some adrenoceptor antagonists have also been shown to improve prognosis in patients following myocardial infarction (Norwegian Multicentre Study Group, 1981; Hjalmarson *et al.*, 1981), and a possible mechanism may be inhibition of platelet function. A fuller discussion of the anti-platelet properties of adrenoceptor antagonists follows in subsequent chapters.

Cyclo-oxygenase Inhibitors

Cyclo-oxygenase occupies a pivotal role in arachidonic acid metabolism, converting arachidonic acid to endoperoxides which are then converted by thromboxane synthetase in the platelet to thromboxane A_2 . In the vessel wall the endoperoxides formed from arachidonic acid are converted to prostacyclin. Cyclo-oxygenase is inhibited by numerous non-steroidal anti-inflammatory drugs such as aspirin (Smith and Willis, 1971; Vane, 1971),

indomethacin (Roth, Stanford and Majerus, 1975), and the newer non-steroidal anti-inflammatory agents such as ibuprofen (Mustard and Packham, 1978).

Aspirin irreversibly acetylates cyclo-oxygenase. Indomethacin blocks cyclo-oxygenase activity but the mechanism is unclear and may be related to reduced Ca^{2+} availability (Northover, 1978). Ibuprofen causes reversible inhibition of cyclo-oxygenase probably competing for the same site as aspirin (Parks, Hoak and Czervionke, 1981).

Sulphinpyrazone is a uricosuric agent which is claimed to have antithrombotic properties, it also reversibly blocks cyclo-oxygenase (Ali and McDonald, 1978).

Aspirin and sulphinpyrazone have been the most extensively used cyclo-oxygenase inhibitors in clinical studies, and have been evaluated in the secondary prevention of myocardial infarction (Aspirin Myocardial Information Study Research Group, 1980; Coronary Drug Project Research Group, 1980; Elwood and Sweetnam, 1980; Anturane Reinfarction Trial Research Group, 1980; Anturan Reinfarction Italian Study Group, 1982). The results of these studies have not been entirely consistent, however, they have shown at least a trend towards reducing the incidence of non-fatal myocardial re-infarction and total mortality.

More recently a combination of dipyridamole and aspirin has been shown to be effective in reducing non-fatal myocardial infarction and cardiac deaths, especially when combined with an adrenoceptor

antagonist (Persantine-Aspirin Reinfarction Study II Research Group, 1985). Although the combination of aspirin and dipyridamole has not been shown to be statistically any better than aspirin alone in the secondary prevention of myocardial re-infarction (Persantine-Aspirin Reinfarction Study Research Group, 1980).

Aspirin has also been shown to be effective in the secondary prevention of cerebrovascular disease, while sulphinyprazole had no effect on its own and a combination of both was not significantly better than aspirin alone (Canadian Co-operative Study Group, 1978). Strangely the benefits of aspirin therapy in this study were confined to males!

Aspirin and dipyridamole have also been shown to be of prophylactic value in pregnancies at high risk of intra-uterine growth retardation and pregnancy induced hypertension (Beaufils et al., 1985).

There is, however, an aspirin dilemma as the beneficial effects of aspirin on platelet function may be offset by a similar inhibitory effect on vascular prostacyclin production (Preston et al., 1981). Clearly this is undesirable. To avoid this problem lower doses of aspirin have been used in an attempt to selectively block platelet aggregation and thromboxane A₂ formation. Platelet thromboxane A₂ production has been shown to be completely inhibited by 20 mg of aspirin a day (Patrignani,

Filabozzi and Patrono, 1982), while prostacyclin metabolite excretion was unaffected, implying that this dose of aspirin had no effect on vascular prostacyclin production.

However, even complete prevention of thromboxane A₂ production does not necessarily reduce platelet aggregation as has been shown with selective thromboxane synthetase inhibitors (Bertele et al., 1981).

A further method of avoiding the effect of aspirin on vascular prostacyclin production is to administer the drug less frequently. The platelet cyclo-oxygenase is permanently acetylated and as the platelet is anucleate it cannot synthesise any further enzyme. In contrast, vascular endothelial cells can synthesise protein and therefore can replace the inactivated enzyme (Jaffe and Weksler, 1979), and maintain prostacyclin production. Since aspirin blocks platelet aggregation and thromboxane A₂ production for at least 24 hours (Preston et al., 1981; Patrignani et al., 1982), there is no need to give the drug more than once a day.

Oral administration of low dose aspirin might also produce pharmacologically active drug concentrations only in the portal circulation and not in the systemic circulation as aspirin is metabolised in the liver. Since platelet cyclo-oxygenase is irreversibly inhibited by aspirin, effective inhibition of platelet function would occur following exposure to aspirin as the platelet passes through the portal circulation, while systemic vascular prostacyclin production would remain unaffected

due to the lower aspirin concentration in the systemic circulation. A recent study (Rosenkranz and Frolich, 1985) has shown such effects to occur after a single dose of 100 mg of aspirin.

Thromboxane Synthetase Inhibitors

The use of thromboxane synthetase inhibitors is theoretically a very attractive method for inhibiting platelet function. They selectively block conversion of endoperoxides to thromboxane A_2 in platelets. Furthermore the endoperoxides may also be diverted to the endothelium and be converted to prostacyclin, as vascular tissue has been shown to be able to utilise platelet derived endoperoxides to form prostacyclin (Marcus et al., 1980). Both these features would be desirable in inhibiting thrombosis.

Imidazole and its derivatives selectively block thromboxane synthetase (Needleman et al., 1977). One such compound is dazoxiben, and it has been shown to effectively inhibit thromboxane synthetase in human platelets, although it is not clear whether vascular prostacyclin production is enhanced (Tyler, Saxton and Parry, 1981; Randall et al., 1981). Despite blocking thromboxane formation these drugs do not abolish aggregation (Vermylen et al., 1981; Bertele et al., 1981) and this suggests that endoperoxides may themselves be able to induce aggregation without conversion to thromboxane A_2 .

2.8.2 Drugs which Increase Platelet Cyclic Adenosine Monophosphate

Cyclic AMP is formed from ATP by adenylate cyclase, and broken

down to 5' AMP by phosphodiesterase. Increased levels of cAMP lower intraplatelet free Ca^{2+} and thereby inhibit platelet function. Drugs can therefore influence cAMP by promoting its formation by stimulating adenylate cyclase, or inhibiting its breakdown, by acting on phosphodiesterase.

Drugs Stimulating Adenylate Cyclase

Prostaglandins D_1 and E_1 , and prostacyclin increase adenylate cyclase activity and thereby reduce Ca^{2+} availability and inhibit aggregation (Gorman, Bunting and Miller, 1977). The order of potency of these prostaglandins to inhibit aggregation are prostacyclin, the most potent, then prostaglandin D_2 then prostaglandin E_2 .

Prostacyclin infusions have been used successfully in peripheral vascular disease (Belch et al., 1983a), Raynaud's phenomenon (Belch et al., 1983b) and severe pregnancy induced hypertension (Belch et al., 1985), where both its vasodilator and anti-platelet effects have been of value.

Phosphodiesterase Inhibitors

Inhibition of phosphodiesterase prevents degradation of cAMP resulting in cAMP accumulation. Dipyridamole and the theophyllines are inhibitors of phosphodiesterase (Moncada and Korbout, 1978). Dipyridamole on its own has little effect on platelet aggregation in vitro (Cucuianu, Nishizawa, and Mustard, 1971), but when combined with aspirin inhibits adhesion to collagen (Cazenave et al. ., 1978) and damaged endothelium (Kinlough-Rathbone et al., 1978).

Dipyridamole in combination has been assessed in the secondary prevention of myocardial infarction (Persantine Aspirin Reinfarction Study Research Group, 1980; Persantine Aspirin Reinfarction Study II Research Group, 1985), and has also been shown to be of value in maintaining graft patency following coronary artery bypass grafts (Chesebro et al., 1982).

Dipyridamole was also initially thought to stimulate vascular prostacyclin production (Vermylen, Chamone and Verstraete, 1979). This has been recently shown not to occur (Deckmyn et al., 1984), however dipyridamole does delay exhaustion of prostacyclin production by irritated vessels (Deckmyn et al., 1984). Exhaustion of tissue cyclo-oxygenase following trauma may be due to an accumulation of peroxides which inactivate cyclo-oxygenase. Dipyridamole is an anti-oxidant and acts as a co-factor for peroxidase which keeps peroxide levels low and thereby protects cyclo-oxygenase allowing prostacyclin synthesis to continue for a longer period (Deckmyn et al., 1984).

2.8.3 Drugs Blocking Calcium Availability

Ca²⁺ channel blocking agents can reduce platelet Ca²⁺ availability and drugs such as verapamil and nifedipine have inhibitory effects on platelet aggregation (Ikeda et al., 1981; Han, Boatwright and Ardlie, 1983). They have also been shown to have a synergistic action with prostacyclin in inhibiting platelet function (Ikeda et al., 1981). A further and fuller discussion of calcium channel blocking agents is given in chapter 9.

2.9 Dietary Lipids and Platelet Function

Eicosapentaenoic acid is the major fatty acid in salt water fish, which is the staple diet of the Eskimo people. Greenland Eskimos have a very low incidence of cardiovascular disease and pregnancy induced hypertension. It has been suggested that this may be due to a diet rich in eicosapentaenoic acid which may result in a beneficial effect on platelet function (Dyerberg and Bang, 1979).

When arachidonic acid is replaced by eicosapentaenoic acid in the diet it also replaces arachidonic acid in the platelet membrane. Eicosapentaenoic acid is metabolised to thromboxane A_3 which has little pro-aggregatory effect. In the vessel wall eicosapentaenoic acid is converted to prostaglandin I_3 which has similar effects to prostacyclin, being a potent vasodilator and inhibitor of platelet aggregation (Dyerberg et al., 1978). Volunteer studies have confirmed that such a dietary modification will reduce platelet aggregation and thromboxane A_2 production (Siess et al., 1980; Thorngren and Gustafson, 1981). These studies suggest that dietary modification may be an exciting new therapy in the prevention of vascular disease.

CHAPTER 3

PREGNANCY INDUCED HYPERTENSION

3.1 CARDIOVASCULAR CHANGES DURING NORMAL PREGNANCY

Normal pregnancy is associated with a number of alterations in the cardiovascular system which start to occur early in pregnancy. Cardiac output increases by around 40% during the first trimester and this increase is maintained during the third trimester (Lees et al., 1967). However, if cardiac output is measured in the supine position a fall in output is noted in the third trimester (Ueland et al., 1969) as the venous return to the heart is obstructed by the gravid uterus.

Blood pressure starts to fall in the first trimester, reaches a nadir in mid-pregnancy, then slowly rises during the third trimester to levels compatible with those in the non-pregnant state (MacGillivray et al., 1969).

As blood pressure is determined by cardiac output and total peripheral resistance, the decrease in blood pressure must be due to a fall in the latter. Since these changes occur early in pregnancy, they must reflect a change in systemic vascular resistance, as the utero-placental circulation is not sufficiently large to account for such a reduction in peripheral resistance at this stage of pregnancy.

The fall in peripheral resistance in normal pregnancy is associated with a relative insensitivity to the pressor effects of exogenous angiotensin II (Gant et al., 1973), which is detectable as early as 8 weeks gestation and reaches a peak in mid-pregnancy. The mechanism underlying this insensitivity may be prostaglandin dependent. The vasodepressor prostaglandin,

prostaglandin E₂, enhances the insensitivity to angiotensin II (Broughton-Pipkin et al., 1982), while prostaglandin synthetase inhibitors such as indomethacin, have the opposite effect (Everett et al., 1978a). In addition, it has recently been shown that exogenous prostacyclin infusions will reduce the sensitivity to angiotensin II in normal pregnancy (Broughton-Pipkin et al., 1984). Thus, the pressor effects of angiotensin II may be balanced in normal pregnancy by prostaglandin E₂ and prostacyclin which have vasodepressor effects. However, it is possible that the progesterone metabolite, 5 α -dihydroprogesterone, which is known to enhance the insensitivity to angiotensin II (Everett et al., 1978b) may also be involved.

3.2 DEFINITIONS OF HYPERTENSION IN PREGNANCY

There are three possible varieties of hypertension which can occur in pregnancy. Firstly, there are women who were hypertensive prior to pregnancy, remain hypertensive during pregnancy and are still hypertensive after pregnancy. These women have "chronic hypertension" and usually this is so called "essential hypertension", which is often inherited and has no defined underlying pathology - although some women with chronic hypertension may have an underlying problem such as renal disease. Secondly, there are women who were normotensive prior to pregnancy, develop hypertension during pregnancy and then become normotensive again within a few weeks of delivery. These women have pregnancy induced hypertension (PIH). Thirdly, there are women who become hypertensive due to a new medical problem such as phaeochromocytoma, Conn's syndrome, or Cushing's

syndrome, which arises coincidentally during a pregnancy, however, this is very rare. This chapter is concerned only with pregnancy induced hypertension.

The terminology of hypertension induced by pregnancy has become somewhat confusing. Pre-eclampsia is a term which is often loosely applied to patients with hypertension induced by pregnancy, but pre-eclampsia means different things to different people and therefore can lead to further confusion. It is a useful term in that it highlights the end-point of the disease, namely eclampsia, however, traditionally it is associated with 3 classical features, hypertension, proteinuria and oedema. Since proteinuria is really a measure of disease severity (MacGillivray, 1961) and oedema is regularly a feature of normal pregnancy (Thomson, Hytten and Billewicz, 1967), many obstetricians now use the term "pregnancy induced hypertension" (PIH) thereby avoiding the association with proteinuria and oedema which is implied by pre-eclampsia.

Blood pressure in pregnancy is distributed continuously in the population, and the dividing line between normal and abnormal is somewhat arbitrary and artificial. Most authorities, however, will use a threshold of 140/90 mm of mercury or a rise in blood pressure of greater than 20 mm of mercury over the measurement taken at 8 - 10 weeks gestation as the dividing line between "normotension" and "hypertension". There is some justification for this as the perinatal mortality rate has been shown to increase when maternal diastolic blood pressure exceeds 90 mm of mercury (Butler and Alberman, 1969; Page and Christianson, 1976).

Recording of blood pressure is also a problem as it is subject to observer error. In addition, blood pressure in pregnancy is highly variable both during waking and sleeping (Redman et al., 1976a), consequently the average of several measurements taken under standard conditions should be used to accurately assess the patient. It is also important to use phase IV, muffling of the Korotkoff sounds to assess diastolic blood pressure, as phase V (disappearance of Korotkoff sounds) often does not exist in pregnancy. Pregnancy creates a hyperdynamic circulation and the Korotkoff sounds may therefore continue down to "zero" cuff pressure.

Proteinuria is also important in defining PIH, but essentially reflects a more severe form of the disease (MacGillivray, 1961). It has been shown that if proteinuria and hypertension are both present then the perinatal mortality rate, and the incidence of intra-uterine growth retardation are increased (MacGillivray, 1981).

Proteinuria is easier to quantitate than hypertension; providing that a urinary tract infection has been excluded, the presence of greater than 0.3 grams of protein per 24 hours is considered significant.

3.3 AETIOLOGY OF PREGNANCY INDUCED HYPERTENSION

Over the past 100 years innumerable hypotheses have been advanced to explain the aetiology of PIH, and it has long been termed the "disease of theories" (MacGillivray, 1981). This statement

remains true today as the primary cause of PIH remains obscure. It is clear, however, that the trigger for the condition lies within the uterus, as delivery is rapidly followed by disappearance of the signs and symptoms of the disease, and by the return to normal of the biochemical markers of the disease.

A number of risk factors have emerged from the study of this condition. PIH is much more common in primigravidae and a previous pregnancy, or even an abortion, offers some protection against the disease (MacGillivray, 1958). The risk increases slightly with age, but is not affected by social class (Baird, 1977). There is also a familial tendency, suggesting some genetic susceptibility (Chesley et al., 1968). Women with chronic hypertension are more likely to develop "superimposed" PIH than are normal pregnant women (Chesley et al., 1947), and women with underlying renal disease are especially at risk (Felding, 1969).

Conditions associated with large placentas, such as multiple pregnancy, diabetes and hydatidiform mole also put the patient at risk of PIH (MacGillivray, 1981), and there are racial and geographical variations, with PIH being more common in the Far East (Davies, 1971).

A variety of biological "cascades" are activated in PIH, including the coagulation system, the renin-angiotensin-aldosterone system, the kinins, the prostaglandins, and the complement cascade. However, these seem to be secondary phenomena, triggered by a stimulus which remains unknown

(MacGillivray, 1981). A current theory is that some immunological factor initiates the activation of these systems but how this occurs remains obscure.

3.4 EPIDEMIOLOGY OF PIH

Owing to differing diagnostic criteria, it is difficult to provide accurate figures for the true incidence of PIH. However, PIH is a relatively common disease of pregnancy, and may be seen in as many as 15% of pregnancies (Chamberlain et al., 1978; Butler and Bonham, 1963). Complications associated with hypertension were the largest single cause of maternal deaths in England and Wales in the years 1973 - 1975 (Report on Confidential Enquiries into Maternal Deaths in England and Wales 1973 - 1975 (1979)), and PIH is associated with one in seven perinatal deaths (McIlwaine et al., 1984). The effect of hypertension alone on the fetus has been the subject of conflicting reports. The 1970 British Births Survey (Chamberlain et al., 1978) suggested that pregnancies complicated by hypertension alone had a perinatal mortality rate which was not significantly different from that of normal pregnancy, and was in fact numerically lower. While Page and Christianson (1976) showed an increased fetal risk with even the mildest elevation of blood pressure. There is no doubt, however, that when hypertension and proteinuria co-exist both the perinatal mortality rate and the incidence of intrauterine growth retardation are significantly increased (MacGillivray, 1981). Prematurity also contributes to the increased perinatal morbidity seen in PIH (Lin et al., 1982).

3.5 CLINICAL FEATURES OF PIH

PIH is a hypertensive disorder of the second half of pregnancy which, once established, will progress steadily until delivery. It is virtually always asymptomatic, with hypertension or proteinuria being picked up by antenatal screening. Sometimes, however, it may develop and rapidly become severe and in this rapidly progressive form the patient usually feels unwell. Headache, irritability, blurred vision, restlessness and epigastric discomfort are serious symptoms that usually herald the onset of seizures.

Oedema is found in 85% of women with proteinuric PIH (Thomson et al., 1967) and may be severe. The oedema fluid is an ultrafiltrate of plasma and is associated with reduced plasma albumen and oncotic pressure, and with retention of sodium and potassium. However, it has little value as a specific diagnostic sign as oedema occurs frequently in normal pregnancy (Thomson et al., 1967; Robertson, 1971). In addition, in a prospective study of oedema in pregnancy, patients with oedema had an incidence of PIH which was similar to patients without oedema (Robertson, 1971).

3.6 PATHOPHYSIOLOGY OF PIH

3.6.1 The Cardiovascular System in PIH

Hypertension is an early feature of PIH and is caused by vasoconstriction associated with a reduced plasma volume (Gallery et al., 1979; Chesley, 1972) and a normal or reduced cardiac output (Smith, 1970). Vascular reactivity is increased so that the normal insensitivity to exogenous angiotensin II is lost and this may be detectable prior to the onset of the hypertension (Gant et

al., 1973). This loss of vascular resistance may be associated with a deficiency of vasodepressor prostaglandins, such as prostacyclin which has also been documented in PIH (Goodman et al., 1982; Downing et al., 1980; Remuzzi et al., 1980).

Severe hypertension causes arterial damage and this has been demonstrated both in animals (Goldby and Beilin, 1972) and in the clinical situation (Johansson et al., 1974). This arterial damage explains the convulsions seen in untreated severe PIH and is also the cause of cerebral haemorrhage, which is the commonest cause of death in PIH (Report on Confidential Enquiries into Maternal Deaths 1973 - 1975 (1979), 1976 - 1978 (1982)).

3.6.2 The Kidney in PIH

Proteinuria in PIH is associated with disease severity (MacGillivray, 1961). The proteinuria is moderately selective in terms of size of the filtered proteins (Maclean et al., 1972), but can be heavy with more than 5 grams of protein per day being lost. It is due to a glomerular protein leak, signifying glomerular involvement in the disease process. Renal biopsy shows a non-inflammatory glomerular lesion. There is swelling and vacuolation of the glomerular endothelial cells which occlude the glomerular lumen and this has been termed "glomerular endotheliosis". The epithelial cells are also swollen although their foot processes remain intact (Pollak and Nettles, 1960). Fibrin deposits within and under the endothelial cells have also been noted (Vassali et al., 1963). Complete resolution of these changes can be seen as early as four weeks post-partum.

Other aspects of renal function are also impaired. There is reduced uric acid clearance implying tubular dysfunction (Chesley and Williams, 1945), leading to increased plasma uric acid concentrations. Increased plasma uric acid is an early sign of PIH and precedes the development of proteinuria and any increase in urea and creatinine (Redman et al., 1976b). It is also useful in differentiating between PIH and chronic hypertension, as in the latter there is no change in on uric acid levels (Redman et al., 1976b).

Oedema is a feature of normal pregnancy but can be more marked in PIH due to capillary leakage, and reduced plasma albumen concentration which results in a lower osmotic pressure. Renal retention of sodium also occurs (Chesley et al., 1958) and contributes to the oedema. Complications of renal impairment and fluid retention include ascites, pulmonary oedema (Strauss et al., 1980) and acute renal failure with tubular and even cortical necrosis (Smith et al., 1968). In view of these complications it is important to carefully monitor renal function in PIH.

3.6.3 The Placenta in PIH

In PIH the terminal segments of the uterine spiral arteries within the placenta become obstructed by fibrin and platelet aggregates (Zeek and Assali, 1950) and this explains the reduced utero-placental blood flow, placental infarcts and placental dysfunction. It is thought likely that many of the maternal changes seen in PIH are secondary to placental ischaemia, as animal models of the disease depend on procedures which cause placental ischaemia (Cavanagh et al., 1977). The trigger for

this placental damage is unknown but the lesions do not seem to be due to hypertensive injury and an immune aetiology has been suggested (Redman, 1980).

3.6.4 The Liver in PIH

Liver dysfunction can be seen in PIH and can be detected by an elevation of circulating hepatic enzymes (Shukla et al., 1978). This may progress to clinical jaundice and hepatic failure (Killam et al., 1975; Davies et al., 1980). Histologically periportal haemorrhage and necrosis is seen (Sheehan and Lynch, 1973), and is associated with microvascular thrombosis.

3.6.5 Coagulation, Fibrinolysis and Platelets in PIH

There is now considerable evidence suggesting that some degree of disseminated intravascular coagulation (DIC) occurs in PIH. While this is unlikely to be the primary cause of the disease, it does explain many of the features of PIH, such as the microvascular obstruction seen in the placenta, kidney and liver (Howie, 1977).

Deposition of fibrin in patients with eclampsia has long been known to occur (Schmorl, 1893; McKay et al., 1953; Govan, 1954). More recently, pathological examination of biopsy material has shown fibrin deposition and fibrinoid necrosis in liver, placenta and kidney in patients with PIH (Howie, 1977). The circulating platelet count is known to be reduced in PIH (Trudinger, 1976; Redman, Bonnar and Beilin, 1978) and this is due to a reduced platelet life-span (Rakoczi et al., 1979). An inverse relationship between platelet count and fibrinogen - fibrin

degradation products (FDP's) has been noted, suggesting that the fall in platelet count is due to the increased platelet consumption seen in DIC (Howie, Prentice and McNicol, 1971). The platelet specific protein beta-thromboglobulin has also been found to be increased in PIH (Redman et al., 1977a; Douglas et al., 1982; Socol et al., 1985) and this is a marker of platelet activation. Furthermore, beta-thromboglobulin has been shown to correlate with proteinuria and serum creatinine (Socol et al., 1985), linking platelet activation with renal microvascular damage in PIH.

The platelet content of 5-hydroxytryptamine is reduced in PIH (Whigham et al., 1978). This is a vasoactive amine released when platelets aggregate, and its loss indicates platelet aggregation and stimulation of the platelet release reaction in vivo. Low platelet 5-hydroxytryptamine levels have also been associated with loss of platelet responsiveness to various aggregating agents (Whigham et al., 1978). The explanation suggested for these findings is that platelets are activated in the microcirculation of the placenta, kidney and liver, release their products such as beta-thromboglobulin and 5-hydroxytryptamine and then re-enter the circulation in an "exhausted" state, unable to respond normally to aggregating agents and having lower levels of 5-hydroxytryptamine (Howie, 1977). In support of this hypothesis, it had previously been noted that placentas from patients with PIH had high levels of 5-hydroxytryptamine (Senior et al., 1963), possibly of platelet origin.

A relative macrothrombocytosis is also seen in PIH and this is due to an increased entry of large, young platelets into the circulation (Giles and Inglis, 1981) and this is in keeping with accelerated platelet consumption.

Platelets have also been shown to be less sensitive to the anti-aggregatory effects of prostacyclin in PIH (Briel, Kieback and Lippert, 1984), and this may contribute to the platelet consumption seen in this disease, especially as deficiency of prostacyclin production may coexist (Downing et al., 1980; Goodman et al., 1982; Remuzzi et al., 1980).

Increased platelet thromboxane A₂ production ex vivo has also been shown to occur in PIH complicated by intra-uterine growth retardation (Wallenburg and Rotmans, 1982). Since thromboxane A₂ is a potent vasoconstrictor and platelet aggregating agent (Hamberg et al., 1975), it may also contribute to the pathogenesis of PIH. The role of platelets in the pathophysiology of PIH may also be emphasised by the recent success of anti-platelet therapy in the treatment of pregnancies at high risk of PIH (Beaufils et al., 1985).

An increased ratio of factor VIII related antigen to factor VIII coagulant activity has been shown to be characteristic of PIH (Redman et al., 1977b) and also to correlate with disease severity (Redman et al., 1977b). This is probably due to factor VIII consumption and it is thought that this reflects low grade DIC (Bonnar and Shepherd, 1981).

Soluble fibrin complexes are also known to be increased in PIH (Howie et al., 1971; McKillop et al., 1976; Edgar et al., 1977) and again this supports the concept of low grade DIC occurring in PIH. In normal pregnancy fibrinolytic activity has been shown to decline steadily and to increase following delivery (Stirling et al., 1984; Bonnar, McNicol and Douglas, 1970). This decline in fibrinolytic activity is accompanied by an increase in fibrinogen - fibrin degradation products (FDP's) from mid-pregnancy (Stirling et al., 1984).

In PIH, serum FDP's are known to be increased compared to normal pregnancy (Bonnar, McNicol and Douglas, 1971; Howie et al., 1971). Urinary FDP's, which are not usually found during normal pregnancy, are increased in PIH (Howie et al., 1971) and rise further after delivery (Howie et al., 1971; Condie and Ogston, 1976). Fibrinolytic activity may be further reduced in PIH compared to normal pregnancy (Bonnar et al., 1971). While fibrinolytic activity may be impaired in PIH, it cannot be totally abolished in view of the presence of FDP's. In addition, plasminogen levels have been shown to be reduced in PIH (Howie et al., 1971) and this would suggest consumption of plasminogen in keeping with some degree of fibrinolytic activity. These studies suggest that there is excessive deposition of fibrin in PIH which is only partially cleared due to impaired fibrinolysis. After delivery the fibrinolytic activity returns to normal and the fibrin is then broken down and excreted in the urine, explaining the higher levels of FDP's seen in the urine following delivery.

All these changes in the coagulation system and platelet function in PIH, support the concept that DIC occurs in this condition. A "coagulation index" of serum FDP's, platelet count and plasma factor VIII has been shown to correlate with a "clinical index" of disease severity (Howie et al., 1976) and this highlights the association of DIC with PIH.

3.7 PROSTACYCLIN AND THROMBOXANE A₂ IN PREGNANCY AND PIH

Prostacyclin is a potent, vasodilator, inhibitor of platelet aggregation (Moncada et al., 1976) and a stimulator of renin secretion (Patrono et al., 1982). The pathological features of PIH are virtually a mirror image of these, as it is associated with vasoconstriction, platelet consumption and low renin secretion (Brown et al., 1965). Furthermore, women with PIH are very sensitive to exogenous angiotensin II infusions when compared to normal pregnant women (Chesley, 1966; Talledo, Chesley and Zuspan, 1968; Gant et al., 1973). This insensitivity to angiotensin II seen in normal pregnancy can be abolished if women are treated with a cyclo-oxygenase inhibitor such as indomethacin (Everett et al., 1978), and enhanced by prostacyclin infusion (Broughton-Pipkin et al., 1984) or PGE₂ infusion (Broughton-Pipkin et al., 1982). These experiments suggest that in normal pregnancy angiotensin II may be balanced by the action of vasodepressor prostaglandins such as prostacyclin. A deficiency of prostacyclin might therefore result in the sensitivity to angiotensin II seen in PIH and many investigators have now studied prostacyclin in this condition.

Reduced prostacyclin production from both placental and umbilical artery tissue has been documented in PIH by a variety of techniques, including bioassay for prostacyclin-like activity (Remuzzi et al., 1980), thin layer chromatography and gas chromatography-mass spectrometry (Downing et al., 1980) and radioimmunoassay (Carreras et al., 1981) for 6-keto-PGF_{1α}, the stable hydration product of prostacyclin. This has been confirmed in a more recent study which showed a reduction in prostacyclin production from placental tissue in PIH compared with normal pregnancy (Walsh, Behr and Allen, 1985). Makila, Viinikka and Ylikorkala (1984) have proposed that prostacyclin deficiency in pregnancy is specific to PIH as they documented reduced prostacyclin production from umbilical artery in pregnancies complicated by PIH, but not in pregnancies complicated by chronic hypertension or intra-uterine growth retardation. This, however, is at variance with other studies which have shown deficient prostacyclin production from placental cells (Jogee, Myatt and Elder, 1983) and umbilical artery (Stuart et al., 1981) to occur in intra-uterine growth retardation. Maternal vascular prostacyclin production has also been shown to be reduced in PIH (Bussolino et al., 1980).

The umbilical artery lacks any type of innervation, it is therefore likely that blood flow is controlled by humoral means (Tuvemo, 1980). Prostacyclin and PGE₁ relax umbilical artery while thromboxane A₂ constricts it (Tuvemo, 1980). Prostacyclin is the major prostaglandin produced by umbilical artery (Ritter et al., 1982; Tuvemo, 1980) and umbilical artery produces significantly more prostacyclin than vessels of comparable size

and weight taken from healthy adults (Remuzzi et al., 1979). Makila et al (1983) have recently shown that umbilical artery blood flow measured ultrasonically in vivo correlated significantly with the vessels' ability to produce prostacyclin in vitro and confirmed that prostacyclin production was lower in pregnancies complicated by PIH. These findings would be in keeping with prostacyclin being involved in the maintenance of the low pressure - high flow feto-placental circulation and prostacyclin deficiency might result in reduced flow and subsequent intrauterine growth retardation.

The prostacyclin content of amniotic fluid in late pregnancy has also been studied. In the normal situation amniotic fluid 6-keto-PGF_{1α} correlates with gestational age (Ylikorkala, Makila and Viinikka, 1981a) in the third trimester and increases during labour following the onset of uterine contractions (Makarainen and Ylikorkala, 1984; Mitchell et al., 1979a) suggesting a possible role for prostacyclin in parturition.

In PIH, the prostacyclin-like activity of amniotic fluid is reduced (Bodzenta, Thomson and Poller, 1980) and Ylikorkala et al (1981a) have documented reduced amniotic fluid 6-keto-PGF_{1α} in pregnancies complicated by severe PIH but not in those with mild disease. During labour amniotic fluid 6-keto-PGF_{1α} is significantly lower in PIH as compared with normal pregnancy and the maximal rise in 6-keto-PGF_{1α} is also less in PIH (Makarainen and Ylikorkala, 1984).

Maternal prostacyclin status has also been studied in PIH. The first report of reduced maternal plasma 6-keto-PGF_{1α} in PIH came from Lewis et al., in 1981. They showed that the level of this prostacyclin metabolite fell as the disease progressed in a women with severe PIH, however, the patient had been started on aspirin as an anti-platelet agent immediately before the levels started to fall and it is therefore difficult to know whether this was an effect of the disease or an effect of the treatment. More recently, significantly lower plasma levels of 6-keto-PGF_{1α} have been recorded in women with eclampsia, or impending eclampsia, than in normal pregnant women (Moodley, Norman and Reddi, 1984), and this was associated with lower levels of PGE and PGF also. Significantly lower 6-keto-PGF_{1α} levels have also been recorded in maternal and umbilical venous plasma in pregnancies complicated by severe PIH as compared with normotensive pregnancies (Yamaguchi and Mori, 1985) and this difference disappeared following delivery. Urinary prostacyclin metabolite excretion has also been shown to be reduced in women with PIH (Goodman et al., 1982).

Reports on maternal prostacyclin status in PIH have not all been consistent. Ylikorkala, Kirkinen and Viinikka (1981b) found no difference in plasma 6-keto-PGF_{1α} levels in PIH compared to normal pregnancy and prostacyclin metabolites have also been found to be increased in PIH (Strickland et al., 1984; Koullapis et al., 1982).

Remuzzi et al (1981b) have studied the ability of plasma to stimulate prostacyclin production from vascular tissue. They found that plasma from women with PIH had a much greater ability to stimulate prostacyclin production than plasma from women in the third trimester of normal pregnancy and they concluded that this was consistent with deficient prostacyclin production in PIH.

These various studies suggest, both from indirect and direct evidence, that prostacyclin deficiency may be involved in the pathophysiology of PIH although the evidence is not entirely consistent. Further studies are therefore required to define changes in levels of prostacyclin in PIH. It is unlikely that prostacyclin deficiency is the primary cause of PIH - it is more likely to be a secondary phenomenon - as prostacyclin infusions used therapeutically do not "cure" the disease, although they do delay disease progression, reduce platelet consumption and help to lower blood pressure (Fidler et al., 1981; Belch et al., 1985). However, in view of prostacyclin's potent biological actions its deficiency may be of major importance in the pathophysiology of PIH and replacement of prostacyclin may be a useful therapeutic option. The use of other drugs which can cause vasodilatation and reduce platelet consumption might also be of value in preventing the consequences of prostacyclin deficiency.

Despite these observations in the abnormal situation there is little information on maternal plasma prostacyclin metabolites in normal pregnancy. In 1980 Lewis et al., published a cross sectional study on 22 pregnancies showing increased levels of

6-keto-PGF_{1α} in the third trimester as compared with normal non-pregnant and early pregnancy values. Bolton et al., (1982) in the only prospective longitudinal study of prostacyclin metabolites in pregnancy found a peak at 18 - 22 weeks gestation, although they did not sample in the first trimester. Other cross sectional studies have shown no difference in prostacyclin throughout pregnancy (Mitchell, 1981; Ylikorkala and Viinikka, 1981). Goodman et al., (1982) studied urinary metabolites of prostacyclin in the second and third trimesters of normal pregnancy and found a five-fold increase in urinary metabolites of prostacyclin in comparison to non-pregnant women.

The values given by these studies for plasma 6-keto-PGF_{1α}, using radioimmunoassays, were between 100 and 500 pg/ml and were compatible with values for normal non-pregnant subjects obtained by radioimmunoassay (Mitchell, 1978) and by gas chromatography-mass spectrometry (Hensby et al., 1979). These findings were in keeping with the belief that prostacyclin was a circulating hormone in man (Moncada et al., 1978). In 1982, however, Blair et al., published work using negative ion gas chromatography-mass spectrometry which showed that the absolute value for 6-keto-PGF_{1α} was below 5 pg/ml, and they concluded that the levels were too low to allow prostacyclin to function as a circulating hormone in man as previously thought. This, however, does not preclude a role for prostacyclin as a local hormone which is important in the regulation of platelet - vessel wall interaction (Patrono et al., 1984).

In view of these findings, however, there was a need to re-examine the role of prostacyclin both in normal pregnancy and PIH.

Thromboxane A₂ is a potent platelet aggregating agent and vasoconstrictor. An excess of such a substance might contribute to the platelet consumption and vasoconstriction seen in PIH, however, there is little information on thromboxane A₂ in pregnancy. Ylikorkala and Viinikka (1980) measured TxB₂ - the stable hydration product of thromboxane A₂ - in normal pregnancy and found increased levels of both plasma and serum TxB₂ compared to non-pregnant control subjects but could show no relationship to gestation while Mitchell et al., (1978) showed increased levels at term as compared to "late pregnancy" (28 - 36 weeks gestation).

There is no demonstrable change in TxB₂ in amniotic fluid of pregnancies complicated by PIH (Ylikorkala et al., 1981a; Makarainen and Ylikorkala, 1984) and Yamaguchi and Mori (1985) demonstrated no significant increase in plasma TxB₂ in pregnancies complicated by PIH. Makila et al., (1983) studied umbilical artery thromboxane A₂ production in pregnancy. They found that umbilical artery produced very little thromboxane A₂ relative to prostacyclin and there was no increase in thromboxane A₂ production in umbilical artery from pregnancies complicated by PIH. Furthermore, thromboxane A₂ production, unlike prostacyclin production, was unrelated to umbilical artery blood flow as determined ultrasonically prior to delivery. More recently (Walsh, 1985) placentas taken from pregnancies complicated by PIH

have been shown to produce more TxA_2 and less PGI_2 than placentas from normal pregnancies. Wallenburg and Rotmans (1982) have shown that ex vivo platelet thromboxane A_2 production is elevated in PIH complicated by intrauterine growth retardation, thus both placental and platelet derived thromboxane A_2 may contribute to the disease process.

The use of drugs which can reduce thromboxane A_2 production and platelet aggregation may therefore be of benefit in the treatment of PIH and studies are required to explore further the role, of thromboxane A_2 in PIH and the possible benefits of drugs which would inhibit its formation.

3.8 TREATMENT OF PIH

PIH is a major cause of both maternal and fetal mortality and morbidity. The fetus is at risk of growth retardation, death, and prematurity from untimely delivery (Chamberlain et al., 1978; Page and Christianson, 1976; Lin et al., 1982). The mother is at risk of vascular, renal, hepatic and neurological damage as discussed above.

PIH is essentially a curable form of hypertension as delivery will remove the disease. The aims of treatment therefore are to control blood pressure, prolong the pregnancy sufficiently to avoid the complications of prematurity, prevent progression of the disease and avoid hypertensive (and hypotensive) crises, especially during labour.

3.8.1 Bed Rest

Traditionally, obstetricians have advocated bed rest for the treatment of PIH. Theoretically it is used to try and reduce blood pressure, increase cardiac output (Euland et al., 1969) and increase sodium excretion (Redd et al., 1968). However, it may also be associated with reduced cardiac output due to the pressure of the gravid uterus on the great vessels (Lees et al., 1967), and lower utero-placental blood flow (Abitbol, 1977) which would be detrimental to the patient.

Only two controlled studies of bedrest have been performed. The first showed no benefit from bedrest in non-proteinuric hypertension (Mathews, 1977) and the second showed some benefit in severe proteinuric hypertension with intra-uterine growth retardation (Mathews et al., 1982). Overall therefore, the use of bedrest in the treatment of PIH remains unproven, at least in the less severe forms of the disease.

3.8.2 Sedation

Sedation, like bed rest, has formed a traditional part of the management of PIH. The use of anticonvulsants in the treatment of impending eclampsia is obviously logical, however, there has been no proven benefit from chronic sedation in the management of PIH (Mathews, 1977).

3.8.3 Anti-hypertensive Drugs

The use of drugs to control blood pressure in pregnancy is gradually increasing, due to the increasing knowledge about the drugs and their safety in pregnancy.

Methyldopa was assessed in hypertension occurring in pregnancy, as long ago as 1968 (Leather et al., 1968) and has become one of the most widely used anti-hypertensive agents in PIH. The most comprehensive study of its use in pregnancy was performed by Redman et al., (1976c). They concluded that it was safe for use in pregnancy and that it appeared to reduce fetal loss, especially from mid-trimester abortions. The children of the women who took part in this study were followed up after seven years, and there was no obvious difference between the children from the treatment group, and those from the control group in terms of physical and mental handicaps, behaviour, vision, hearing and intellectual ability (Cockburn et al., 1982), thus emphasising the drug's safety. Methyldopa, however, is associated with some adverse effects such as lassitude, depression and postural hypotension. These side effects were troublesome enough to cause 15% of the women assessed by Redman et al., (1976c) to be withdrawn from the study (Redman, Beilin and Bonnar, 1977b).

Adrenoceptor antagonists are increasingly being used in the treatment of PIH. Initially their use in pregnancy was associated with some degree of hesitation, as they have possible depressant effects on the fetal circulation and uterine tone. In addition, initial studies had suggested that they might be associated with adverse effects such as neonatal hypoglycaemia, intra-uterine growth retardation and increased fetal loss. Most of these studies, however, have been anecdotal and have not been confirmed in prospective studies (Rubin, 1981). Adrenoceptor

antagonists have now been studied fairly extensively in PIH, and they have been shown to be both safe and effective in controlling blood pressure (Gallery et al., 1979b; Lubbe and Hodge, 1981; Symonds et al., 1982; Walker et al., 1982; Walker, Greer and Calder, 1983; Rubin et al., 1983; Gallery, Ross and Gyory, 1985). They are also relatively free of side effects, making them acceptable to patients. There are a variety of adrenoceptor antagonists available which have varying receptor specificity and varying degrees of intrinsic sympathomimetic activity and most of these have been assessed to some extent in pregnancy. Oxprenolol, a non-selective β -receptor antagonist with intrinsic sympathomimetic activity, has been compared with methyldopa in the treatment of hypertension in pregnancy. The patients studied were mostly chronic hypertensives. Both drugs had similar effects on blood pressure control, but the oxprenolol group had a better outcome in terms of fetal and placental weight (Gallery et al., 1979b).

More recently Gallery et al., (1985) have published the final results of this study, where 183 patients with hypertension in pregnancy were randomly allocated to receive either oxprenolol or methyldopa. Control of blood pressure was equivalent in both groups, but fetal growth was increased in the oxprenolol group. However, with increasing duration of treatment the differences between the groups diminished, so that after ten weeks treatment no difference in fetal growth was found. The authors have suggested that the early effect is due to oxprenolol producing

peripheral vasodilatation, and they suggest that over time the same effect on fetal growth can be achieved by long term control of blood pressure as seen with the methyldopa group.

The largest study of an adrenoceptor antagonist in PIH was performed with atenolol, a selective β_1 -receptor antagonist (Rubin et al., 1983). This was a double blind placebo controlled study in 120 women with PIH. Atenolol effectively controlled blood pressure and reduced the subsequent development of proteinuria, suggesting a possible beneficial effect on the disease process. There was no difference in fetal and neonatal complications such as intra-uterine growth retardation, neonatal hypoglycaemia or hyperbilirubinaemia between the two groups. Respiratory distress was seen only in the control group, however, neonatal bradycardia was more common in the atenolol group, although there was no effect on blood pressure. The children from this study have all been followed up for one year and atenolol has not been shown to have any adverse effects on their development (Reynolds et al., 1984).

Labetalol is a unique adrenoceptor antagonist as it has both non-selective β -adrenoceptor antagonist and selective α_1 adrenoceptor antagonist properties. This means that it can directly cause peripheral vasodilation and this may help to reduce blood pressure and maintain renal and uterine blood flow. It has been shown to be effective in controlling blood pressure (Symonds et al., 1982; Redman, 1982; Walker et al., 1983; Walker et al., 1982) and may have a beneficial effect on platelet consumption (Walker et al., 1982). Labetalol has also been

compared with atenolol in PIH (Lardoux et al., 1983). Both drugs controlled blood pressure but birth weights were significantly higher in the labetalol group, possibly due to increased utero-placental blood flow.

It would seem from these studies that adrenoceptor antagonists are both safe and effective in the treatment of PIH, and may have some beneficial effects on the disease process. Whether any particular adrenoceptor antagonist is more effective than the others is not yet known and further studies are required in this field.

Hydralazine is a vasodilator which can be used both orally or parenterally in PIH and is often used in the treatment of hypertensive crises (Lubbe, 1984). It has side effects of headache and tachycardia which can be avoided by concomitant use of an adrenoceptor antagonist. At higher doses, however, it can cause a lupus-like syndrome (Lubbe, 1984). Hydralazine is also frequently used as a "second line" drug to augment the effects of adrenoceptor antagonists and methyldopa when satisfactory control is not achieved with a single agent.

Diazoxide, a potent vasodilator, and labetalol have also been used effectively in the acute hypertensive situation where parenteral therapy is required (Lubbe, 1984). Both agents are potent but severe hypotension can occur especially with diazoxide.

Magnesium sulphate remains a popular drug in North America for control of impending eclampsia. It is effective, but has depressant effects on both the maternal and fetal cardiovascular and respiratory systems. It causes peripheral vasodilatation and also has effects on the central and peripheral nervous systems (Lubbe, 1984).

Recently calcium channel blocking drugs have become available and have proven useful in the management of hypertension in non-pregnant subjects. One such agent, nifedipine, has recently been assessed in PIH (Walters and Redman, 1982) and was found to be an effective anti-hypertensive agent with no notable side effects. Further studies are required to determine the place of calcium channel blocking agents in PIH.

An interesting development in the treatment of severe PIH has been the use of prostacyclin infusions (Belch et al., 1985; Fidler et al., 1981). This is a logical step as PIH is associated with vasoconstriction and platelet consumption, while prostacyclin is a potent vasodilator and anti-platelet agent. It has also been shown that prostacyclin production may be impaired in PIH (Remuzzi et al., 1980, Downing et al., 1980; Goodman et al., 1982). Thus far it has only been used in patients with severe disease but it has been successful both in lowering blood pressure and reducing platelet consumption. It is difficult to use as it is unstable, requires to be given parenterally, and its dose is limited by vasodilator side effects. However, results from the studies cited are sufficiently encouraging to warrant further investigation.

3.8.4 Anti-platelet Drugs in PIH

Another recent development in the treatment of PIH is the use of anti-platelet drugs. In view of the role of platelets in the pathogenesis of PIH this is an attractive therapeutic option, especially when anti-platelet agents have been used with some success in arteriosclerotic vascular disease (see chapter 2). Beaufils et al., (1985) have recently published the results of a study looking at the effects of an aspirin - dipyridamole combination in 102 pregnancies at high risk of PIH and growth retardation.

They found that the incidence of PIH, fetal death and intra-uterine growth retardation were lower in the treated group compared with the control group who received no treatment. In addition, the platelet count was protected in the treatment group and no adverse effects were noted.

Clearly, further studies of anti-platelet drugs are required in PIH, but this study at least offers a great deal of encouragement to pursue this potentially very exciting line of research. It may be that a combination of an anti-platelet agent and an antihypertensive agent would be beneficial in the treatment of PIH.

CHAPTER 4**METHODOLOGY**

4.1 MEASUREMENT OF PROSTACYCLIN AND THROMBOXANE

4.1.1 General Considerations in the Measurements of PGI₂ and TxA₂ Metabolites

The normal physiological concentrations of PGI₂ and TxA₂ are very low, consequently highly sensitive and specific assays are required to measure these substances in biological samples. Radioimmunoassay (RIA) and gas-chromatography-mass-spectrometry therefore provide the only techniques sensitive enough to measure these substances. Measurement of PGI₂ metabolites in plasma has been, and remains, a subject of great controversy. Initial work on 6-keto-PGF_{1α} by radioimmunoassay (RIA) suggested that levels in normal subjects were of the order 70 - 100 pg/ml (Mitchell, 1978), and this was supported by gas-chromatography mass-spectrometry (GCMS) work which suggested similar levels (Hensby et al., 1979). In 1982, however, Blair et al., published work using negative ion GCMS which showed that the absolute values for 6-keto-PGF_{1α} in peripheral plasma were below 5 pg/ml, confirming the earlier work of Christ-Hazelhof and Nugteren (1981) using the technique of gas chromatography with electron capture detection. It was concluded that PGI₂ could not be a circulating hormone in man as previously thought. This, however, does not preclude a role for PGI₂ functioning as a local hormone in the regulation of platelet-vessel wall interaction (Patrono, Preston and Vermylen, 1984). Changes in levels of its stable metabolites must reflect changes in production, it is still therefore valuable to measure these metabolites in an attempt to elucidate the role of prostacyclin in disease. RIA has also been discredited by varying 'normal' values obtained by RIA. Such differences are

due to variations in methodology and antibody sensitivity (Vinnikka and Ylikorkala, 1982). While RIA's cannot give absolute values for PGI₂ metabolites, they can still produce accurate comparative values.

Much improved RIA's have been developed recently with sensitivities and normal levels much closer to those obtained by GCMS (McLaren et al., 1985). RIAs therefore can still yield much valuable and accurate data provided proper controls are included and validation performed (Salmon, 1983). They have many advantages over GCMS, as they are relatively sensitive, specific and can cope with large number of samples. For these reasons RIA is probably the method of choice from routine measurements (Belch et al., 1983; Salmon, 1983).

Measurement of plasma TxB₂ also has methodological problems, as platelet activation at the time of venepuncture can easily produce artifactual results. In addition, RIA of TxB₂, like that of PGI₂ metabolites, can only give comparative rather than absolute values for TxB₂.

4.1.2 Problems with Extraction in RIA

Extraction of plasma and serum samples is a debatable subject in the field of RIA, as many of the procedures involved can themselves be a source of error. The main reason for extraction is to remove unwanted substances which might interfere with the assay. However, the opposite may hold true. In unextracted plasma the interfering substances may remain bound to albumen and are thus harmless, while extraction would strip the albumen

molecules of many substances which could disturb the subsequent antigen/antibody binding (Kindahl and Granstrom, 1980). Free fatty acids especially will remain in high concentrations after extraction, and unless extensive purification is performed will interfere with the assay (Gold and Edgar, 1978). These extraction evaporation and purification steps can also add further impurities (Greaves and Preston, 1982; Kindahl and Granstrom, 1980), which can cause marked and unacceptable non-specific interference with the assay.

In view of these problems, the assays described below were developed on unextracted plasma and have been shown to be accurate, sensitive and reproducible.

4.1.3 PGI₂M Assay Method

Venous blood was taken without stasis into an anticoagulant mixture consisting of 3.8% w/v trisodium citrate containing 3×10^{-5} molar indomethacin and 10^{-4} molar adenosine (9 volumes of blood to 1 volume anticoagulant), and then centrifuged at 4°C for 20 minutes at 2500 g. Plasma was stored at -70°C until the assay was performed. Plasma immunoreactive 6-keto-PGF_{1α} (PGI₂M), was measured on unextracted plasma by radioimmunoassay as previously described (McLaren et al., 1985; Belch et al., 1983). The antibody used was a polyclonal antibody raised in rabbits by Dr. F.E. Preston of the Department of Haematology, Royal Hallamshire Hospital, Sheffield. The cross reactivities for this antibody are shown in table 4.1. The buffer used was 0.1M phosphate buffer pH 7.0 containing 0.1% bovine serum albumen (The protein being added to prevent adsorption losses onto walls of tubes and pipette

TABLE 4.1

Cross reactivities of PGI₂M antibody with
available prostaglandins and metabolites

	<u>% Cross-Reactivity</u> (50% B/B ₀ Replacement)
6-keto-PGF _{1α}	100
13,14-dihydro-6-keto-PGF ₁	1.1
PGF _{1α}	0.35
PGD ₂	0.25
PGF _{2α}	0.20
PGE ₁	0.075
13,14-dihydro-6,15-diketo-PGF _{1α}	0.075
6,15-diketo-PGF _{1α}	0.075
13,14-dihydro-PGF _{2α}	0.020
PGA ₂	0.015
PGA ₁	0.004
Thromboxane B ₂	0.004
13,14-dihydro-PGE ₁	0.004
PGB ₁	0.002
13,14-dihydro-PGE ₂	<0.001
13,14-dihydro-15-keto-PGF _{2α}	<0.001
13,14-dihydro-15-keto-PGE ₁	<0.001
Arachidonic acid	<0.001

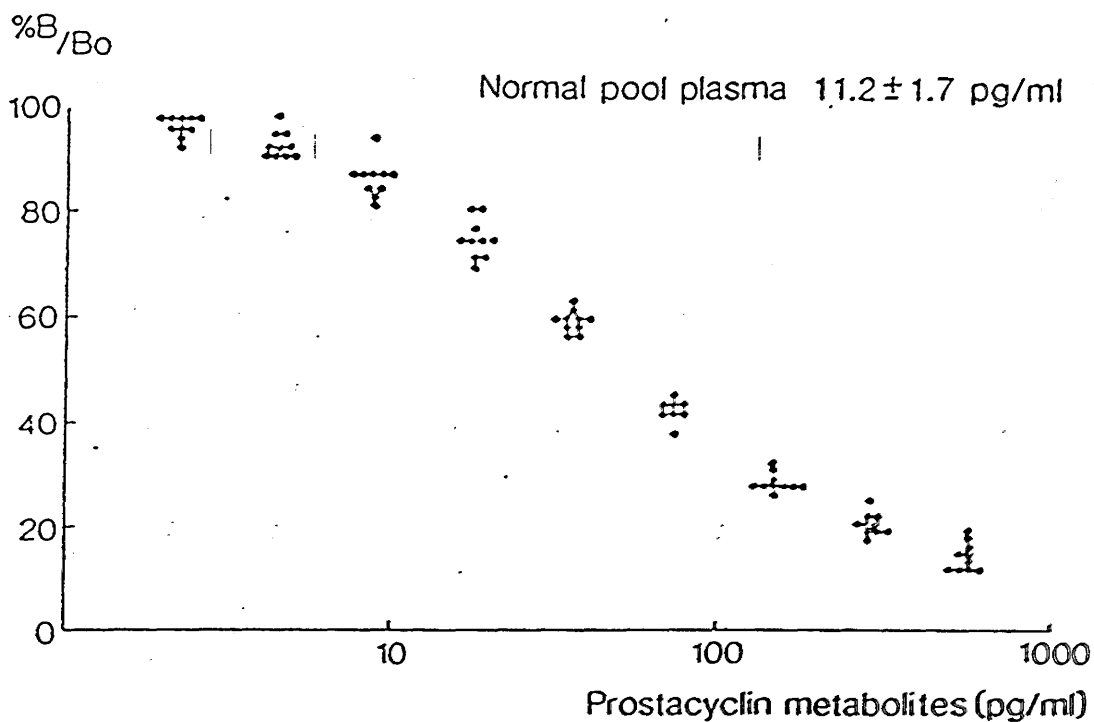


FIGURE 4.1

**Prostacyclin Metabolite Assay
Standard Curve Over Ten Assays**

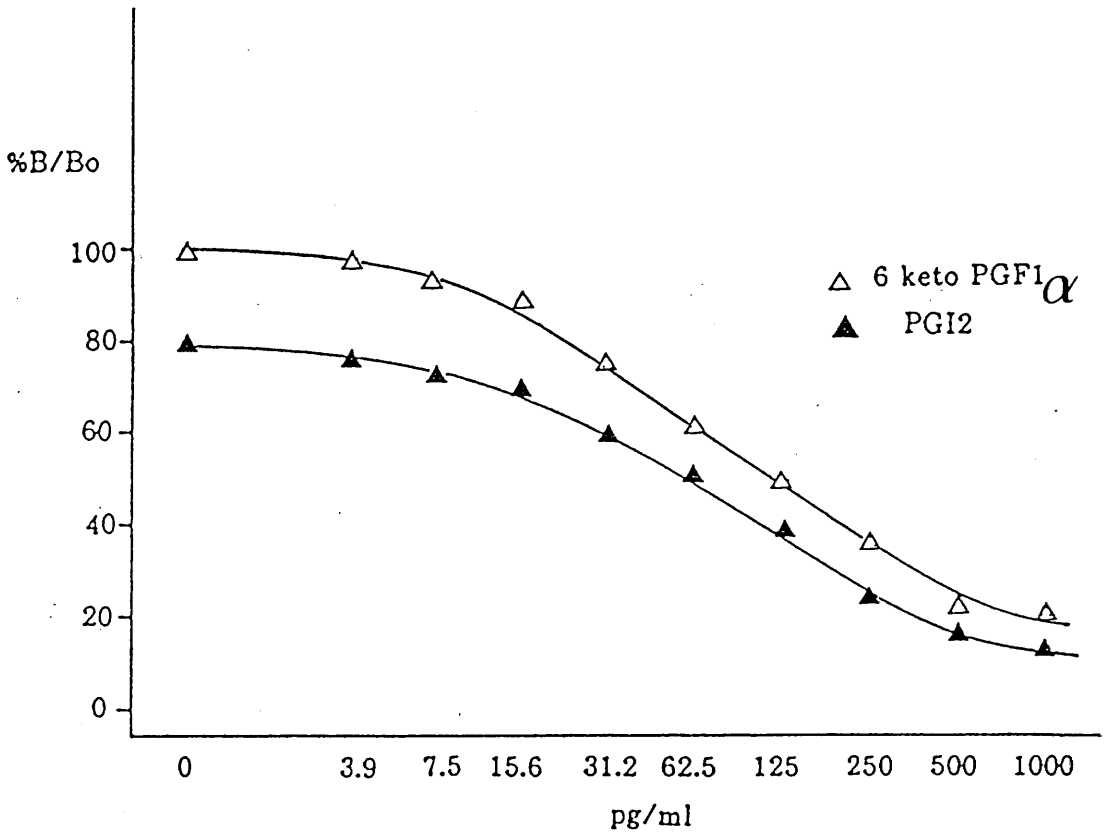


FIGURE 4.2

Standard curve for 6-keto-PGF₁α compared to
standard curve for hydrolysed PGI₂.

TABLE 4.2

PGI₂ Metabolite Concentrations During Infusions of PGI₂

<u>Time</u>	<u>Dose of PGI₂ (ug)</u>	<u>PGI₂ metabolites (pg/ml)</u>
0	0	5
15	4	50
30	8	70
45	12	139
60	16	220
15 min post-infusion	0	5

tips). Separation was performed using acid washed charcoal in assay buffer. All measurements were performed in duplicate and the mean value recorded.

The lower limit of sensitivity of the assay was 5 pg/ml, recovery of added 6-keto-PGF_{1α} was 95 ± 9 (SD)%; the intra-assay variation was 4% and interassay variation 4.9%.

The standard curves over 10 assays are shown in figure 4.1.

The assay was further validated by hydrolysing known quantities of PGI₂ in buffer, and comparing this with the same quantities of 6-keto-PGF_{1α} measured by the same assay (McLaren *et al.*, 1985; Belch *et al.*, 1983), this produced a parallel standard curve which is shown in figure 4.2. Validation was also performed *in vivo* by measuring PGI₂M in a normal volunteer infused with PGI₂ (McLaren *et al.*, 1985) where measured plasma PGI₂M correlated well with the rate of the infusion (table 4.2). Furthermore, subjects given non-steroidal anti-inflammatory agents had undetectable PGI₂M levels after treatment.

4.1.4 TxB₂ Assay Method

Venous blood was prepared as for the PGI₂M assay described above. TxB₂ was measured by RIA on unextracted plasma. Antibody was obtained from Upjohn Diagnostics Limited, Kalamazoo, Michigan, U.S.A., and this was a polyclonal antibody raised in rabbits. The cross reactivities for this antibody are shown in table 4.3. The method used was that of Granstrom and Kindahl (1978). The assay buffer was again 0.1M phosphate buffer pH 7.0 with 0.1% bovine

TABLE 4.3

Cross reactivities for TxB₂ antibody with available
prostaglandins and metabolites

	<u>% Cross-reactivity</u> (50% B/B ₀ Replacement)
TxB ₂	100.0 (by definition)
PGD ₂	1.2
PGF _{2α}	.15
13,14-diH-15-keto TxB ₂	.13
6β.PGI	.10
PGE ₁	.05
PGE ₂	.01
PGF _{1α}	.01
11-epi PGE ₁	.01
13,14-diH-6,15-diketo PGF _{1α}	.007
PGF _{1β}	.007
8-ISO-PGE ₂	.006
13,14-diH PGE ₂	.005
13,14-diH PGE ₁	.004
13,14-diH PGF _{2α}	.004
di-Homo-6-keto PGF _{1α}	.004
6-keto PGE ₁	.003
PGF _{2β}	.003
13,14-diH-15-keto PGE ₂	.003
15-keto PGE ₂	.002
13,14-diH PGF _{1α}	.002
6,15-di-keto PGF _{1α}	.001
PGB ₁	.001
13,14-diH-15-keto PGF _{2α}	< .001
15 keto PGF _{1α}	< .001
15-Epi PGE ₂	< .001
13,14-diH-15-keto PGF _{1α}	< .001
15-keto PGF _{2α}	< .001

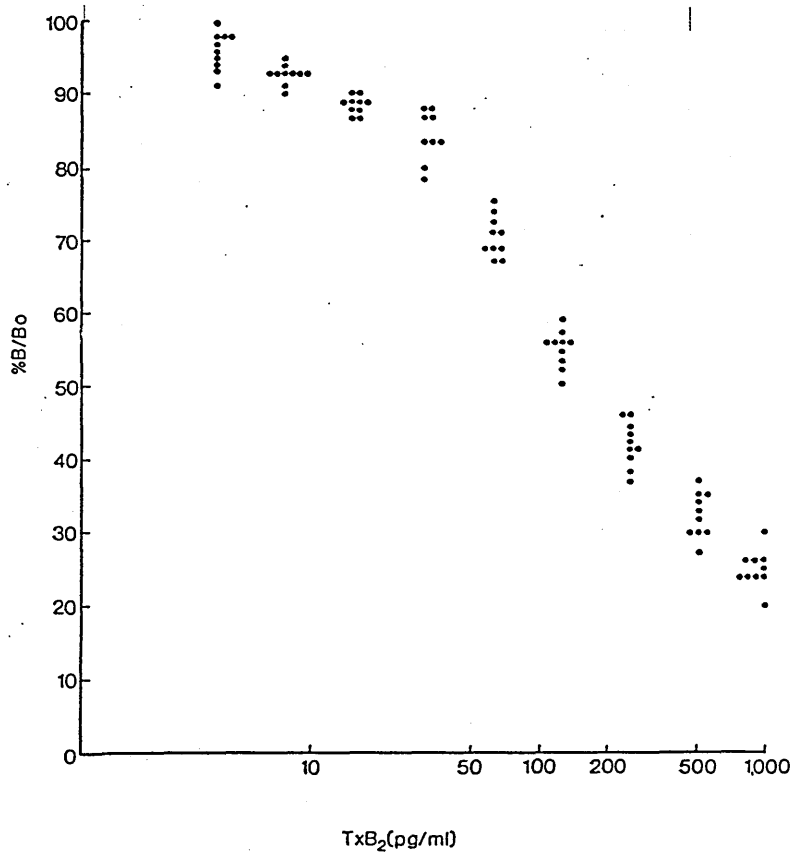


FIGURE 4.3

TxB₂ Assay: standard curve over 10 assays using ³H tracer.

serum albumen to prevent adsorption losses. Separation was performed with acid washed charcoal in assay buffer. All measurements were performed in duplicate and the mean value recorded. The lower limit of sensitivity was 10 pg/ml; the intra-assay variation was 4% and inter-assay variation was 10%. Recovery of added TxB₂ was 91.2 ± 5.5 (SD)%. The standard curves over 10 assays are shown in figure 4.3.

4.2 PLATELET AGGREGATION STUDIES IN PLATELET RICH PLASMA

4.2.1 Platelet Aggregation in Platelet Rich Plasma

The most commonly used method for studying platelet shape change and aggregation in platelet rich plasma (plasma containing platelets but not erythrocytes), is the photometric technique described by Born in 1962. This measures changes in light transmission through stirred platelet rich plasma (PRP). Initially, the disc shaped platelets cause oscillations in light transmission through the platelet suspension. Following the addition of an aggregating agent such as collagen these oscillations decrease in amplitude and light transmission falls slightly. During this time the platelets have been shown to change from a disc to a more rounded form with pseudopodia (O'Brien and Heywood, 1966; Born, 1970). This shape change which is due to calcium dependent myosin phosphorylation in the platelet cytoskeleton (Nachmias, Kavalier and Jacobowitz, 1985), is followed by aggregation. As the platelets aggregate the optical density of the plasma decreases, and light transmission increases. The increase in light transmission is proportional to the aggregation response and also varies with the initial platelet count (Born and Cross, 1963). Platelet contents must

therefore be standardised in the various samples of PRP, and this can be achieved by dilution with autologous platelet poor plasma. Most workers standardise the platelet count to 200 or 300 x 10⁹/l platelets.

4.2.2 Conditions Influencing Platelet Aggregation Tests

The anticoagulant used in the study is important, as heparin can itself induce aggregation (Zucker, 1970; Eika, 1972) and EDTA will inhibit aggregation (Zucker and Borrelli, 1962). Trisodium citrate is therefore the anticoagulant of choice. Although differences in citrate concentration can affect ADP induced aggregation (Ts'ao, Lo and Raymond, 1976), collagen induced aggregation has been shown to be the least affected by this (Rossi and Louis, 1977). The pH of the suspending plasma also influences aggregation. Only shape change occurs below a pH of 6.4 or above 10, and maximal aggregation occurs between pH 7.4 and 8.0 (Packham, Kinlough-Rathbone and Mustard, 1976). The rate of stirring influences platelet aggregation as it affects the number of collisions between platelets, and the platelet suspension is usually stirred at 1000 rpm by a magnetic stir bar (Born, 1962; Born and Cross, 1963). Temperature and time of storage are also important. There is little change in platelet function during the first 2 hours of storage at room temperature, while there is a progressive loss of function if storage is at 37°C (Han and Ardlie, 1974). It is conventional to study aggregation at 37°C (Packham et al., 1976).

4.2.3 Measurement of Platelet Aggregation in Platelet Rich Plasma

Various parameters have been used to measure the degree of aggregation. The maximal change in light transmission and the maximal rate of aggregation (maximal rate of change in optical density) being those most commonly adopted (Packham et al., 1976). The latter has been shown to be reliable and to have greater reproducibility for collagen induced aggregation (Rossi and Louis, 1977). It is therefore the method adopted for measuring aggregation in the following studies.

4.2.4 Preparation of Platelet Rich Plasma

Venous blood was collected without stasis from healthy volunteers, using a 21 gauge butterfly cannula and anticoagulated with 3.2% trisodium citrate (9:1 volume:volume). No volunteer had taken drugs of any kind for a minimum of 2 weeks prior to venesection. Platelet rich plasma (PRP) was obtained by centrifugation at 150 g for 15 minutes at room temperature, and platelet poor plasma (PPP) by further centrifugation at 1500 g for 10 minutes. The PRP platelet count was determined using a Coulter thrombocounter (Coulter Electronics Ltd., England) and standardised to $300 \times 10^9/l$ by dilution with autologous PPP if required. All experiments were completed within 2 hours of venepuncture.

4.2.5 Effect of Drugs on Platelet Aggregation

Varying concentrations of drug or vehicle, the latter acting as control were added to 200 ul aliquots of PRP. The volume of drug or vehicle solution added was kept constant at 100 ul giving a

total volume of 300 μ l in the aggregometer cuvette, and a final platelet concentration of $200 \times 10^9/l$. Treated PRP was incubated at 37°C for 5 minutes. Platelet aggregation was performed after the method of Born (1962) using a Malins photometric 2 channel platelet aggregometer (Malins Electronics Ltd., U.K.) coupled to a pen recorder. The PRP was stirred at a constant 1000 rpm. Calibration was performed using stirred PRP with vehicle as 0% light transmission, and PPP with vehicle as 100% light transmission. Collagen was used as the aggregating agent at final concentrations of 1 μ g/ml and 2 μ g/ml. Maximum rate of aggregation was determined by measuring the maximum gradient of the aggregation curve (change in optical density/time) and expressed as a percentage of the control (PRP with vehicle) rate of aggregation. All drugs were dissolved in 0.1 M phosphate buffer pH 7.0 (pH 7.0 was required to facilitate dilution of some of the drugs assessed).

4.3 THROMBOXANE GENERATION FROM PLATELET RICH PLASMA

4.3.1 Thromboxane A₂ Generation

The main product of the cyclo-oxygenase pathway in platelets is TxB₂ which is a potent vasoconstrictor and platelet aggregating agent (Hamberg *et al.*, 1975). It is released when platelets aggregate and the quantity produced is dependent on platelet concentration and the type and dose of the aggregating agent used (Siess, Roth and Weber 1981). The aim of the following methodology was to provide a reliable method for determining the effect of drugs on platelet TxB₂ production.

4.3.2 Effect of Different Aggregating Agents and Their Concentrations on Thromboxane B₂ Production from PRP

Platelet rich plasma (PRP) was prepared and standardised as above, from healthy donors none of whom had taken any drugs for a minimum of two weeks prior to venesection. The PRP was incubated with the vehicle which was to be used in the later studies (0.1 M phosphate buffer pH 7.0) for 10 minutes at 37°C. The volume of vehicle was kept constant with 100 ul of vehicle being added to each 200 ul aliquot of PRP. The final platelet concentration was therefore $200 \times 10^9/l$. Varying doses of collagen, ADP and arachidonic acid were used to induce aggregation.

Exactly 4 minutes after adding the aggregating agent the reaction was halted by adding 100 ul of absolute alcohol. The samples were then frozen in dry ice, and stored at -70°C. Thromboxane A₂ was measured by radioimmunoassay of its stable hydration product thromboxane B₂ (TxB₂) as described above. Prior to radio-immunoassay the samples were thawed, centrifuged at 1500 g for 10 minutes at 4°C to remove cell debris, and diluted 1:300 using assay buffer (0.1 M phosphate buffer pH 7.0 with 0.1% bovine albumen) for collagen and ADP induced aggregation, and 1:400 for arachidonic acid induced aggregation. Results for TxB₂ generation are expressed as ng/ml/ 10^7 platelets to compensate for minor variations in the final platelet count. Rate of aggregation was also determined for these experiments and expressed as change in optical density/time (arbitrary units).

4.3.3 Effect of Indomethacin on Thromboxane A₂ Generation

To confirm that the method could measure inhibition of TxB₂ production, indomethacin an inhibitor of platelet cyclo-oxygenase was studied in this model.

Platelet rich plasma was prepared and standardised as before, and incubated with either vehicle (0.1 M phosphate buffer pH 7.0 or indomethacin in final drug concentrations of 0.1 and 1.0 ug/ml, for 10 minutes at 37°C. The volume of drug/vehicle was kept constant with 100 ul being added to each 200 ul of PRP. Aggregation was induced with either 4 ug/ml collagen (final concentration) or 0.5 mM arachidonic acid (final concentrations). These concentrations were used as they provided maximal aggregation and TxB₂ production. ADP was not assessed as it induced relatively little TxB₂ generation compared to arachidonic acid and collagen (see figure 5.1), confirming the findings of previous studies (Siess et al., 1977; Koh et al., 1980).

Exactly 4 minutes after adding the aggregating agent the reaction was halted by adding 100 ul of absolute alcohol. The samples were then frozen in dry ice, and stored at -70°C. Thromboxane A₂ was measured by radioimmunoassay of its stable hydration product thromboxane B₂ (TxB₂) as described above. Prior to radioimmunoassay the samples were thawed, centrifuged at 1500 g for 10 minutes at 4°C to remove cell debris, and diluted 1:300 using assay buffer for collagen induced aggregation and 1:400 for TxB₂

generation was expressed as ng/ml/10⁷ platelets. Rate of aggregation was also determined and expressed as change in optical density/time (arbitrary units).

4.3.4 Effect of Various Antihypertensive Agents on TxA₂ Generation

PRP was prepared as before from healthy donors, and incubated with drug or vehicle at 37°C for 10 minutes. The volume of drug/vehicle was again kept constant with 100 ul being added to each 200 ul of PRP. Aggregation was then performed using 4 ug/ml collagen (final concentration) as the aggregating stimulus. Exactly 4 minutes after adding collagen the reaction was halted by adding 200 ul of absolute alcohol. The samples were then frozen in dry ice, and stored at -70°C. Thromboxane A₂ was measured by radioimmunoassay of its stable hydration product TxB₂ as described above (Chapter 4.1.4). Prior to radioimmunoassay the samples were thawed, centrifuged at 1500 g for 10 minutes at 4°C to remove cell debris, and diluted 1:300 using assay buffer.

The dose of collagen was chosen to provide maximal aggregation so that there was no difference in the rate of aggregation between drug treated or control PRP. This was done to exclude any difference in rate of aggregation being responsible for any change in TxB₂ generation. Drugs found to have an inhibitory effect on TxB₂ generation were assessed further to determine whether this inhibitory effect could be overcome by using exogenous arachidonic acid as the aggregating stimulus. The experiment was repeated as before with 0.5 mM arachidonic acid replacing collagen as the aggregating agent. Following

arachidonic acid aggregation, TxB₂ was assayed as before except that a dilution of 1:400 with assay buffer was performed. Results for TxB₂ generation are expressed as ng/ml/10⁷ platelets to compensate for minor variations in the final platelet count. Rate of aggregation was also determined for all these experiments, and expressed as percentage of control. None of the drugs assessed had any demonstrable cross reactivity with the assay. Exogenous arachidonic acid also had no demonstrable cross reactivity.

4.4 SYNERGISM OF PROSTACYCLIN WITH ADRENOCEPTOR ANTAGONISTS IN INHIBITING PLATELET AGGREGATION IN PLATELET RICH PLASMA

PGI₂ is a potent inhibitor of platelet aggregation (Moncada et al., 1976). Some of the foregoing studies have shown that some adrenoceptor antagonists have anti-platelet effects. The aim of the following experiments was to determine whether PGI₂ was synergistic with adrenoceptor antagonists, in inhibiting platelet aggregation in PRP.

4.4.1 Effect of Prostacyclin on Platelet Aggregation

Initially dose response curves for the effect of PGI₂ on collagen induced aggregation were constructed to determine what dose to use in the experiments. Platelet rich plasma was prepared and standardised as before. 100 ul of 0.1 M phosphate buffer was added to each 200 ul aliquot of PRP. The final platelet concentration was 200 x 10⁹/l. Varying doses of PGI₂ (Wellcome, U.K.) or its vehicle (glycine buffer pH 10.5, Wellcome U.K.) were added 2 minutes prior to aggregation being performed and incubated at 37°C. The volume of PGI₂ or buffer added was kept constant at 10 ul. Aggregation was induced using 1 ug/ml

and 2 ug/ml collagen (final concentrations). The volume of aggregating agent was also kept constant at 3 ul. Maximal rate of aggregation was measured and expressed as a percentage of the control aggregation.

4.4.2 Effect of Adrenoceptor Antagonists and Prostacyclin on Platelet Aggregation

200 ul aliquots of platelet rich plasma were prepared as before and incubated with 100 ul of either drug, in final concentrations of 1 ug/ml or 12.5 ug/ml, or vehicle alone (0.1 M phosphate buffer pH 7.0). After 8 minutes incubation at 37°C, PGI₂ at a final concentration of 100 pg/ml or its vehicle (glycine buffer pH 10.5) was added (10 ul) and incubation continued for a further 2 minutes. Aggregation was then induced by collagen in a final concentration of 2 ug/ml. Maximal rate of aggregation was measured and expressed as a percentage of the control value.

4.4.3 Synergistic Action of PGI₂-like Activity from Human Umbilical Artery with Adrenoceptor Antagonists in Inhibiting Platelet Aggregation

PGI₂ is the major prostanoid produced by umbilical artery (Ritter *et al.*, 1982). The ability of the PGI₂-like activity of human umbilical artery to act synergistically with adrenoceptor antagonists was examined using this method. Fresh umbilical cords were obtained from normal pregnancies at term. The umbilical arteries were flushed with physiological saline, then dissected out and cut into rings 2 mm wide. The rings were then washed in Ringer's lactate buffer, which had been gassed with a

mixture of 95% air:5% CO₂, for approximately 3 hours at 37°C. Two rings were then placed in each tissue bath containing 2 ml of Ringer's lactate buffer at 37°C. Drugs were dissolved in 0.1 M phosphate buffer pH 7.0. PRP was prepared as before.

50 ul of drug solution or vehicle (buffer alone) was added to each 200 ul aliquot of PRP, and incubated at 37°C for 8 minutes. 50 ul of supernatant from the umbilical artery rings, or 50 ul of Ringer's lactate buffer alone was then added and incubation continued for a further 2 minutes. Aggregation was then performed as before using collagen in a final concentration of 2 ug/ml. Maximal rate of aggregation was measured and expressed as a percentage of the control (control = 200 ul PRP, 50 ul phosphate buffer and 50 ul Ringer's lactate buffer).

4.5 EFFECT OF DRUGS ON PGI₂ PRODUCTION FROM HUMAN UMBILICAL ARTERY

PGI₂ is the main product of the arachidonic acid cascade in vascular tissue (Moncada et al., 1977) and has been shown to be the major prostaglandin produced from umbilical arteries (Ritter et al., 1982). The object of the following method was to determine whether any of the drugs assessed had any effect on PGI₂ production from human umbilical artery.

Fresh umbilical cords were obtained from normal pregnancies at term. The umbilical arteries were flushed with physiological saline, then dissected out and cut into rings 2 mm wide. The artery rings were then washed in Ringer's lactate buffer, which had been gassed with a mixture of 95% air:5% CO₂, for 3 hours at

37°C to exhaust them of spontaneous endogenous PGI₂ production. Two rings (wet weight approximately 25 mg) were then placed into each tube containing 1 ml of pooled plasma, and either the drug or its vehicle to act as control, or 100 ug/ml aspirin. Aspirin was used to confirm that inhibition of PGI₂ production from the artery rings was possible. Pooled plasma was prepared from 6 healthy donors using 3.2% trisodium citrate (9:1 v:v) as the anticoagulant. After 15 minutes incubation at 37°C 750 ul aliquots were removed and PGI₂M measured by RIA as described above. The amount of PGI₂M was corrected for tissue weight, if required, and is expressed as pg/ml/25 mg tissue.

4.6 PLATELET AGGREGATION IN WHOLE BLOOD

4.6.1 Limitations of Platelet Aggregation Studies in Platelet Rich Plasma and Advantages of Studying Aggregation in Whole Blood

The most commonly used method for studying platelet aggregation in vitro is the photometric technique of Born (1962) and O'Brien (1962) which measures aggregation in stirred platelet rich plasma. While this method has proved invaluable in the assessment of platelet function, it has several inherent limitations. Firstly, the whole blood must be centrifuged to produce PRP, separating platelets from red cells and white cells. Red cells are known to influence aggregation by release of ADP (Harrison and Mitchell, 1966; Born and Wehmeier, 1979; Fox et al., 1982; Saniabadi et al., 1984a) and also by a mechanical action on the platelets which facilitates platelet-platelet collisions (Goldsmith, 1971; Turito and Baumgartner, 1975). White cells, conversely, may reduce platelet aggregation by generating PGI₂ (Blackwell et al., 1978; Deckmyn et al., 1983).

Furthermore, platelets are heterogenous with regard to age, size, density and metabolic activities (Karpatkin and Charmatz, 1969; Karpatkin, 1969; Karpatkin, 1978; Mannucci and Sharp, 1967), with larger and denser platelets being metabolically more active (Karpatkin and Charmatz, 1969), than smaller and lighter ones. Consequently, certain sub-populations may be spun down and lost on centrifugation and studies in platelet rich plasma may not be representative of platelet function in whole blood.

In addition the photometric technique using platelet rich plasma is only semi-quantitative. It has been shown by microscopy that after addition of an aggregatory stimulus free platelets form small aggregates prior to any change in light transmission occurring (Born and Hume, 1967). The first visible change in light transmission in platelet rich plasma treated with low dose ADP has been shown to occur at the moment all free platelets disappear (Nichols and Bosmann, 1979) and the presence of a second wave of aggregation, induced by ADP is not related to any change in platelet number (Gear and Lambrecht, 1981) but possibly to formation of larger aggregates or changes in density (Born and Hume, 1967).

In view of these limitations it is of value to study platelet aggregation in whole blood, leaving the whole platelet population in their natural milieu surrounded by red cells and white cells. It has recently become possible to measure platelet aggregation in whole blood using the Clay Adams Ultra Flo 100 whole blood platelet counter (Lumley and Humphrey, 1981), thus avoiding the

problems associated with preparation of platelet rich plasma. The Ultra Flo 100 can count accurately the number of single platelets in whole blood (Day, Young and Helfrich, 1979). It is essentially an impedance type electronic cell counter, which counts both platelets and red cells in a diluted specimen of whole blood differentiating them according to size. The Ultra Flo 100 was used in the following studies on platelet aggregation in whole blood.

4.6.2 Studies on Platelet Aggregation in Whole Blood

Venous blood was obtained without stasis from healthy male and female volunteers using a 21 gauge butterfly cannula, and anticoagulated with 3.2% trisodium citrate (9:1 v:v). No donor had taken any drugs for a minimum of 2 weeks prior to venepuncture. The blood was divided into 0.9 ml aliquots and varying concentrations of drug or vehicle were added, the latter acting as control. The volume of drug or vehicle added was kept constant at 100 ul giving a final volume of 1 ml. Red cell count was determined on one of the aliquots for each donor using the Clay-Adams Haematology Analyser (HA-5), the value obtained was then dialed into the Clay Adams Ultra Flo 100 whole blood platelet counter prior to any platelet count being performed. Each sample was incubated for 30 minutes at 37°C. A 10 ul aliquot was then withdrawn from the sample and rinsed into a prefilled diluent reservoir (Clay Adams) and platelet count determined using the Ultra Flo 100. The value obtained was recorded as the baseline (time 0) platelet count. The aggregating agent was then added and each sample placed in a shaking water bath (Gallenkamp; 200 shakes/min; excursion of tube

holder approximately 5 cm). Further 10 ul aliquots were removed at 1,2,3 and 5 minutes following addition of the aggregating agents, and platelet count determined as before. At all times 2 platelet counts were taken and the mean value recorded and expressed as a percentage of the baseline (time 0) value. Three aggregating agents were used, collagen (Hormone Chemie) in a final concentration of 0.5 ug/ml, ADP (Sigma) in a final concentration of 10 uM and arachidonic acid (Sigma) in a final concentration of 0.5 mM; the volume of all aggregating agents was kept constant at 10 ul.

This technique of platelet aggregation in whole blood using the Clay-Adams Ultra Flo 100 has previously been fully evaluated by Saniabadi et al., (1983a; 1983b; 1984a; 1984b) in our laboratory, and has been shown to be a sensitive and reliable method.

4.6.3 Effect of PGI₂ on Platelet Aggregation in Whole Blood

Blood was prepared as before and incubated for 2 minutes at 37°C with varying concentrations of PGI₂ (Wellcome U.K.) or its vehicle (glycine buffer pH 10.5). The volume of PGI₂ or buffer which was added was kept constant at 10 ul. Aggregation was performed as above using 0.5 ug/ml collagen. A concentration of PGI₂ which had minimal effect on aggregation to collagen in all subjects was thus determined (125 pg/ml PGI₂). This concentration was used in assessing whether PGI₂ was synergistic with adrenoceptor antagonists and a calcium channel blocking agent (nicardipine) in inhibiting platelet aggregation.

4.6.4 Synergistic Effects of Adrenoceptor Antagonists and Nicardipine with PGI₂ in Inhibiting Platelet Aggregation in Whole Blood

Aliquots of whole blood were prepared as before and incubated with either labetalol, pindolol or propranolol at a final concentration of 1 ug/ml, or their vehicles. After 28 minutes incubation at 37°C, PGI₂ in a final concentration of 125 pg/ml or its vehicle was added, and incubation continued for a further 2 minutes. Aggregation studies were then performed as before using 0.5 ug/ml collagen as the aggregating agent.

The possible synergistic effect of the calcium channel blocking agent nicardipine was similarly assessed with the final concentration of nicardipine being 10 ug/ml.

The volume of drug or vehicle added was kept constant at 100 ul, in all experiments.

4.6.5 Synergistic Action of Aspirin with an Adrenoceptor Antagonist or Calcium Channel Blocking Agent in Inhibiting Platelet Aggregation in Whole Blood

Blood from healthy donors, none of whom had taken any drugs for at least 2 weeks before venesection, was anticoagulated with 3.2% trisodium citrate (9:1 v:v). Samples were then incubated with labetalol, nicardipine, or their vehicles (controls) for 25 minutes at 37°C. Aspirin or its vehicle was then added and incubation continued for a further 5 minutes. The concentrations of all three drugs were known from prior assessment to have little effect on aggregation when used alone in all subjects.

studied. Whole blood aggregation was performed with the Clay Adams Ultra Flo 100 whole blood platelet counter, platelet counts being measured 1, 2, 3 and 5 minutes after addition of the aggregating agent (0.5 ug/ml collagen) and expressed as a percentage of the count at time zero.

All aggregation studies in whole blood were completed within 2 hours of venesection.

4.7 GENERATION OF TxB₂ AND PGI₂M FROM WHOLE BLOOD

It is known that whole blood if allowed to clot will produce TxA₂ due to endogenous thrombin induced activation of platelets. This has been shown to be a simple and reproducible capacity related index of platelet TxA₂ production (Alessandrini et al., 1985; Patrono et al., 1980). Whole blood is also known to produce PGI₂ (Blackwell et al., 1978; Deckmyn et al., 1983) from white cells.

The effect of various drugs on production of TxA₂ and PGI₂ from whole blood was determined using the following methodology. Fresh venous blood was obtained without stasis using a 21 gauge butterfly cannula, from healthy male and female volunteers. No donor had taken any medication for a minimum of 2 weeks prior to venepuncture. No anticoagulant was used and 0.9 ml aliquots were immediately placed into glass tubes containing either the drug or its vehicle, the latter acting as control. The volume of drug or vehicle was kept constant at 100 ul. The samples were then allowed to clot spontaneously at 37°C for 30 minutes. The reaction was halted by centrifugation at 3000 rpm for 15 minutes at 4°C. The serum was then assayed for immunoreactive

6-keto-PGF_{1α} (PGI₂M) and TxB₂ using radioimmunoassays which have been described above. Prior to TxB₂ measurement the serum required to be diluted 1:300 with assay buffer.

To confirm that inhibition of PGI₂M and TxB₂ production could be measured using this technique, the experiment was first performed with varying concentrations of indomethacin or its vehicle (0.9% sodium chloride).

CHAPTER 5

A COMPARATIVE STUDY OF THE EFFECT OF ADRENOCEPTOR
ANTAGONISTS ON PLATELET AGGREGATION
AND THROMBOXANE GENERATION

5.1 INTRODUCTION

Platelets have a well established role in the pathogenesis of vascular disease, and platelet reactivity has been shown to be increased in patients with arteriosclerotic disease (Steele et al., 1973; Murphy and Mustard, 1962; Harker and Slichter, 1972; Mehta et al., 1978). Platelets contribute to the disease process both by obstructing the microcirculation when aggregates form, and by release of vasoactive substances such as thromboxane A₂ (TxA₂) which is the major arachidonic acid metabolite in platelets. It is released when platelets aggregate and is a potent platelet aggregating agent and vasoconstrictor (Hamberg et al., 1975) which has been implicated in experimental myocardial infarction (Coker et al., 1981), vasospastic angina (Lewy et al., 1979; Robertson et al., 1981), and ischaemic heart disease (Hirsh et al., 1981).

β -adrenoceptor antagonists are used extensively in the treatment of hypertension and ischaemic heart disease. Propranolol has been shown to reduce platelet aggregation responses in patients with coronary artery disease (Frishman et al., 1974) and hypertension (Campbell et al., 1981a). This has provoked interest in the effect of β -adrenoceptor antagonists on platelet function and platelet aggregation is known to be affected by these drugs (Weksler et al., 1977; Frishman et al., 1978; Small et al., 1982; Campbell et al., 1981b; Rubegni, Provvedi and Bellini, 1974). Although the mechanism is not entirely clear, it is independent of β -adrenoceptor antagonism (Weksler et al., 1977) and may be due to the membrane stabilising activity (MSA) of these compounds (Weksler et al., 1977; Dachany-Prigent et al., 1979; Heinroth et

TABLE 5.1

Properties of adrenoceptor antagonists

Drug	Receptors* Blocked	ISA*	MSA*	Lipid Solubility** (distribution coefficient in octanol-buffer pH 7.4 and 37°C)	Recorded Plasma Levels
Atenolol	β_1	-	-	0.015	0.2 - 0.5 ug/ml***
Labetalol	$\alpha_1, \beta_1, \beta_2$	-	+	11.5	0.4 - 0.7 ug/ml ⁺
Metoprolol	β_1	-	+/-	0.98	0.05 - 0.1 ug/ml***
Pindolol	β_1, β_2	+	+/-	0.82	0.05 - 0.15 ug/ml***
Propranolol	β_1, β_2	-	+	20.2	0.05 - 0.1 ug/ml***
Timolol	β_1, β_2	+	-	1.16	0.005 - 0.1 ug/ml***
Prazosin	α_1	-	-	-	0.23 ug/ml ⁺⁺

* Feely et al. (1983)

** Woods and Robinson (1981)

*** Frishman (1979)

+ unpublished observations

++ Wood et al. (1976)

al., 1983). More recently studies have described an inhibitory effect on TxA_2 production (Campbell et al., 1981a; Campbell et al., 1981b; Heinroth et al., 1983; Mehta and Mehta, 1982; Mehta, Mehta and Ostrowski, 1983). The majority of studies have concentrated on propranolol despite the large numbers of α and β -adrenoceptor antagonists available. Furthermore, adrenergic blocking agents differ widely in their receptor selectivity, possession of MSA, and intrinsic sympathomimetic activity (ISA), and lipophilicity. The aim of this study was to compare the effects of a wide range of adrenoceptor antagonists with varying ISA, MSA, lipophilicity and receptor specificity (table 5.1) on platelet aggregation and TxA_2 generation.

5.2 MATERIALS AND METHODS

The materials and methods for the following in vitro studies using platelet rich plasma (PRP) are fully described in Chapter 4. Venous blood from normal volunteers was obtained and PRP prepared as described in Chapter 4.2.4.

The effect of adrenoceptor antagonists on platelet aggregation was studied using the method described in Chapter 4.2.5. Collagen was used as the aggregating agent at a final concentration of 1 $\mu\text{g}/\text{ml}$. Drugs showing an inhibitory effect on 1 $\mu\text{g}/\text{ml}$ collagen induced aggregation, were also assessed with 2 $\mu\text{g}/\text{ml}$ collagen.

The effects of adrenoceptor antagonists on TxB_2 production from PRP were studied using the methodology described in Chapter 4.3.4. Prior to any studies using the drugs, the methodology was validated by constructing dose response curves for TxB_2

production and platelet aggregation in response to collagen, arachidonic acid and adenosine diphosphate (ADP) as described in Chapter 4.3.2 and the minimal concentration of each aggregating agent which produced maximal TxB_2 generation and platelet aggregation was thus determined.

To confirm that this method could measure inhibition of TxB_2 production, indomethacin, an inhibitor of platelet cyclooxygenase, was studied in this system as described in Chapter 4.3.3.

Statistical analysis was by the Wilcoxon rank sum test for paired data and all results are expressed as mean \pm SEM. Correlation was assessed using the Spearman rank test.

5.3 RESULTS

5.3.1 Dose Response Curves for TxB_2 Production and Platelet Aggregation in Response to Arachidonic Acid, Collagen and ADP.

The dose response curves for TxB_2 production and platelet aggregation in response to arachidonic acid, collagen and ADP are illustrated in figure 5.1. As can be seen, all three aggregating agents stimulated TxB_2 production and platelet aggregation in a dose dependent manner. However, ADP induced relatively little TxB_2 production compared to arachidonic acid or collagen, and therefore was not used in any of the subsequent experiments to study the effect of drugs on TxB_2 production.

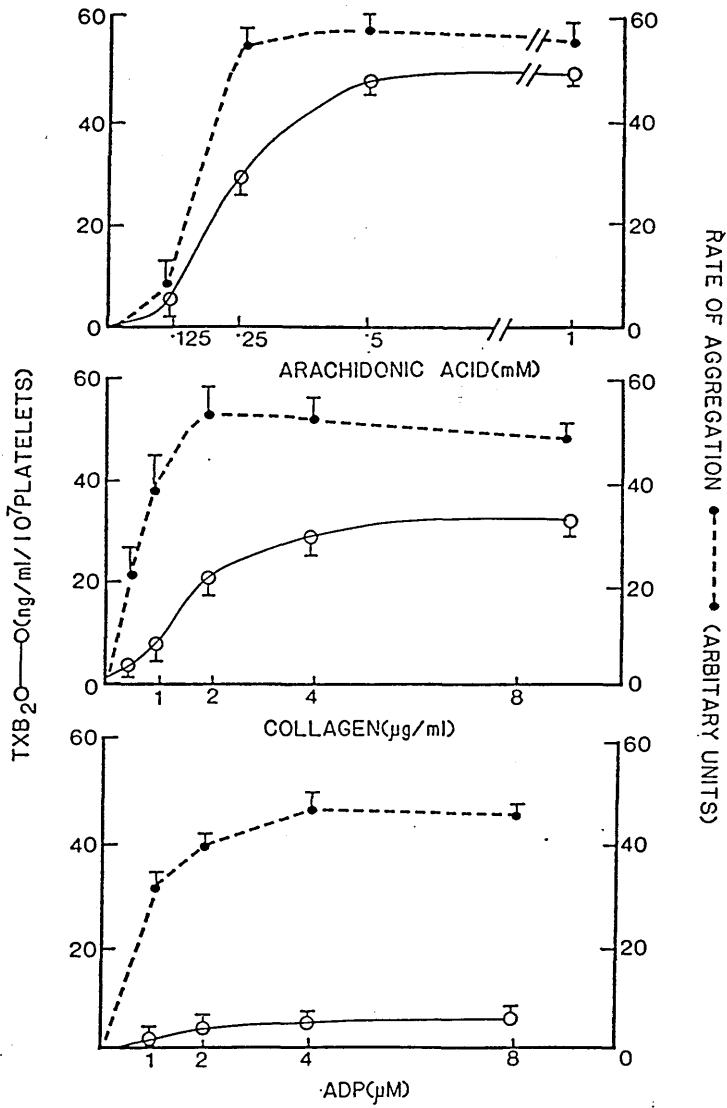


FIGURE 5.1

Dose response curves for Tx_{B2} generation and platelet aggregation in response to arachidonic acid, collagen and ADP. (Each point represent the mean ± SEM of 6 experiments)

Maximal aggregation and TxB₂ production were obtained at a concentration of 4 ug/ml collagen and 0.5 mM arachidonic acid. Increasing the dose of each aggregating agent above these concentrations did not produce any significant effect on either platelet aggregation or TxB₂ production. These concentrations of collagen and arachidonic acid were therefore used to assess the effect of drugs on TxB₂ production in the following experiments.

Under maximal stimulation, arachidonic acid stimulated more TxB₂ production than either of the other aggregating agents.

The dose of arachidonic acid used correlated positively with the rate of aggregation ($r = 0.74$; $p < 0.001$), and with TxB₂ production ($r = 0.97$; $p < 0.001$). Rate of aggregation in response to arachidonic acid correlated with TxB₂ production ($r = 0.72$; $p < 0.001$).

The dose of collagen used correlated with the rate of aggregation ($r = 0.75$; $p < 0.001$) and TxB₂ production ($r = 0.75$; $p < 0.001$). Rate of aggregation in response to collagen correlated with TxB₂ production ($r = 0.72$; $p < 0.001$).

The dose of ADP used correlated with the rate of aggregation ($r = 0.83$; $p < 0.001$) and TxB₂ production ($r = 0.89$; $p < 0.001$). Rate of aggregation in response to ADP correlated with TxB₂ production ($r = 0.74$; $p < 0.001$).

5.3.2 Effect of Indomethacin on TxB₂ Production

The effects of indomethacin on TxB₂ production and platelet aggregation induced by 0.5 mM arachidonic acid and 4 ug/ml collagen are illustrated in figure 5.2.

Indomethacin significantly inhibited both aggregation and TxB₂ production in response to 0.5 mM arachidonic acid at a dose of 0.1 ug/ml, and virtually abolished both aggregation and TxB₂ production at a dose of 1.0 ug/ml. However, both aggregation and TxB₂ production induced by collagen were unaffected by 0.1 ug/ml indomethacin, but were significantly inhibited by the higher dose of indomethacin (1.0 ug/ml).

5.3.3 Effect of Adrenoceptor Antagonists on Platelet Aggregation and Thromboxane Generation

Labetalol, pindolol and propranolol had significant anti-aggregatory actions on both 1 ug/ml and 2 ug/ml collagen induced aggregation and these are illustrated in the form of dose response curves (figure 5.3). The dose response curves were "shifted" to the right when the larger dose of collagen was used (figure 5.3). None of these agents had any effect on the platelet shape change reaction which occurred following addition of collagen as measured by the change in wave form of the aggregation trace. Atenolol, metoprolol and timolol had no demonstrable effect on collagen induced aggregation at drug concentrations up to 100 ug/ml (table 5.2) and prazosin had no effect at concentrations up to 4 ug/ml (table 5.2). Labetalol, pindolol and propranolol also significantly inhibited TxB₂ generation after stimulation with 4 ug/ml collagen (figure 5.4)

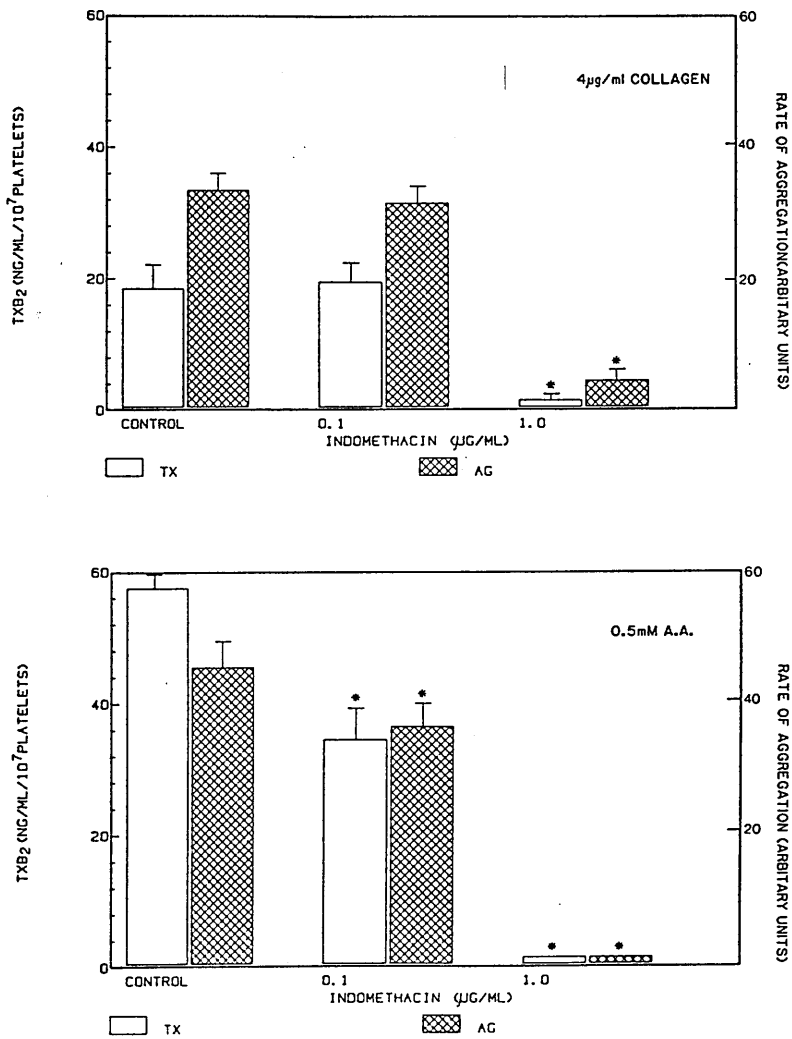


FIGURE 5.2

Effect of indomethacin on platelet aggregation and TxB₂ generation in response to 0.5 mM arachidonic acid and 4 µg/ml collagen. Each bar represents the mean ± SEM of 6 experiments. (Tx = TxB₂; Ag = Rate of Aggregation)

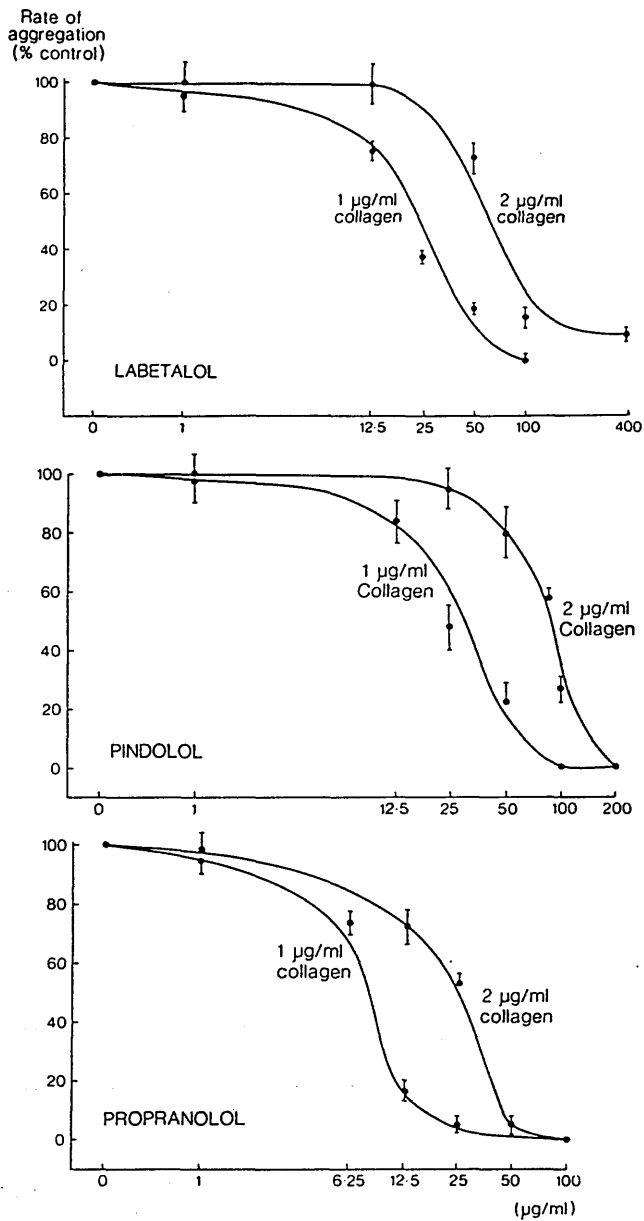


FIGURE 5.3

Effect of labetalol, pindolol and propranolol on platelet aggregation to collagen. Each point represents the mean (\pm SEM) of 5 separate experiments.

TABLE 5.2

Effect of adrenoceptor antagonists on platelet aggregation to 1 ug/ml collagen (all results expressed as mean \pm SEM).

Drug	Concentration ug/ml	n	Rate of Aggregation (% control)
Metoprolol	25	8	90 \pm 8.6
	50	8	95 \pm 12.5
	100	8	92 \pm 6.5
Atenolol	25	8	109 \pm 15.7
	50	8	126 \pm 17.5
	100	8	128 \pm 16.0
Timolol	25	7	103 \pm 2.3
	50	7	116 \pm 7.0
	100	7	110 \pm 7.6
Prazosin*	0.5	6	105 \pm 6.5
	1	6	104 \pm 9.4
	4	6	92 \pm 9.8

*low drug solubility prevented assessment at higher concentrations.

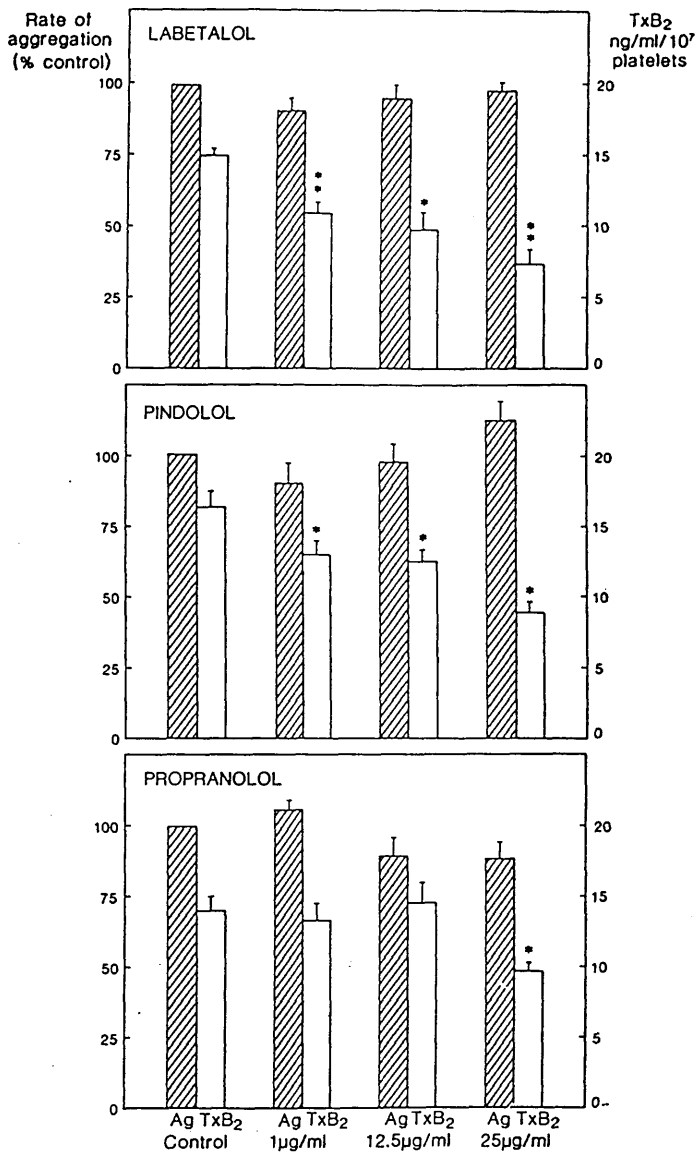


FIGURE 5.4

Effect of labetalol, pindolol and propranolol on platelet aggregation and TxB₂ generation from PRP stimulated with 4 ug/ml collagen. (Mean ± SEM of 8 separate experiments)

* p < 0.05

** p < 0.02

TABLE 5.3

Effect of adrenoceptor antagonists on TxB₂ generation
(all results expressed as mean ± SEM of 8 separate experiments)

Drug	Concentration (ug/ml)	Rate of Aggregation (% control)	TxB ₂ (ng/ml/10 ⁷ platelets)
Metoprolol	control	100 ± 0	17.7 ± 1.00
	1	99 ± 6.4	17.1 ± 0.75
	12.5	101 ± 6.8	18.0 ± 1.01
	25	99 ± 2.1	17.5 ± 0.86
Atenolol	control	100 ± 0	15.8 ± 1.35
	1	114 ± 9.5	17.3 ± 0.95
	12.5	114 ± 13.6	17.5 ± 0.86
	25	119 ± 18.9	15.7 ± 0.92
Timolol	control	100 ± 0	18.6 ± 1.11
	1	111 ± 13.0	17.7 ± 0.62
	12.5	112 ± 12.2	19.8 ± 1.27
	25	111 ± 10.3	19.0 ± 1.63
Prazosin	control	100 ± 0	18.1 ± 0.92
	0.05	107 ± 11.3	18.7 ± 0.74
	0.5	113 ± 8.0	17.4 ± 0.94
	1	105 ± 10.6	19.0 ± 0.85

TABLE 5.4

Effect of labetalol, pindolol and propranolol on platelet aggregation and TxB₂ generation with 0.5 mM/1 arachidonic acid. All results expressed as mean ± SEM of 8 separate experiments.

Drug	Concentration (ug/ml)	Rate of Aggregation (% control)	TxB ₂ (ng/ml/10 ⁷ platelets)
Labetalol	control	100 ± 0	50 ± 2.1
	1	136 ± 17.6	53 ± 1.5
	12.5	119 ± 14.8	54 ± 1.7
	25	105 ± 14.1	52 ± 2.6
Pindolol	control	100 ± 0	47 ± 2.6
	1	103 ± 15.4	43 ± 2.8
	12.5	99 ± 16.1	47 ± 2.7
	25	97 ± 12.5	43 ± 2.7
Propranolol	control	100 ± 0	48 ± 0.8
	1	98 ± 7.4	49 ± 0.6
	12.5	96 ± 3.1	49 ± 0.6
	25	114 ± 6.1	48 ± 0.8

despite there being no inhibitory effect on platelet aggregation in these experiments (figure 5.4). Labetalol and pindolol were equally potent, inhibiting TxB_2 generation at all 3 concentrations assessed - 1 $\mu\text{g}/\text{ml}$, 12.5 $\mu\text{g}/\text{ml}$ and 25 $\mu\text{g}/\text{ml}$, while propranolol only had a significant effect at 25 $\mu\text{g}/\text{ml}$ (figure 5.4). Atenolol, metoprolol, timolol and prazosin had no effect on TxB_2 production (table 5.3) to 4 $\mu\text{g}/\text{ml}$ collagen. Labetalol, pindolol and propranolol failed to inhibit either platelet aggregation or TxB_2 generation in response to 0.5 mM arachidonic acid (table 5.4).

5.4 DISCUSSION

5.4.1. Validation of TxB_2 Generation Method

The dose response curves for TxB_2 generation and platelet aggregation in response to arachidonic acid, collagen and ADP show that both the type and the dose of the aggregating agent used will influence these responses.

Following ADP induced aggregation only small amounts of TxB_2 were produced despite maximal platelet aggregation occurring. This is in keeping with previous studies (Siess et al., 1981; Koh et al., 1980), and suggests that ADP induced aggregation is largely independent of TxA_2 production, although a small amount of TxA_2 may be required to produce irreversible aggregation in response to ADP (see chapter 2.2.2).

As expected, arachidonic acid was the most potent stimulator of TxB_2 production. It induces platelet aggregation solely by TxA_2 formation, as it is the substrate for the platelet cyclo-oxygenase pathway, the end product of which is TxA_2 .

Collagen induced aggregation also produced relatively large amounts of TxB_2 . Collagen induces aggregation by activating phospholipase A_2 in the platelet membrane which in turn releases arachidonic acid from the cell membrane phospholipids, and this is subsequently transformed to TxA_2 (see chapter 2.2.2) although high doses of collagen can induce aggregation independently of TxA_2 (Vargaftig et al., 1981).

As aggregation in response to collagen and arachidonic acid was maximal prior to TxB_2 production reaching its peak, the platelets biosynthetic capacity for TxA_2 production is much higher than is necessary to produce aggregation and this seems to be especially true for collagen.

The experiments with indomethacin, an inhibitor of cyclo-oxygenase, confirmed that this method can measure inhibition of TxB_2 production stimulated by arachidonic acid and collagen.

5.4.2 Effect of Adrenoceptor Antagonists on Platelet Aggregation and TxA_2 Generation

These studies have shown that labetalol, pindolol and propranolol have significant anti-aggregatory effects, and confirm that this effect is not due to α or β blockade, as timolol (β_1 , β_2),

atenolol (β_1), metoprolol (β_1), and prazosin (α_1) had no inhibitory effect on either platelet aggregation to collagen or on TxA_2 generation. This is in agreement with studies on propranolol (Campbell et al., 1981a; Weksler et al., 1977) which showed that both d(+) and l(-) isomers of propranolol had inhibitory effects on platelet aggregation while the d(+) isomer had only 1% of the β receptor blocking activity of l(-) propranolol. Since both isomers possess MSA, Weksler et al., (1977) suggested that MSA was responsible for this anti-aggregatory effect. The results of the present study also suggest that MSA and lipophilicity, which are closely related, may be responsible, as all 3 drugs which were found to possess anti-aggregatory qualities had this feature in common. Although MSA and lipophilicity do not entirely explain this as metoprolol, which has MSA and is more lipid soluble than pindolol, had no effect.

This study has also demonstrated that labetalol, pindolol and propranolol have an inhibitory effect on TxA_2 production in PRP stimulated with collagen. This effect was independent of any inhibitory effect on platelet aggregation, as there was no difference in the rate of aggregation between drug treated PRP and controls. Inhibition of TxA_2 production by these drugs could be overcome by using exogenous arachidonic acid as the aggregating stimulus. Normally when platelets aggregate in response to collagen, phospholipase A_2 (PLA_2) is activated and this in turn acts on platelet membrane phospholipid, in the presence of Ca^{2+} , to produce free arachidonic acid which is converted by cyclo-oxygenase to PGH_2 , and then by thromboxane

synthetase to TxA_2 (Bakhle, 1983). Thus, it appears that labetalol, pindolol, and propranolol may inhibit TxA_2 by acting at the phospholipase A_2 step. There are 3 possible mechanisms by which this enzyme can be inhibited (Blackwell and Flower, 1983), firstly by binding to the enzyme, secondly by binding to substrate, and thirdly by interfering with Ca^{2+} availability which is essential for PLA_2 activity. Weksler et al., (1977) demonstrated that propranolol interfered with Ca^{2+} availability probably by interfering with internal shifts at intracellular sites. This interference with Ca^{2+} availability may therefore be the underlying mechanism for the effect of these drugs on platelets. Neither labetalol, pindolol nor propranolol had any effect on platelet shape change in response to collagen. This is in agreement with the results of Weksler et al., (1977) who showed that propranolol did not inhibit shape change induced by ADP, epinephrine, thrombin or collagen. However, shape change has recently been shown to be due to Ca^{2+} dependent myosin phosphorylation (Nachmias et al., 1985). It is therefore surprising that shape change was not affected by these drugs, unless there are other mechanisms whereby shape change can proceed independently of Ca^{2+} . The failure to inhibit shape change does not therefore support the hypothesis that these drugs inhibit platelet function by interfering with Ca^{2+} availability as proposed by Weksler et al., (1977).

Campbell et al., (1981a) have shown that hypertensive patients treated with propranolol had reduced TxA_2 generation from their PRP ex vivo. It has been suggested that this is not due to MSA, but that reduced aggregation was secondary to reduced TxA_2 .

production (Campbell et al., 1981b). These studies (Campbell et al., 1981a; Campbell et al., 1981b) showed that inhibition of aggregation and TxA₂ production occurred hand in hand, therefore they could not exclude the possibility that the reduced TxA₂ production may have been secondary to reduced aggregation, rather than vice versa. The experiments in the present study were designed to exclude inhibition of TxA₂ production being secondary to reduced platelet aggregation, by using a high dose of collagen, and low concentrations of the drugs so that the drugs had no inhibitory action on aggregation per se. This study demonstrated that despite the same degree of aggregation occurring in both drug treated and control PRP, labetalol, pindolol and propranolol significantly reduced TxA₂ generation. Thus, inhibition of TxA₂ by these drugs was independent of any effect on platelet aggregation at the drug concentrations assessed.

It has been suggested that the anti-aggregatory effects of β -adrenoceptor antagonists are not present in vivo, as MSA occurs only at concentrations much higher than those obtained in vivo (Weksler et al., 1977). There are, however, several studies documenting an effect on platelet aggregation ex vivo (Mehta et al., 1978; Frishman et al., 1974; Campbell et al., 1981a; Small et al., 1982; Mehta and Mehta, 1982) and propranolol has been shown to reduce circulating aggregates in vivo (Green, Rossi and Haring, 1982). Platelets therefore may be more sensitive in vivo than in vitro to the effects of these agents. This may be due to accumulation of these drugs within the platelet, which has been shown to occur with propranolol (Weksler et al., 1977). These

present in vitro studies demonstrated that the IC50 concentrations for labetalol, pindolol and propranolol, for aggregation induced by collagen, were above the therapeutic range of plasma concentrations (table 5.1). It is difficult, however, to relate aggregation induced by 1 or 2 ug/ml collagen in vitro to the in vivo situation where the aggregatory challenge is likely to be much lower. As the dose response curves for these drugs is "shifted" to the left when smaller concentrations of collagen are used they may well possess this anti-aggregatory effect in vivo. These studies have also shown that labetalol at a concentration of 1 ug/ml has an inhibitory effect on TxA₂ generation in vitro. This drug concentration is only 1.5 - 2 times the mean concentrations of labetalol which have been observed in patients with pregnancy induced hypertension (unpublished observations), so these drugs may well have an effect in vivo.

Timolol and metoprolol have been shown to improve prognosis in patients following myocardial infarction (Hjalmarson et al., 1981; Norwegian Multicentre Study Group, 1981). The mechanism behind this is unclear. An attractive hypothesis is reduction of TxA₂ production by these β -adrenoceptor antagonists especially with the growing volume of evidence implicating TxA₂ in ischaemic heart disease. The present results suggest that neither timolol nor metoprolol were effective in inhibiting TxA₂ generation in response to collagen, and therefore do not support this hypothesis, and suggest that the cardioprotective effect on these agents is mediated by some other mechanism.

In conclusion these studies suggest that some adrenoceptor antagonists are able to inhibit both platelet aggregation to collagen, and TxA_2 generation. The latter effect was shown to be independent of inhibition of aggregation. The mechanism of these effects may be due to inhibition of platelet PLA_2 , and may be related to the MSA and lipophilicity of these agents. Whether these inhibitory effects on platelet aggregation and TxA_2 generation are clinically relevant will depend on long term prospective studies. However, there are good theoretical reasons to use drugs which possess these properties in patients with vascular disease where platelet activation may play a role in the pathophysiology.

CHAPTER 6

EFFECT OF ADRENOCEPTOR ANTAGONISTS ON UMBILICAL ARTERY
PROSTACYCLIN PRODUCTION AND SYNERGY WITH PROSTACYCLIN AND
UMBILICAL ARTERY PROSTACYCLIN-LIKE ACTIVITY IN INHIBITING
PLATELET AGGREGATION

6.1 INTRODUCTION

Prostacyclin (PGI₂) is the major prostaglandin produced by vascular tissue (Moncada et al., 1977; Ritter et al., 1982). It is a potent vasodilator and inhibitor of platelet aggregation (Moncada et al., 1976) which is thought to function as a local hormone in the regulation of the platelet-vessel wall interaction (Patrono et al., 1984). It may also be important in the local control of vascular tone and blood flow as there are specific PGI₂ receptors in vascular tissue (MacDermot et al., 1981). Thromboxane A₂ (TxA₂) is the major product of arachidonic acid in platelets, and has actions directly opposed to those of PGI₂, as it is a potent vasoconstrictor and platelet aggregating agent (Hamberg et al., 1975). PGI₂ and TxA₂ oppose each other through regulation of platelet adenylate cyclase activity (Tateson et al., 1977) and it has been proposed that a balance exists between these two substances (Bunting et al., 1977). Consequently, an imbalance between these two substances might contribute to the pathophysiology of diseases associated with vasoconstriction and platelet aggregation and subsequent thrombotic vascular occlusion.

Vascular PGI₂ production can be readily stimulated by a variety of mild stimuli to the vessel wall (Ritter et al., 1983). It is thought that following vascular injury the extent of platelet activation and thrombus formation is limited and vascular integrity maintained by PGI₂ production. Failure of this mechanism might therefore result in excessive platelet consumption, intravascular thrombosis and vasoconstriction.

Pregnancy induced hypertension (PIH) is a disease associated with platelet consumption (Redman et al., 1978) and vasoconstriction. Since reduced PGI₂ production both from fetal (Remuzzi et al., 1980; Downing et al., 1980; Walsh et al., 1985) and maternal (Bussolino et al., 1980) vascular tissue has been documented in this disease, PGI₂ deficiency may contribute to the disease process (see chapter 3.7).

Adrenoceptor antagonists such as labetalol are being increasingly used in the treatment of PIH (Lubbe, 1984). The studies described in chapter 5 have shown that some adrenoceptor antagonists can inhibit platelet aggregation and TxA₂ production, and suggest that the latter effect may be accomplished through inhibition of phospholipase A₂ the enzyme which provides arachidonic acid substrate for both PGI₂ and TxA₂ formation (see chapter 1.2). Consequently, these drugs may inhibit vascular PGI₂ production as well as platelet TxA₂ production, and this is potentially harmful especially in a disease such as PIH where PGI₂ production may already be compromised.

The aims of this study were two-fold, firstly to determine whether adrenoceptor antagonists had any effect on vascular PGI₂ production and secondly to determine whether these drugs might act synergistically with PGI₂ to inhibit platelet aggregation. The latter effect would be assessed both with pure PGI₂ and also with the PGI₂ directly produced from vascular tissue.

6.2 MATERIALS AND METHODS

The materials and methods used in the following experiments have been fully described in chapter 4.

The effects of the adrenoceptor antagonists labetalol, pindolol and propranolol on umbilical artery PGI₂ production were determined using the method described in chapter 4.5. Vascular PGI₂ production was measured as the stable PGI₂ metabolite 6-keto-PGF_{1α} (PGI₂M) using the radioimmunoassay described in chapter 4.1.3.

In order to determine whether adrenoceptor antagonists were synergistic with PGI₂ in inhibiting platelet aggregation, dose response curves for the effect of PGI₂ on collagen induced aggregation in platelet rich plasma were first constructed. Platelet rich plasma was prepared as described in chapter 4.2.4, and the effect of PGI₂ on aggregation induced by 1 ug/ml and 2 ug/ml collagen was assessed as described in chapter 4.4.2. The dose response curves obtained allowed the concentration of PGI₂ for use in the subsequent experiments to be determined.

The possible synergistic effects of PGI₂ and adrenoceptor antagonists on platelet aggregation were studied using the method described in chapter 4.4.3.

The possible synergistic effects of PGI₂-like activity from human umbilical artery and adrenoceptor antagonists were determined using the method described in chapter 4.4.4.

The doses of adrenoceptor antagonists used were known from previous studies (see chapter 5, figure 5.3) to have minimal effects on platelet aggregation to 2 ug/ml collagen when used alone.

Statistical analysis was by the Wilcoxon rank sum test for paired data.

6.3 RESULTS

6.3.1 Effect of Adrenoceptor Antagonists on Umbilical Artery PGI₂M Production

The results for the effects of pindolol, propranolol and labetalol on PGI₂M production from human umbilical artery are shown in table 6.1. Aspirin (100 ug/ml) was used to confirm that PGI₂M production could be inhibited and significantly reduced PGI₂M production in all experiments compared to the controls.

Each of the 3 adrenoceptor antagonists was assessed at 2 drug concentrations, 1 ug/ml and 100 ug/ml. None of the drugs had any effect on PGI₂M production at the lower concentration. Pindolol significantly inhibited PGI₂M production at the higher dose and was as effective as aspirin (100 ug/ml) in reducing PGI₂M production. Neither labetalol or propranolol, however, had any significant effect on PGI₂M production, at the higher concentration, although there was a trend towards lower levels of PGI₂M with 100 ug/ml labetalol.

TABLE 6.1

Effect of Adrenoceptor Antagonists on PGI₂M Production from Umbilical Artery. All values are mean ±SEM pg/ml PGI₂M.

	Labetalol	Pindolol	Propranolol
Control	790.0 ±28.1	762.8 ±44.9	771.4 ±37.4
1 ug/ml drug	762.8 ±17.2	634.3 ±51.9	792.8 ±52.3
100 ug/ml drug	721.4 ±59.9	571.4* ±48.3	750.0 ±41.3
Aspirin 100 ug/ml	621.4* ±36.5	585.7* ±41.1	614.3* ±43.5

* p<0.05 compared to control n = 7 in all experiments.

(Pool plasma PGI₂M concentration was 6 pg/ml prior to incubation with the artery rings.)

6.3.2 Dose Response Curves for the Effect of PGI₂ on Collagen Induced Platelet Aggregation

The dose response curves for the effect of PGI₂ on platelet aggregation are shown in figure 6.1 and a representative aggregation trace is shown in figure 6.2. As can be seen, aggregation induced by 2 ug/ml collagen was virtually totally inhibited by 400 pg/ml PGI₂, and aggregation induced by 1 ug/ml collagen was abolished by 200 pg/ml PGI₂. The concentration of PGI₂ causing 50% inhibition of aggregation was approximately 50 pg/ml for 1 ug/ml collagen, and 200 pg/ml for 2 ug/ml collagen (figure 6.1).

6.3.3 Synergistic Effects of Adrenoceptor Antagonists and PGI₂ on Platelet Aggregation

The results for these experiments are shown in figure 6.3 and a representative aggregation tracing is shown in figure 6.4. Propranolol, pindolol and labetalol were all assessed at 2 drug concentrations, 1 ug/ml and 12.5 ug/ml. These concentrations were known from previous studies to have minimal effects on platelet aggregation induced by 2 ug/ml collagen when used alone (see Chapter 5, figure 5.3). PGI₂ was used in a final concentration of 100 pg/ml which was known from the above studies to cause around 20% inhibition of aggregation to 2 ug/ml collagen when used alone (see figure 6.1). As can be seen at these concentrations of drug and PGI₂ which on their own produced no significant effect on platelet aggregation a combination of both produced an additive or synergistic effect, significantly inhibiting aggregation at both drug concentrations assessed. All 3 drugs behaved similarly in this respect.

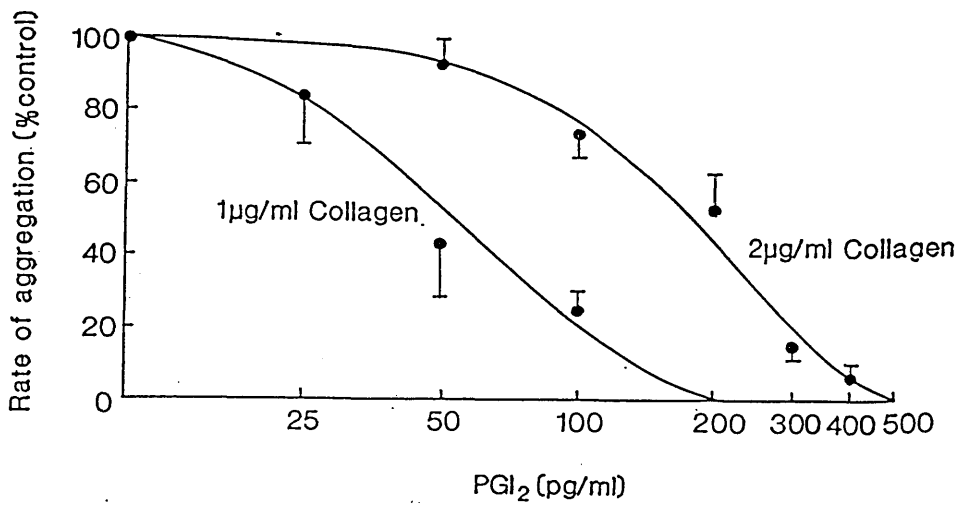


FIGURE 6.1

Dose response curves for the effect of PGI₂ on platelet aggregation induced by collagen. All points represent the mean \pm SEM of 5 separate experiments.

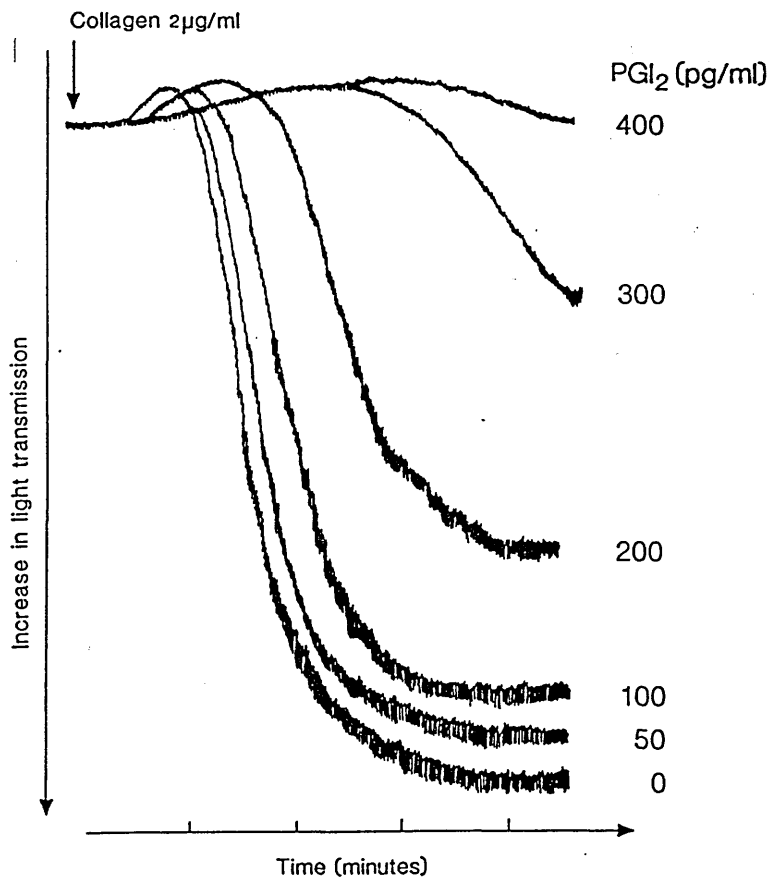


FIGURE 6.2

Representative aggregation tracing of the effect of PGI₂ on platelet aggregation induced by 2 µg/ml collagen.

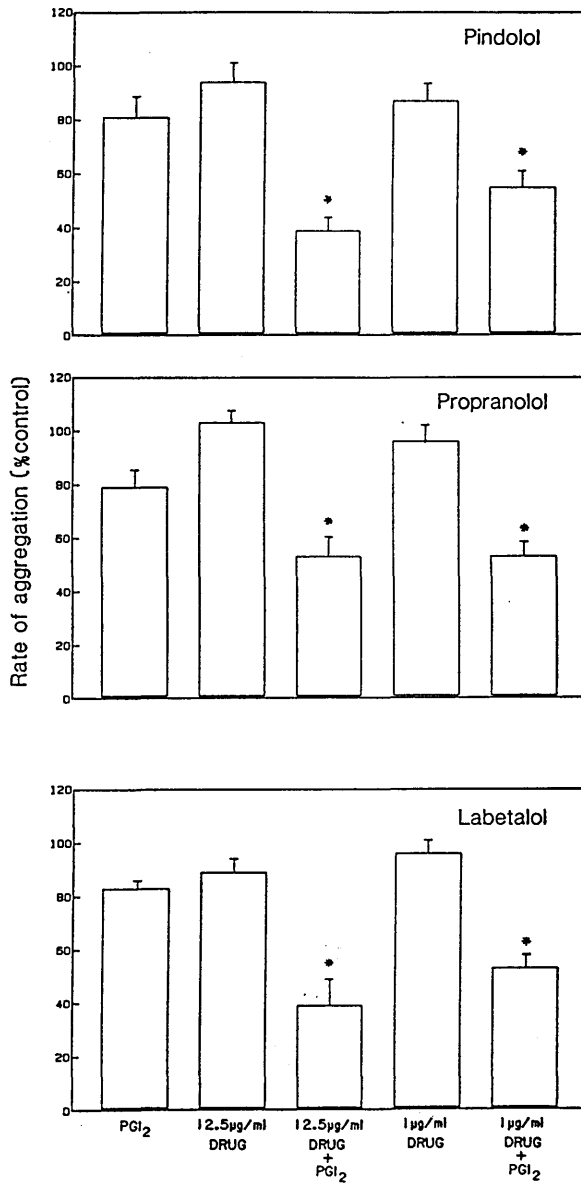


FIGURE 6.3

Synergistic effect of adrenoceptor antagonists and PGI₂ on platelet aggregation. All values are mean \pm SEM of 7 separate experiments. The dose of PGI₂ used was 100 pg/ml for all experiments.

*p < 0.05 compared to control, PGI₂ alone, and drug alone.

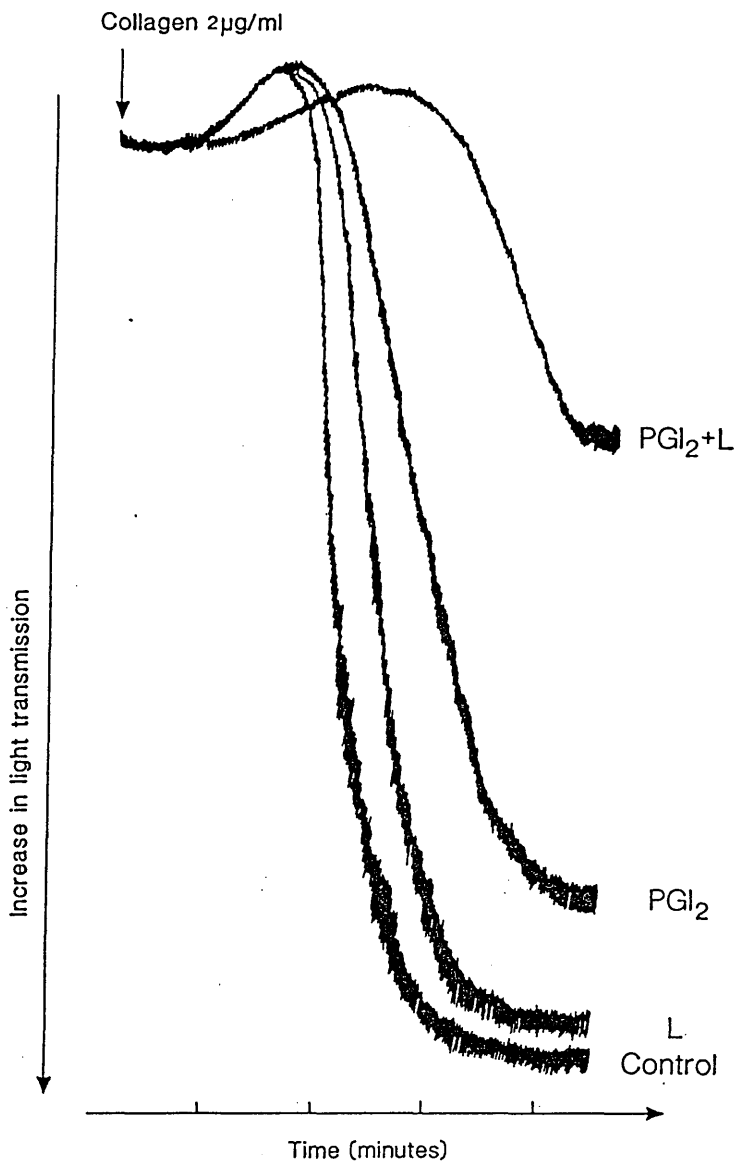


FIGURE 6.4

Representative aggregation tracing showing the inhibitory effects of PGI₂ and labetalol on platelet aggregation.

- Control = labetalol vehicle plus PGI₂ vehicle.
- L = labetalol 12.5 ug/ml plus PGI₂ vehicle.
- PGI₂ = labetalol vehicle plus PGI₂ 100 pg/ml.
- PGI₂ + L = labetalol 12.5 ug/ml plus PGI₂ 100 pg/ml.

6.3.4 Synergistic Action of PGI₂-like Activity from Human Umbilical Artery with Adrenoceptor Antagonists in Inhibiting Platelet Aggregation

The results from this part of the study are shown in figure 6.5. Pindolol, propranolol and labetalol were all assessed at a concentration of 12.5 ug/ml, which when used alone had minimal effects on platelet aggregation induced by 2 ug/ml collagen. The supernatant from umbilical artery also had no significant effect on platelet aggregation when used alone. However, when a combination of umbilical artery supernatant and an adrenoceptor antagonist was used aggregation to 2 ug/ml collagen was significantly inhibited compared to the control, adrenoceptor antagonist alone and umbilical artery supernatant alone.

6.4 DISCUSSION

These studies have shown that adrenoceptor antagonists have no significant effect on vascular PGI₂ production in vitro at low doses, but may inhibit vascular PGI₂ production if high doses of the drugs are used, as seen with pindolol (100 ug/ml). This potential inhibitory effect is unlikely therefore to occur in vivo as mean plasma levels for all of these agents when used therapeutically are considerably below 1 ug/ml (see table 5.1). This finding is compatible with that of Campbell et al., (1981b) who showed that propranolol had no effect on PGI₂ production from vascular endothelial cells in vitro. However, propranolol has been shown to stimulate PGI₂ production from a guinea pig heart

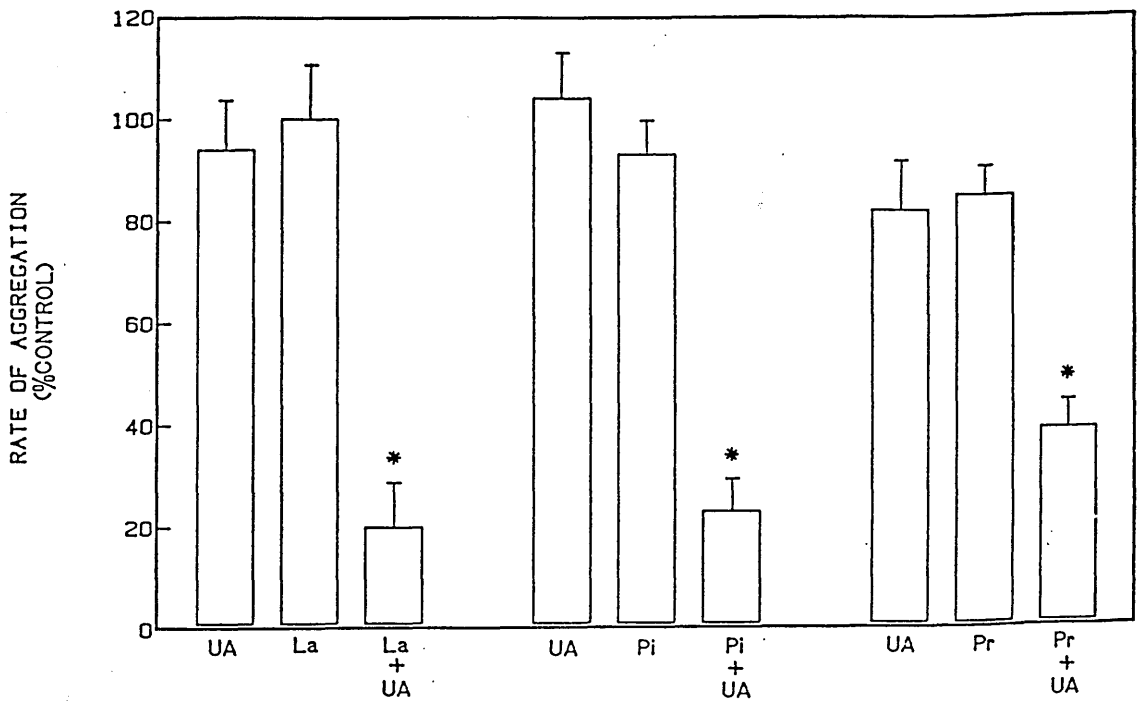


FIGURE 6.5

Synergistic action of PGI₂-like activity from human umbilical artery with adrenoceptor antagonists in inhibiting platelet aggregation. All values are mean ± SEM of 7 experiments.

UA = umbilical artery supernatant.

La = labetalol 12.5 ug/ml

Pi = pindolol 12.5 ug/ml

Pr = propranolol 12.5 ug/ml

* p < 0.05 compared to control, umbilical artery supernatant alone and drug alone.

preparation (Forster, 1980) which does not agree with either the results of the present study or those of Campbell et al., (1981b).

It has also been proposed that adrenoceptor antagonists exert their anti-hypertensive effect by stimulating vasodepressor prostaglandin production (Duraó et al., 1977), as inhibitors of prostaglandin synthesis such as indomethacin have been shown to reduce the anti-hypertensive effect of propranolol (Duraó et al., 1977). If the anti-hypertensive effects of these drugs are mediated through increased prostaglandin production, in view of the result of the present study, it is unlikely to be due to any direct stimulatory effect on vascular PGI₂ production.

Umbilical vessels lack any form of innervation (Tuvemo, 1980) and consequently the control of blood flow must be regulated by humeral means (Tuvemo, 1980). PGI₂ is the major prostaglandin produced by umbilical artery (Ritter et al., 1982), and umbilical artery produces significantly more PGI₂ than adult vessels of comparable size and weight (Remuzzi et al., 1979). PGI₂ may therefore be important in the regulation of umbilical artery blood flow. Makila et al., (1983) have shown that umbilical artery blood flow in vivo correlates with the vessels' ability to produce PGI₂, and they have also shown both to be reduced in PIH. Since reduced umbilical artery blood flow may lead to fetal compromise and intra-uterine growth retardation, it is important to know whether anti-hypertensive agents used in the treatment of PIH have any detrimental effects on PGI₂ production. The adrenoceptor antagonists assessed in this study are unlikely to

produce any significant effect on PGI₂ production at therapeutic doses, but it would be worthwhile measuring umbilical artery blood flow in vivo before and after treatment with an adrenoceptor antagonist in PIH. It is also conceivable that these drugs might even enhance umbilical artery blood flow through other mechanisms, as labetalol for example has a direct vasodilator effect mediated by its α_1 adrenoceptor antagonist action.

In the studies described in chapter 5, labetalol, pindolol and propranolol were shown to have an inhibitory effect on platelet aggregation and platelet TxA₂ production, the latter effect possibly being due to inhibition of phospholipase A₂ activity. This occurred at drug concentrations as low as 1 ug/ml. Since these drugs did not reduce vascular PGI₂ production at this same drug concentration platelets must be more sensitive than blood vessels to the inhibitory effects of these drugs on prostaglandin production. This feature may prove useful in vivo as TxA₂ production may be suppressed while vascular PGI₂ production remains unaffected.

This study has also shown that labetalol, pindolol and propranolol are synergistic with PGI₂, including the PGI₂-like activity produced by umbilical artery, in inhibiting platelet aggregation.

Some in vitro studies have suggested that the anti-platelet effects of adrenoceptor antagonists are only present at supra-pharmacological drug concentrations and therefore could not occur

in vivo. However, several studies have shown a significant antiplatelet effect of these drugs ex vivo (Mehta et al., 1978; Campbell et al., 1981a) and circulating platelet aggregates have been shown to be reduced in vivo by propranolol (Green et al., 1982). This discrepancy between in vivo and in vitro studies suggests that platelets in vivo may be more sensitive to the effects of adrenoceptor antagonists. This might be explained by the synergy between these drugs and PGI₂ in inhibiting platelet aggregation which might occur in the in vivo situation.

These synergistic effects might also be beneficial in the treatment of diseases associated with reduced PGI₂ production, such as PIH, where the combination of the drug and the remaining endogenous PGI₂ might have sufficient anti-platelet effects to compensate for the relative PGI₂ deficiency.

In conclusion, these studies have shown that adrenoceptor antagonists, despite having an inhibitory effect on platelet prostaglandin production, have no inhibitory effect on vascular PGI₂ production at least at low drug concentrations. Furthermore, these drugs act synergistically with PGI₂ to inhibit aggregation. This effect may be useful in the treatment of disorders where PGI₂ production is reduced such as in PIH.

CHAPTER 7

INHIBITION OF PLATELET AGGREGATION IN WHOLE BLOOD BY
ADRENOCEPTOR ANTAGONISTS AND SYNERGISM WITH
PROSTACYCLIN AND ASPIRIN

7.1 INTRODUCTION

Platelet reactivity has been shown to be increased in patients with arteriosclerotic vascular disease (Steele et al., 1973; Murphy and Mustard, 1962; Harker and Slichter, 1972; Mehta, Mehta and Pepine, 1978), and platelet consumption is a well recognised feature of pregnancy induced hypertension (Redman et al., 1978; Trudinger, 1976; Rakoczi et al., 1979).

Adrenoceptor antagonists are widely used in the treatment of atherosclerotic vascular disease, and their use in pregnancy induced hypertension is rapidly becoming established (Gallery et al., 1979b; Lubbe and Hodge, 1981; Symonds et al., 1982; Walker et al., 1982; Walker, Greer and Calder, 1983; Rubin et al., 1983; Lardoux et al., 1983; Gallery et al., 1985). These drugs are known to have an inhibitory effect on platelet aggregation (Frishman et al., 1974; Campbell et al., 1981a; Campbell et al., 1981b; Weksler et al., 1977; Mehta et al., 1983) and have been shown to reduce circulating aggregates in patients with coronary artery disease (Green et al., 1982). It is possible therefore that some of the beneficial effects of these drugs may be mediated through an inhibitory effect on platelet function.

Aspirin has long been known to inhibit platelet aggregation (O'Brien, 1968) and has been used as an antiplatelet agent in the secondary prevention of myocardial infarction (Aspirin Myocardial Infarction Study Research Group 1980; Elwood and Sweetnam, 1980; Anturan Reinfarction Trial Research Group 1980; Persantin-Aspirin Reinfarction Study II Research Group 1985; Persantine Aspirin Reinfarction Study Research Group, 1980) and threatened

stroke (Canadian Co-operative Study Group 1978). More recently a combination of aspirin and dipyridamole has been shown to be beneficial in pregnancies at high risk of pregnancy induced hypertension and intra-uterine growth retardation (Beaufils et al., 1985).

Most previous studies on the effect of adrenoceptor antagonists on platelets have concentrated on propranolol despite the wide range of adrenoceptor antagonists available, and all studies have been performed in platelet rich plasma (PRP), by traditional turbidometric techniques (Born, 1962). It has recently become possible to study platelet aggregation in whole blood using the Clay-Adams Ultra Flo 100 whole blood platelet counter. This may be a more physiological method than turbidometric techniques as it leaves platelets in their natural milieu, surrounded by red and white cells which can themselves influence platelet behaviour by release of ADP (Saniabadi et al., 1984a) and prostacyclin (PGI₂) (Deckmyn et al., 1983; Blackwell and Flower, 1978), respectively. In addition, a direct counting technique of single platelets is employed rather than the indirect method of change in optical density used with PRP (see chapter 4.6.1).

The aim of this study was to determine the effects of adrenoceptor antagonists on platelet aggregation in whole blood. As these drugs vary not only in their receptor specificity but also in their lipid solubility, possession of membrane stabilising activity (MSA), and possession of intrinsic sympathomimetic activity (ISA), a variety of drugs which differed in

TABLE 7.1

Properties of Adrenoceptor Antagonists

Drug	Specificity*	ISA*	MSA*	Lipid solubility** (Distribution co-efficient for octanol/buffer pH 7.4 and 37°C)
Atenolol	β_1	-	-	0.015
Labetalol	$\alpha_1\beta_1\beta_2$	-	+	11.5
Pindolol	$\beta_1\beta_2$	+	+/-	0.82
Propranolol	$\beta_1\beta_2$	-	+	20.2

* See Feely, de Vane and MacLean, 1983.

** See Woods and Robinson, 1981.

these respects were studied (table 7.1). Labetalol was also assessed in combination with aspirin to determine whether the drugs might act synergistically to inhibit platelet aggregation.

7.2 MATERIALS AND METHODS

7.2.1 Whole Blood Aggregation Studies

Venous blood was obtained without stasis from healthy male and female volunteers using a 21 gauge butterfly cannula. The blood was prepared and platelet aggregation studies performed in whole blood as described in chapter 4.6.2. The following drugs were assessed: atenolol, labetalol and propranolol (using 0.9% sodium diloride as vehicle) and pindolol (using 0.1 M phosphate buffer pH 7.0 as vehicle). The vehicle alone was used in control experiments. Collagen (0.5 ug/ml final concentration), ADP (10 uM final concentration) and arachidonic acid (0.5 mM final concentration) were used as the aggregating agents.

7.2.2 Effect of PGI₂ on Platelet Aggregation in Whole Blood

A dose response curve for the effect of PGI₂ on whole blood platelet aggregation induced by collagen (0.5 ug/ml) was obtained using the methods described in chapter 4.6.3. A concentration of PGI₂ which had minimal effect on aggregation to collagen in all subjects was thus determined (125 pg/ml PGI₂). This concentration was used in assessing whether PGI₂ and adrenoceptor antagonists were synergistic in inhibiting platelet aggregation in whole blood.

7.2.3 Synergistic Effect of Adrenoceptor Antagonists and PGI₂ in Inhibiting Platelet Aggregation in Whole Blood

These experiments were performed as described in chapter 4.6.4. Collagen (0.5 ug/ml final concentration) was used as the aggregating agent. Labetalol, pindolol and propranolol at a final concentration of 1 ug/ml were assessed with PGI₂ in a final concentration of 125 pg/ml. The vehicle for each drug was used alone in the control experiments.

7.2.4 Synergistic Action of Aspirin and Labetalol in Inhibiting Platelet Aggregation in Whole Blood

This experiment was performed as described in chapter 4.6.5. Collagen (0.5 ug/ml) was used as the aggregating agent. Labetalol was used in a final concentration of 1 ug/ml, and aspirin in a final concentration of 5 ug/ml. The drug vehicles alone were used in the control experiments. The concentrations of both drugs used were known from prior study to have minimal effects on aggregation when used alone.

7.2.5 Statistical Analysis

Statistical analysis was by the Wilcoxon paired rank sum test for all experiments.

7.3 RESULTS

Labetalol, pindolol and propranolol inhibited platelet aggregation in whole blood stimulated with 0.5 ug/ml collagen in a dose dependent manner (figure 7.1).

Labetalol, pindolol and propranolol also inhibited 0.5 mM arachidonic acid induced aggregation in a dose dependent manner (figure 7.2) but much higher concentrations of drug were required to produce a significant effect as compared to aggregation induced by collagen.

These three drugs also produced a dose dependent reversal of 10 μ M ADP induced aggregation (figure 7.3). This dose of ADP was sufficient to cause irreversible aggregation in the control experiments of all subjects studied. However, only labetalol and propranolol, at high concentration, had any effect on aggregation to 10 μ M ADP.

Atenolol had no significant effect on aggregation induced by collagen, arachidonic acid or ADP, at drug concentrations up to 1000 μ g/ml (figure 7.4).

A dose response graph for the effect of PGI₂ on 0.5 μ g/ml collagen induced aggregation was obtained (figure 7.5). A concentration of 125 pg/ml PGI₂ had no significant effect on aggregation while 1000 pg/ml caused virtually complete inhibition of aggregation.

Labetalol, pindolol and propranolol were synergistic with PGI₂ in inhibiting aggregation induced by 0.5 μ g/ml collagen (figure 7.6), at concentrations of the drugs which on their own had minimal effects on platelet aggregation.

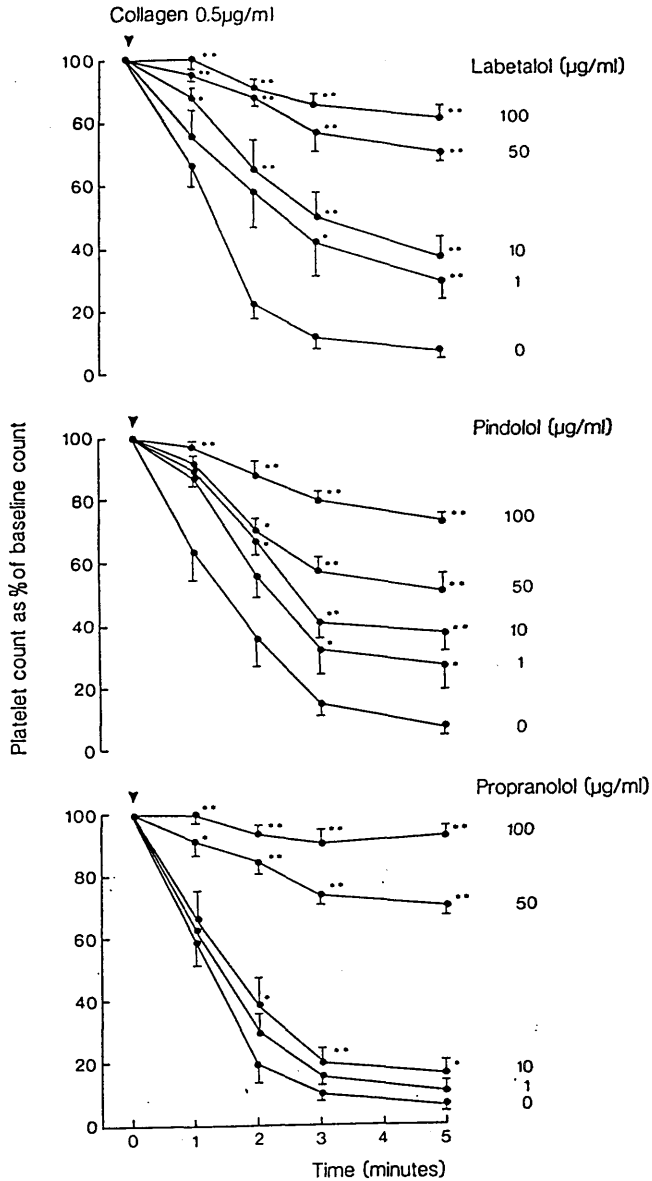


FIGURE 7.1

Effect of labetalol, pindolol and propranolol on platelet aggregation in whole blood stimulated with 0.5 ug/ml collagen. All points represent mean \pm SEM (n = 8 for labetalol, n = 7 for propranolol and pindolol)

* p < 0.05)
 ** p < 0.02) compared to control

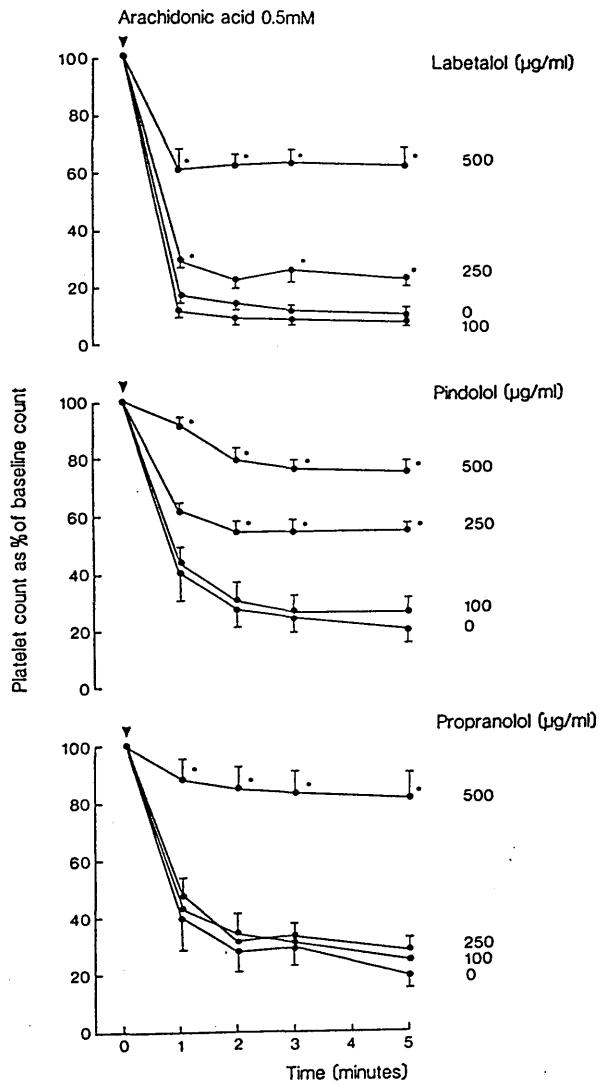


FIGURE 7.2

Effect of labetalol, pindolol and propranolol on platelet aggregation in whole blood stimulated with 0.5 mM arachidonic acid. All points represent the mean \pm SEM of 5 experiments.

* $p < 0.05$ compared to control

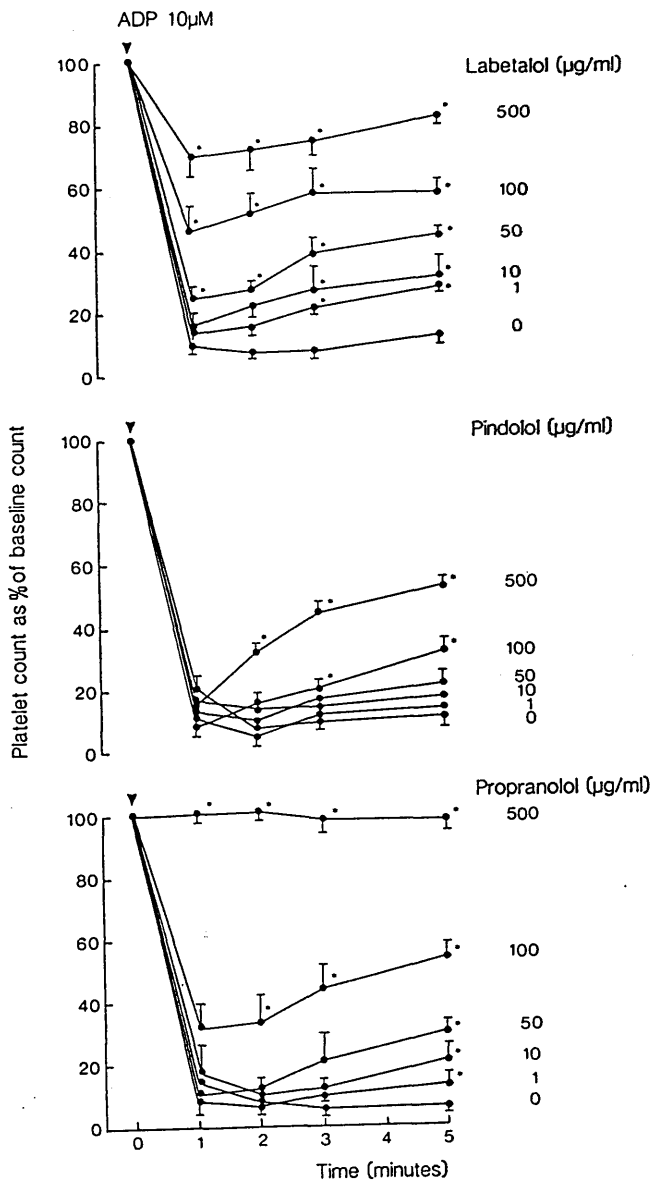


FIGURE 7.3

Effect of labetalol, pindolol and propranolol on platelet aggregation in whole blood stimulated with 10 μ M ADP. All points represent the mean \pm SEM of 5 experiments.

* <0.05 compared to control.

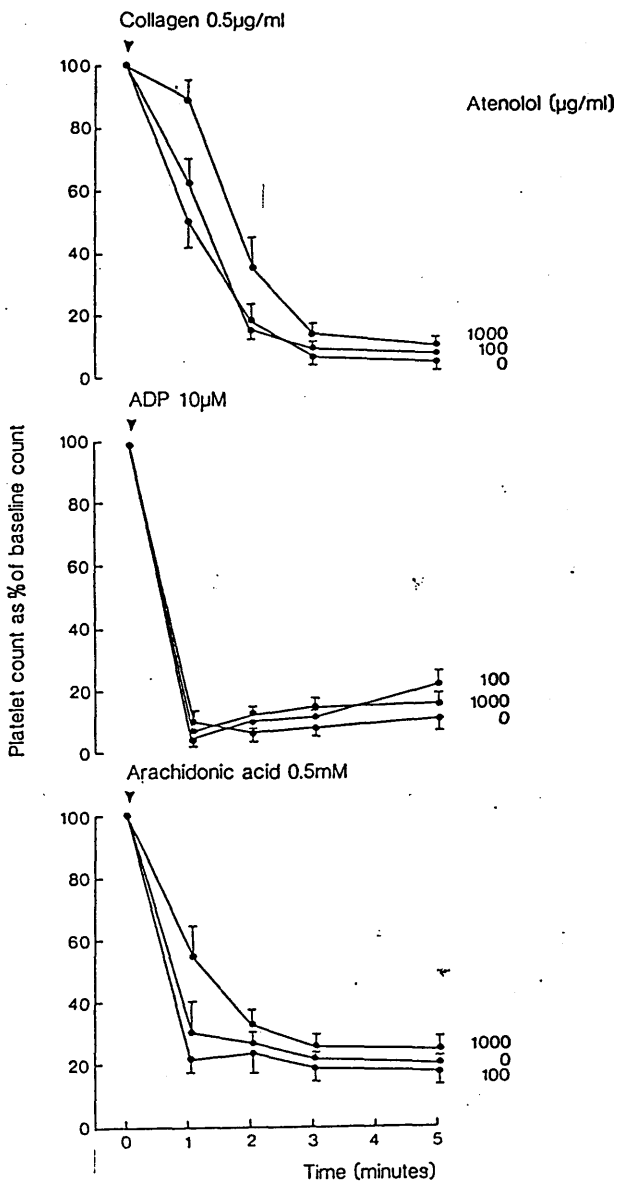


FIGURE 7.4

Effect of atenolol on platelet aggregation in whole blood stimulated by 0.5 ug/ml collagen, 10 uM ADP and 0.5 mM arachidonic acid.

All points represent mean ± SEM of 5 experiments.

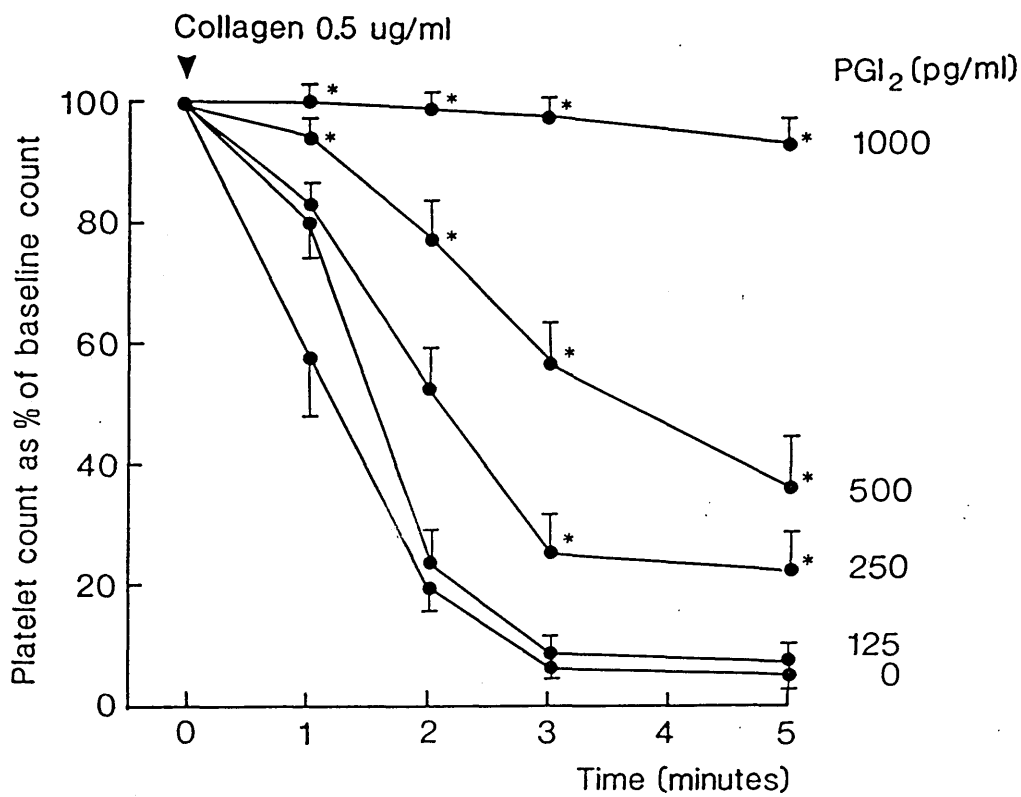


FIGURE 7.5

Effect of PGI₂ on platelet aggregation in whole blood stimulated with 0.5 ug/ml collagen.

All points represent the mean \pm SEM of 6 experiments.

* p < 0.05 compared to control.

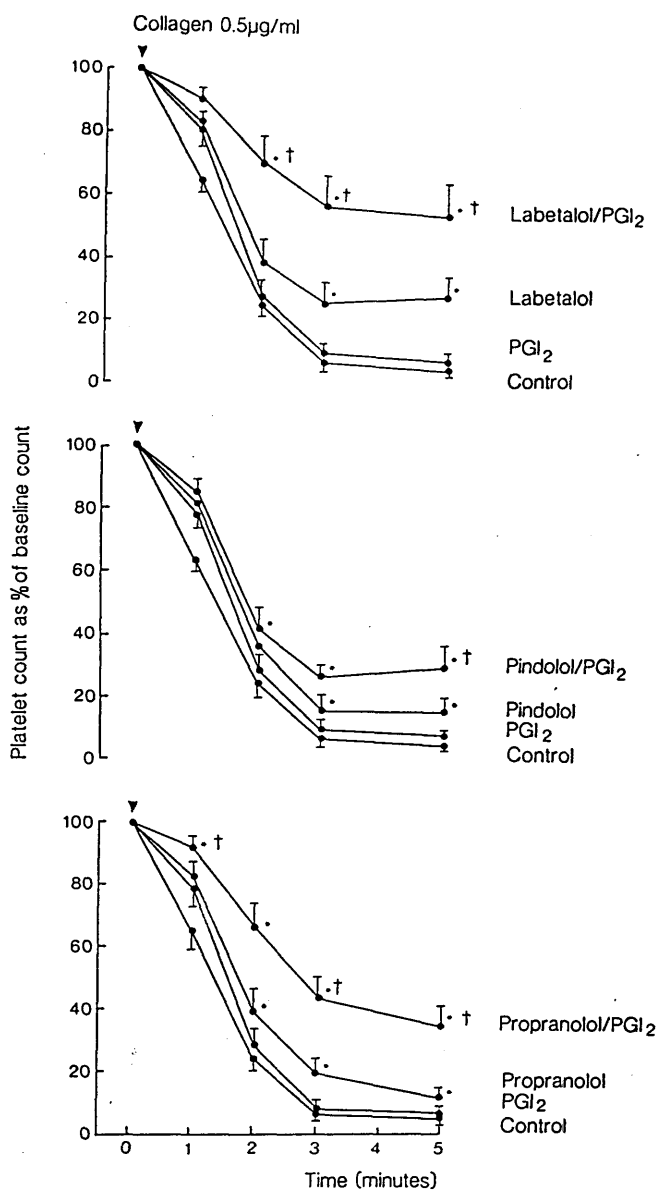


FIGURE 7.6

Synergistic effect of adrenoceptor antagonists and PGI₂ in inhibiting platelet aggregation in whole blood stimulated by 0.5 ug/ml collagen. (PGI₂ concentration = 125 pg/ml; labetalol, pindolol and propranolol concentration = 1 ug/ml).

All points represent mean ± SEM of 6 experiments.

* p < 0.05 compared to control

+ p < 0.05 compared to adrenoceptor antagonist alone, and PGI₂ alone.

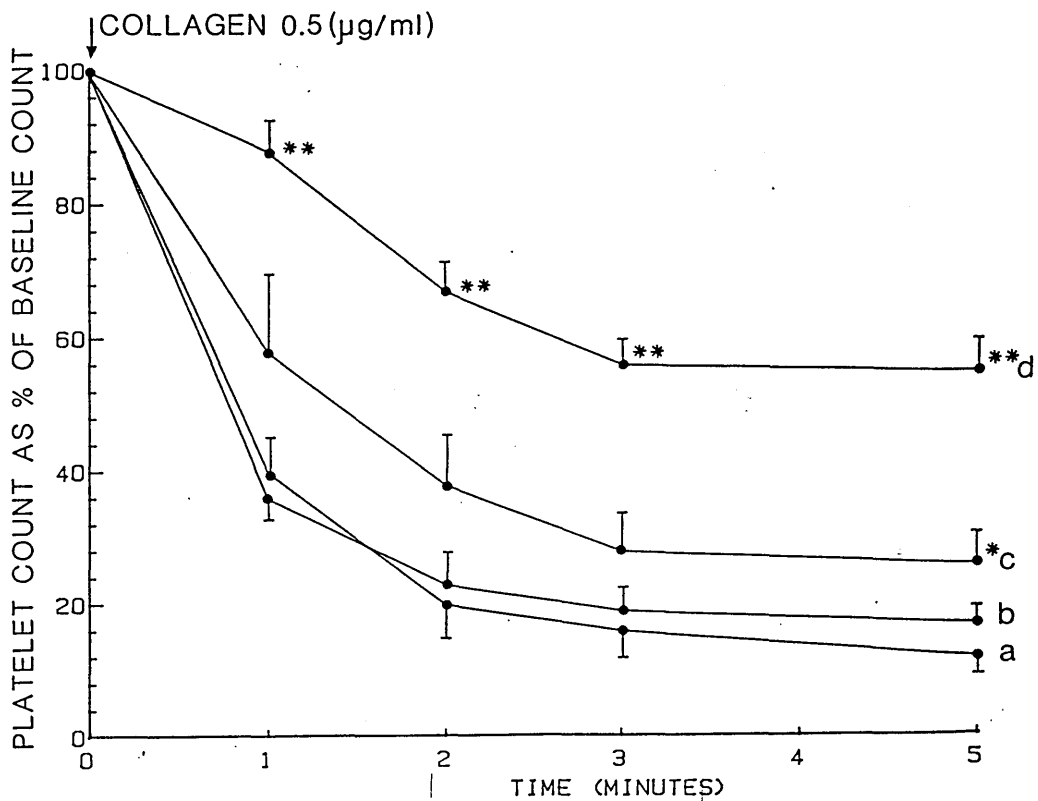


FIGURE 7.7

Effect of labetalol and aspirin on whole blood platelet aggregation (Mean \pm SEM of 7 experiments)

a = control

b = 5 $\mu\text{g/ml}$ aspirin

c = 1 $\mu\text{g/ml}$ labetalol

d = 5 $\mu\text{g/ml}$ aspirin and 1 $\mu\text{g/ml}$ labetalol

* $p < 0.05$ compared with control (a)

** $p < 0.02$ compared with a, b and c.

Labetalol was also found to be synergistic with aspirin in inhibiting platelet aggregation (figure 7.7). As can be seen, at concentrations of labetalol and aspirin which on their own produced minimal effects on platelet aggregation, a combination of both the drugs resulted in a significantly greater inhibition of platelet aggregation.

7.4 DISCUSSION

The results of this study show that some adrenoceptor antagonists have a dose dependent inhibitory effect on platelet aggregation in whole blood. Previous studies using PRP have shown that this effect is independent of β -receptor blockade (see Chapter 5), and suggested that this may be due to MSA (Weksler *et al.*, 1977; Heinroth *et al.*, 1983) which is closely related to lipid solubility. This study is in agreement with these findings as atenolol, which has no MSA and is very poorly lipid soluble (table 7.1), had no effect on aggregation.

As collagen induced aggregation was inhibited and ADP induced aggregation was reversed, we would suggest that these drugs may inhibit thromboxane A₂ (TxA₂) production, as TxA₂ is a major mediator of collagen induced aggregation, and the second phase of ADP induced aggregation (Vargaftig *et al.*, 1981). These drugs only inhibit arachidonic acid induced aggregation at high concentrations, suggesting that arachidonic acid may bypass at least in part the block to aggregation. This would localise the inhibitory action to the phospholipase A₂ step which releases membrane bound arachidonic acid following stimulation by agents such as collagen.

This study has also shown that adrenoceptor antagonists act synergistically with PGI₂ to inhibit platelet aggregation in whole blood stimulated with 0.5 ug/ml collagen. Some in vitro studies have suggested that the concentration of these drugs required to inhibit aggregation are supra-pharmacological and therefore could not occur in vivo. However, several studies have shown a significant effect ex vivo, and circulating aggregates have been shown to be reduced in vivo by propranolol (Green et al., 1982). This discrepancy suggests that platelets in vivo may be more sensitive than platelets in vitro to the anti-platelet effect of adrenoceptor antagonists. This might be explained by the synergy between PGI₂ and these drugs in inhibiting aggregation, which may occur in the in vivo situation. The lowest concentration of labetalol which on its own had a significant effect was 1 ug/ml; this is approximately 1.5 - 2 times greater than mean plasma levels which have been recorded in women with pregnancy induced hypertension given labetalol (unpublished observations) so this effect could possibly occur in vivo.

This study has shown that labetalol and aspirin act synergistically to inhibit platelet aggregation in vitro. This may be due to sequential inhibition of the enzymes involved in TxA₂ production as adrenoceptor antagonists may inhibit phospholipase A₂ (see chapter 5), while aspirin irreversibly inhibits cyclo-oxygenase (Vane, 1971; Smith and Willis, 1971). Such a drug combination may well prove useful in vivo as both adrenoceptor antagonists and aspirin have been shown to be of

some benefit when used alone in the secondary prevention of myocardial infarction (Hjalmarson et al., 1981; Norwegian Multicentre Study Group 1981; DeGaetano et al., 1982). Recently the results of the Persantin - Aspirin Reinfarction Study II Research Group (1985) have become available. These showed that a combination of the anti-platelet agents aspirin and dipyridamole improved prognosis following myocardial infarction, and that this effect was further enhanced if the patient was also on a beta-adrenoceptor antagonist. The mechanism behind this protective effect may be the synergistic action of aspirin and adrenoceptor antagonists on platelet function which has been documented in vitro in this present study.

In its role as an anti-platelet agent, aspirin has been given in low doses to try to avoid suppressing vascular prostacyclin production which is potentially harmful (de Gaetano et al., 1982). Use of an aspirin/adrenoceptor antagonist combination may allow even lower doses of aspirin to be used which might, in combination with the adrenoceptor antagonist, effectively inhibit platelet aggregation and TxA₂ production, while sparing vascular PGI₂ production. However, further studies are required to ensure that this combination does not also act synergistically to inhibit vascular PGI₂ production in vivo.

Such a combination of an anti-hypertensive agent with anti-platelet properties and low dose aspirin may also be of benefit in the treatment of pregnancy induced hypertension, since platelet aggregation may play a major role in the pathophysiology of this disease. This requires urgent clinical investigation.

In conclusion, this study has shown that some adrenoceptor antagonists can inhibit platelet aggregation in whole blood and can act synergistically with PGI₂ and aspirin. Whether these properties will prove useful in the clinical situation remains to be established but there are at least good theoretical reasons to use an adrenoceptor antagonist with anti-platelet properties.

CHAPTER 8

**INHIBITION OF THROMBOXANE AND PROSTACYCLIN PRODUCTION
IN WHOLE BLOOD BY ADRENOCEPTOR ANTAGONISTS**

8.1 INTRODUCTION

Thromboxane A₂ (TxA₂) is the major product of arachidonic acid metabolism in platelets and is a potent vasoconstrictor and platelet aggregating agent (Hamberg et al., 1975). It has been implicated in the pathogenesis of myocardial infarction (Coker et al., 1981), arrhythmias (Coker and Parratt, 1983), vasospastic angina (Lewy et al., 1979; Robertson et al., 1981) and in angina in patients with ischaemic heart disease (Hirsh et al., 1981). Increased ex vivo production of TxA₂ has also been documented in pregnancy induced hypertension (PIH) complicated by intra-uterine growth retardation (Wallenburg and Rotmans, 1982). Since PIH is associated with vasoconstriction and platelet consumption, TxA₂ may well play a role in its pathophysiology.

Prostacyclin is the major product of arachidonic acid metabolism in vascular tissue (Moncada et al., 1977), but can also be produced by white cells in the blood (Blackwell et al., 1978; Deckmyn et al., 1983). It has actions directly opposed to TxA₂ as it is a potent vasodilator and inhibitor of platelet aggregation (Moncada et al., 1976). It is thought that a balance exists between these two substances to maintain vascular integrity (Bunting et al., 1983).

Beta-adrenoceptor antagonists are widely used in the treatment of hypertension and ischaemic heart disease, and they are also being increasingly used in the treatment of PIH (see chapter 3.8.3). They have also been shown to improve prognosis following myocardial infarction (Hjalmarson et al., 1981; Norwegian

TABLE 8.1

Properties of adrenoceptor antagonists

Drug	Receptor Specificity*	ISA*	MSA*	Lipid solubility** (distribution co-efficient for octanol/buffer pH 7.4 and 37°C)
Atenolol	β_1	-	-	0.015
Labetalol	$\alpha_1\beta_1\beta_2$	-	+	11.5
Pindolol	$\beta_1\beta_2$	+	±	0.82
Propranolol	$\beta_1\beta_2$	-	+	20.2

* see Feely, de Vane and MacLean, 1983.

** see Woods and Robinson, 1981.

Multicentre Study Group 1981), and may reduce platelet consumption and disease progression in PIH (Walker et al., 1982). The mechanism behind these beneficial effects is unclear but an attractive hypothesis would be inhibition of platelet thromboxane production. There have been several studies showing that beta-adrenoceptor antagonists can reduce platelet aggregation and TxA₂ production from platelet rich plasma stimulated by thrombin and arachidonic acid (Campbell et al., 1981a; Campbell et al., 1981b; Heinroth et al., 1983; Brandt et al., 1984; Mehta, Mehta and Ostrowski, 1983) both in vitro and in vivo, although most of these studies have concentrated on propranolol. There has been little work, however, on the effect of these drugs on TxB₂ and PGI₂ production from whole blood, which may be more physiological as it avoids the separation of platelets from red and white cells which can themselves influence platelet behaviour by release of ADP (Saniabadi et al., 1984a) and PGI₂, respectively.

The aim of this study was to determine what effect a variety of adrenoceptor antagonists had on TxA₂ and PGI₂ production from whole blood in vitro. As adrenoceptor antagonists vary not only in their receptor specificity but also in their lipid solubility, possession of membrane stabilising activity (MSA) and intrinsic sympathomimetic activity (ISA), a variety of drugs which differed in these respects (table 8.1) were studied.

8.2 MATERIALS AND METHODS

Venous blood was obtained without stasis from healthy volunteers using a 19 gauge butterfly cannula and the effects of several adrenoceptor antagonists on TxA₂ and PGI₂ production from

spontaneous whole blood clotting in vitro was determined using the method described in chapter 4.7. Serum TxB₂, the stable hydration product of TxA₂, was determined by radioimmunoassay as described in chapter 4.1.4. Serum immunoreactive 6-keto-PGF_{1α} (PGI₂M) the stable product of PGI₂ was also measured by radioimmunoassay as described in chapter 4.1.3.

To confirm that inhibition of PGI₂M and TxB₂ production could be measured using this technique, the experiment was first performed with varying concentrations of indomethacin or its vehicle (0.9% sodium chloride). The following drugs were then assessed; atenolol, labetalol, propranolol (using 0.9% sodium chloride as vehicle), and pindolol (using 0.1M phosphate buffer pH 7.0 as vehicle). All four drugs were assessed at final drug concentrations of 1, 10 and 100 ug/ml, compared with controls (vehicle alone). None of the drugs assessed had any demonstrable cross reactivity with either assay.

Statistical analysis was performed using the Wilcoxon paired rank sum test, and correlation was assessed using the Spearman rank test.

8.3 RESULTS

Indomethacin treated blood showed a dose dependent reduction in serum PGI₂M and TxB₂ (Figure 8.1), confirming that inhibition of production of these substances in whole blood could be measured using this method.

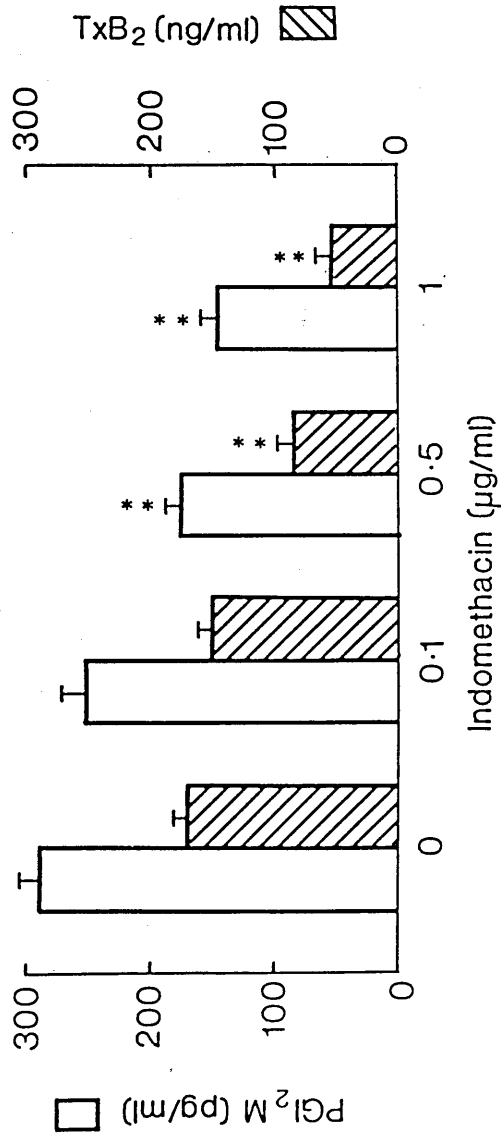


FIGURE 8.1

The effect of indomethacin on PGI₂M and TxB₂ production from whole blood clotting.
 Mean ± SEM of 8 experiments (**p < 0.02).

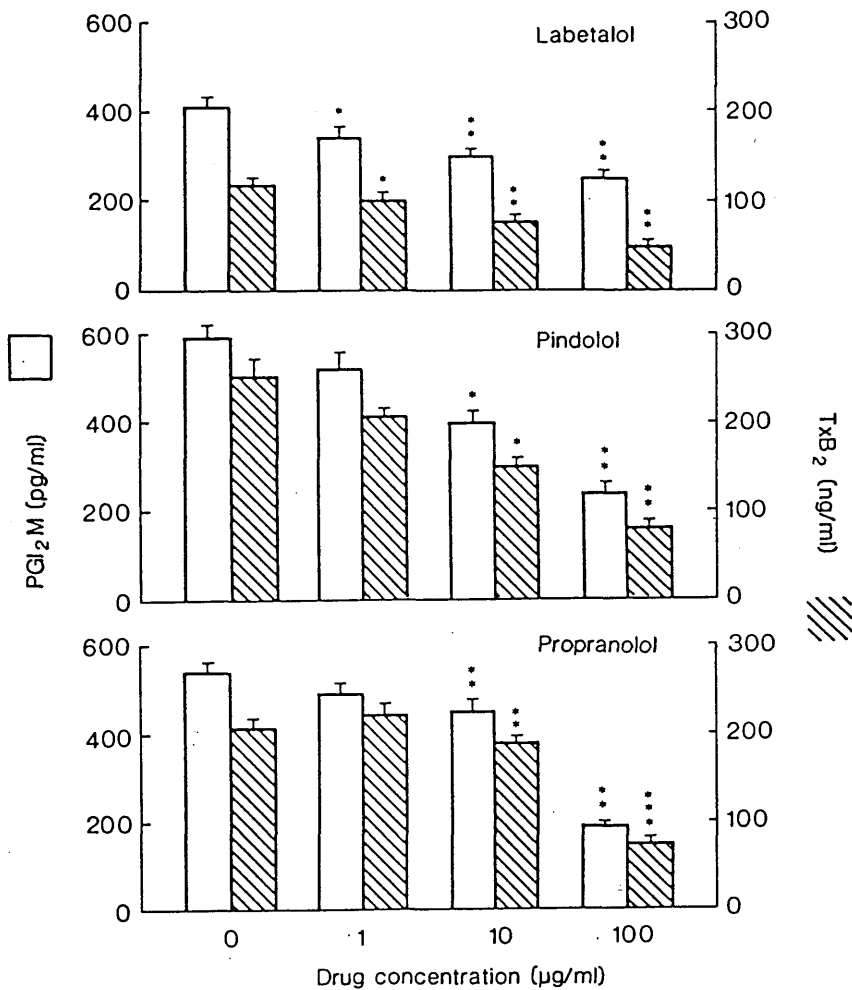


FIGURE 8.2

Effect of labetalol, pindolol and propranolol on TxB₂ and PGI₂M production from whole blood. Mean ± SEM of 8 experiments for labetalol and propranolol. Mean ± SEM of 7 experiments for pindolol (*p < 0.05, **p < 0.02, *** p < 0.001).

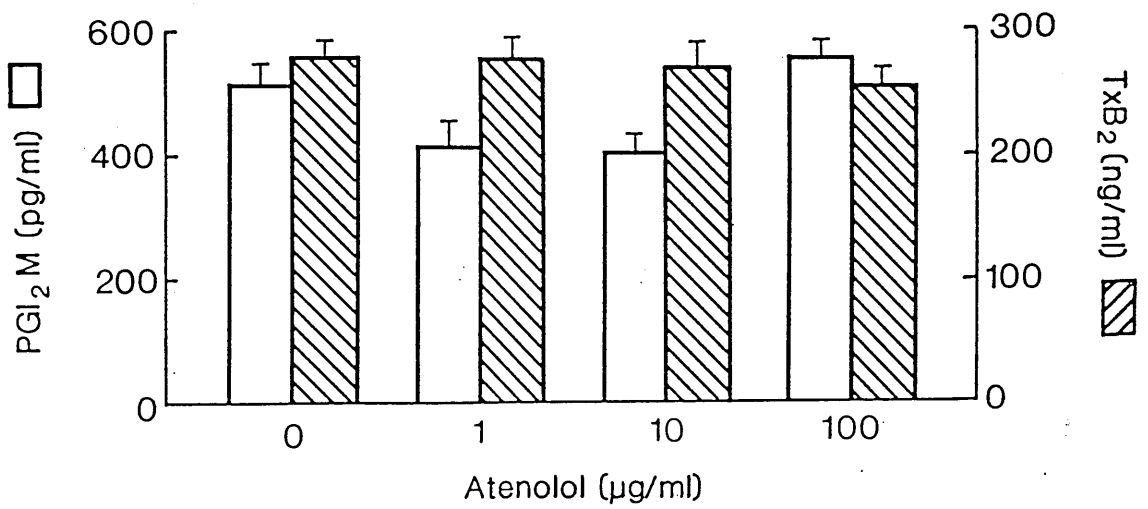


FIGURE 8.3

Effect of atenolol on TxB₂ and PGI₂M production from whole blood clotting. Mean ± SEM of 8 experiments.

Labetalol, pindolol and propranolol all showed a dose dependent reduction in both PGI₂M and TxB₂. Labetalol was the most potent as it significantly inhibited the production of both PGI₂M and TxB₂ at a final drug concentration of 1 ug/ml, while propranolol and pindolol had significant effects at 10 ug/ml (figure 8.2).

Atenolol, however, had no significant effect on either TxB₂ or PGI₂M production from whole blood at concentrations up to 100 ug/ml of the drug (figure 8.3).

The inhibition of TxB₂ production correlated positively with the inhibition of PGI₂M production for labetalol (p <0.002), pindolol (p <0.001), propranolol (p <0.001) and indomethacin (p <0.001). There was no correlation between TxB₂ and PGI₂M in the atenolol experiments.

8.4 DISCUSSION

This study has shown that adrenoceptor antagonists differ in their ability to reduce TxB₂ and PGI₂M production from whole blood in vitro. The inhibition of TxB₂ production in the present study is compatible with studies using platelet rich plasma (Campbell et al., 1981a; Campbell et al., 1981b; Heinroth et al., 1983; Mehta and Mehta, 1984; Mehta et al., 1983) both in vitro and ex vivo. However, the results of this and other studies (Campbell et al., 1981a; Campbell et al., 1981b; Mehta and Mehta, 1984; Mehta et al., 1983) disagree with those of Brandt et al., (1984), who showed that propranolol had no effect on TxB₂ production ex vivo, while atenolol significantly inhibited TxB₂ production.

Since inhibition of PGI₂M and TxB₂ production occurred hand in hand this inhibitory effect may be due to a block at a common point in the pathways for TxA₂ and PGI₂ formation. These substances are synthesised from arachidonic acid which is released from the cell membrane by the action of phospholipases which are activated during platelet activation and blood clotting. The free arachidonic acid is then converted to PGH₂ by the action of cyclo-oxygenase, the pathway then divides and PGH₂ is converted to TxA₂, PGI₂ or other prostaglandins depending on the cell type. Therefore, the inhibitory effects of adrenoceptor antagonists may be at either the phospholipase or cyclo-oxygenase steps. Weksler et al., (1977) have shown that propranolol can interfere with intracellular Ca²⁺ availability in the platelet. Since Ca²⁺ is essential for phospholipase activity (Blackwell and Flower, 1983), this is a possible mechanism of action. However, Heinroth et al., (1983) showed that pindolol but not propranolol inhibited cyclo-oxygenase activity in ram seminal vesicles so that a block at either of these steps is possible.

The results of this study also suggest that membrane stabilising activity or lipid solubility of adrenoceptor antagonists is the property which is required for inhibition of TxB₂ and PGI₂M production. Labetalol, pindolol and propranolol all possess MSA, are lipid soluble and inhibited PGI₂ and TxB₂ production, while atenolol which is very poorly lipid soluble had no effect. This is in agreement with other studies which also suggest that MSA

and lipid solubility, which are closely related, are responsible for the effects of these drugs on platelet aggregation and TxB_2 production (Heinroth et al., 1983; Weksler et al., 1977).

The inhibition of TxA_2 production by these drugs may be clinically beneficial in view of the growing evidence implicating TxA_2 in vascular disease, while the inhibition of PGI_2 production is potentially harmful in view of PGI_2 's vasoprotective effect. However, the effect of these drugs on vascular PGI_2 production may be more important than PGI_2 production from whole blood. It has been shown that propranolol has no effect on PGI_2 production from cultured vascular endothelial cells (Campbell et al., 1981b), so that vessel wall PGI_2 production may be less sensitive to the inhibitory effects of these drugs. This would suggest that the net effect of these drugs might be a reduction in TxA_2 production with sparing of vascular PGI_2 . The effects of labetalol, pindolol and propranolol on umbilical artery PGI_2 production were studied in chapter 6 of this thesis and all 3 drugs were shown to have minimal effects on the amount of PGI_2 produced. This is in keeping with the hypothesis that vascular PGI_2 production is less sensitive than platelet TxA_2 production to the effects of these drugs.

It has been suggested that the inhibitory actions of adrenoceptor antagonists on platelets and TxA_2 production only occurs at drug concentrations higher than those obtained in vivo (Weksler et al., 1977). However, there are several studies documenting an effect ex vivo (Campbell et al., 1981a; Mehta and Mehta, 1984;

Small et al., 1982; Frishman et al., 1974), and propranolol has been shown to reduce circulating platelet aggregates in vivo at therapeutic doses in patients with coronary artery disease (Green et al., 1982). In the present study labetalol had a significant effect at a final concentration of 1 ug/ml which is only 1.5 - 2 times greater than the mean plasma concentrations which have been recorded following oral administration in pregnancy (unpublished observation), therefore these drugs may well have an important therapeutic effect in vivo.

In conclusion, this study suggests that some adrenoceptor antagonists inhibit TxA_2 and PGI_2 production from whole blood. Whether this is clinically useful remains to be established, by in vivo studies. However, there are good theoretical reasons for using adrenoceptor antagonists which can inhibit TxA_2 production, as this substance is implicated in the pathophysiology of several vascular disorders.

CHAPTER 9

EFFECTS OF THE CALCIUM CHANNEL BLOCKING AGENT NICARDIPINE
ON PLATELET FUNCTION IN WHOLE BLOOD, SYNERGISM WITH
ASPIRIN AND PROSTACYCLIN, AND EFFECT ON THROMBOXANE A₂
AND PROSTACYCLIN PRODUCTION

9.1 INTRODUCTION

Platelets are intimately involved in the pathogenesis of vascular disease. They act by obstructing the microcirculation and by release of vasoactive substances which can alter vascular permeability, and cause proliferative changes in the vessel wall (Ross et al., 1974). Thromboxane A₂ (TxA₂) is the major product of the arachidonic acid cascade in platelets (Hamberg et al., 1975). It is released when platelets aggregate and in turn it promotes vasoconstriction and further platelet aggregation (Hamberg et al., 1975), possibly by increasing Ca²⁺ availability (Gorman, 1979). There is growing evidence implicating TxA₂ in ischaemic heart disease and vasospastic angina (Hirsh et al., 1981; Coker et al., 1981; Robertson et al., 1981).

Pregnancy induced hypertension (PIH) is associated with platelet activation and consumption (Trudinger, 1976; Redman et al., 1978; Socol et al., 1985) and platelet aggregates may contribute to the microvascular obstruction and tissue ischaemia seen in organs such as the placenta and kidney in PIH. Release of vasoactive substances from the platelets, such as 5-hydroxytryptamine (Whigham et al., 1978) and thromboxane A₂, may also contribute to the disease process and Wallenburg and Rotmans (1982) have recently documented increased platelet TxA₂ production ex vivo in PIH complicated by intra-uterine growth retardation.

Calcium channel blocking agents (CCB) are of proven value in the treatment of vasospastic angina, effort angina and hypertension (Braunwald, 1982) and have recently been used with some success in the treatment of PIH (Walters and Redman, 1982). Although

they do not yet have an established role in the treatment of this disease, they may be of value not only because they lower blood pressure but also because they produce peripheral vasodilatation which may improve utero-placental blood flow.

CCB's are known to have an inhibitory action on platelet aggregation to a variety of stimuli in vitro (Onoda, Sloane and Honn, 1984; Lippton et al., 1984; Mehta et al., 1983; Addonizio et al., 1982; Han et al., 1983) and an effect has also been documented ex vivo (Dale, Landmark and Myhre, 1983). They are thought to act by blocking Ca^{2+} availability, preventing influx of extracellular Ca^{2+} or mobilisation of intracellular Ca^{2+} stores (Han et al., 1983). Free cytosolic Ca^{2+} is the biochemical intermediary linking stimulus to cellular response, and Ca^{2+} is required for irreversible platelet aggregation (Feinstein, 1978; Rink, Smith and Tsien, 1981; Le Breton et al., 1976).

Prostacyclin (PGI_2), the major prostaglandin produced by vascular endothelium, is a potent vasodilator and platelet inhibitor which may act by increasing Ca^{2+} sequestration within the platelet (see chapter 1.4). Consequently, CCBs and PGI_2 may have a synergistic action on inhibition of platelet aggregation.

All previous studies with CCBs have been performed on platelet rich plasma (PRP) by traditional turbidometric techniques (Born, 1962). It has recently become possible to measure platelet aggregation in whole blood using a whole blood platelet counter, which can count single platelets without separation from other

blood cell types. This may be superior to turbidometric techniques, as platelets are left in their natural milieu with both red and white cells present which can themselves influence aggregation by release of ADP and PGI₂ respectively (see chapter 4.6.1).

The aims of this study were to determine what effect a CCB had on platelet aggregation and TxA₂ and PGI₂ production from whole blood in vitro and also determine whether it had any effect on PGI₂ production from the vessel wall. As a representative example we used nicardipine (supplied by Syntex Research) a drug chemically and pharmacologically similar to nifedipine.

9.2 MATERIALS AND METHODS

The materials and methods used in the following in vitro studies with nicardipine are fully described in chapter 4 (Methodology). The effect of nicardipine on platelet aggregation in whole blood was determined using the method described in chapter 4.6.2. Varying concentrations of nicardipine or its vehicle (physiological saline and hydrochloric acid pH 3.6) were assessed, the latter acting as control. Synergistic effects of nicardipine and PGI₂ or aspirin in inhibiting platelet aggregation in whole blood were determined using the methods described in chapter 4.6.4 and 4.6.5, respectively.

The effect of nicardipine on prostacyclin and thromboxane A₂ generation from whole blood was assessed using the methodology described in chapter 4.7 and the effect of nicardipine on prostacyclin production from umbilical artery was assessed as

described in chapter 4.5. TxA_2 was measured by radioimmunoassay of its stable hydration product TxB_2 , and prostacyclin was measured by radioimmunoassay for immunoreactive 6-keto-PGF $_{1\alpha}$ (PGI $_2$ M). These assays are described in chapter 4.1.4 and 4.1.3, respectively.

Statistic Methods

Statistical analysis was by the Wilcoxon paired rank sum test for all experiments.

9.3 RESULTS

Results for the effect of nicardipine on platelet aggregation in whole blood are shown in figures 9.1 - 9.5. As can be seen, nicardipine produced a dose dependent inhibition of 0.5 ug/ml collagen induced aggregation, and produced a significant effect at concentrations as low as 1-10 ug/ml (figure 9.1). Nicardipine, however, had no effect on aggregation to 10 uM ADP, but caused significant reversal of aggregation at 200 ug/ml (figure 9.2). It also produced a dose dependent inhibition of aggregation to 0.5 mM arachidonic acid, but was only significant at high concentrations (figure 9.3).

Nicardipine was synergistic with both aspirin and PGI $_2$ at concentrations of the drugs which on their own had minimal effects on platelet aggregation (figures 9.4 and 9.5).

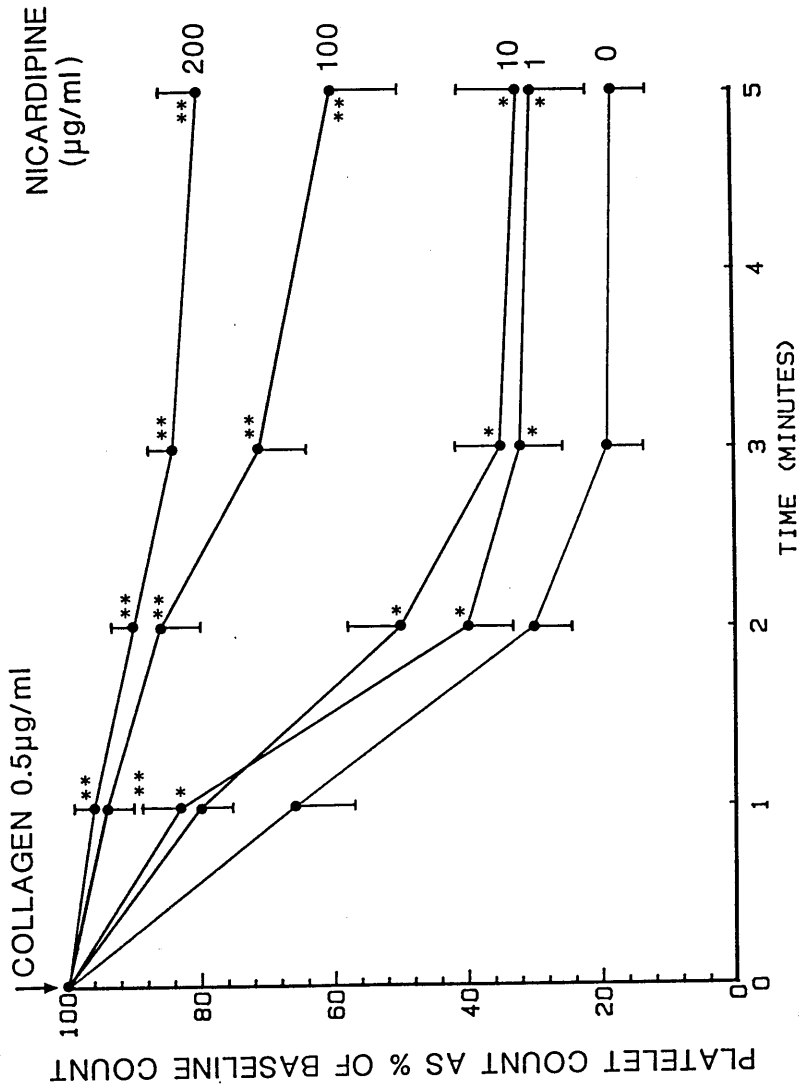


FIGURE 9.1

Effect of nicardipine on collagen induced aggregation.

All points represent the mean \pm SEM of 7 experiments using different donors.

* $p < 0.05$ compared to control

** $p < 0.02$

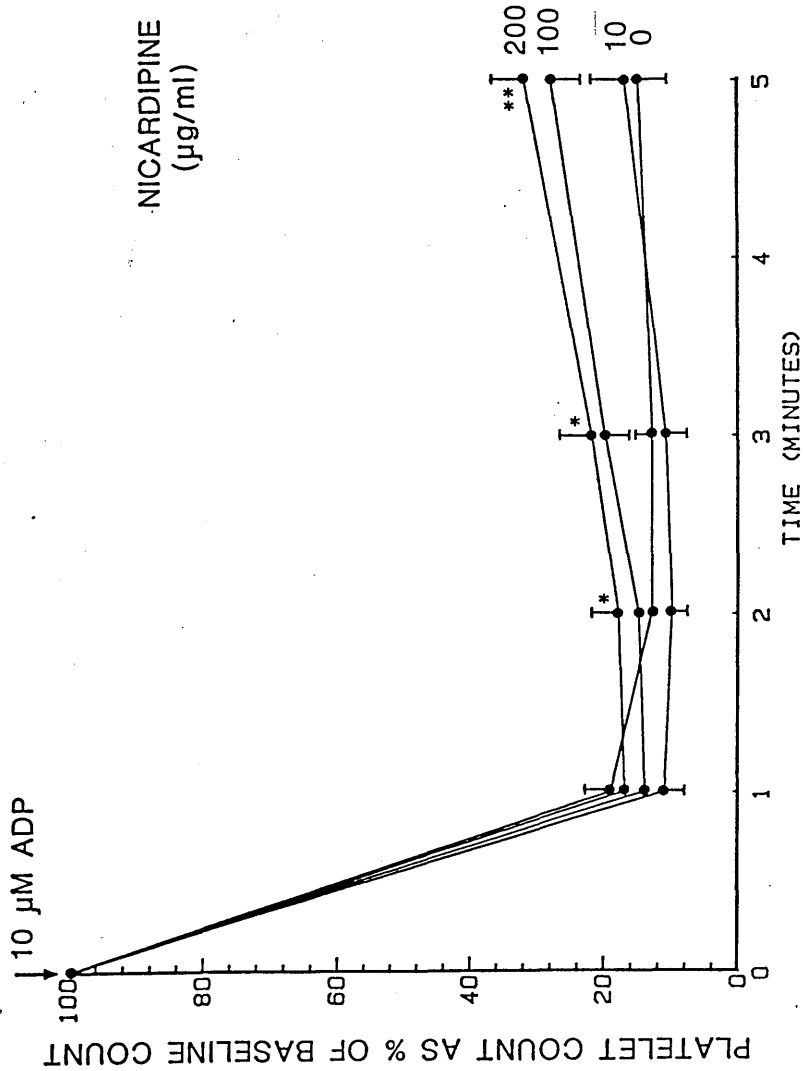


FIGURE 9.2

Effect of nicardipine on aggregation to ADP.

All points represent the mean \pm SEM of 7 experiments using different donors.

* $p < 0.05$ compared to control

** $p < 0.02$

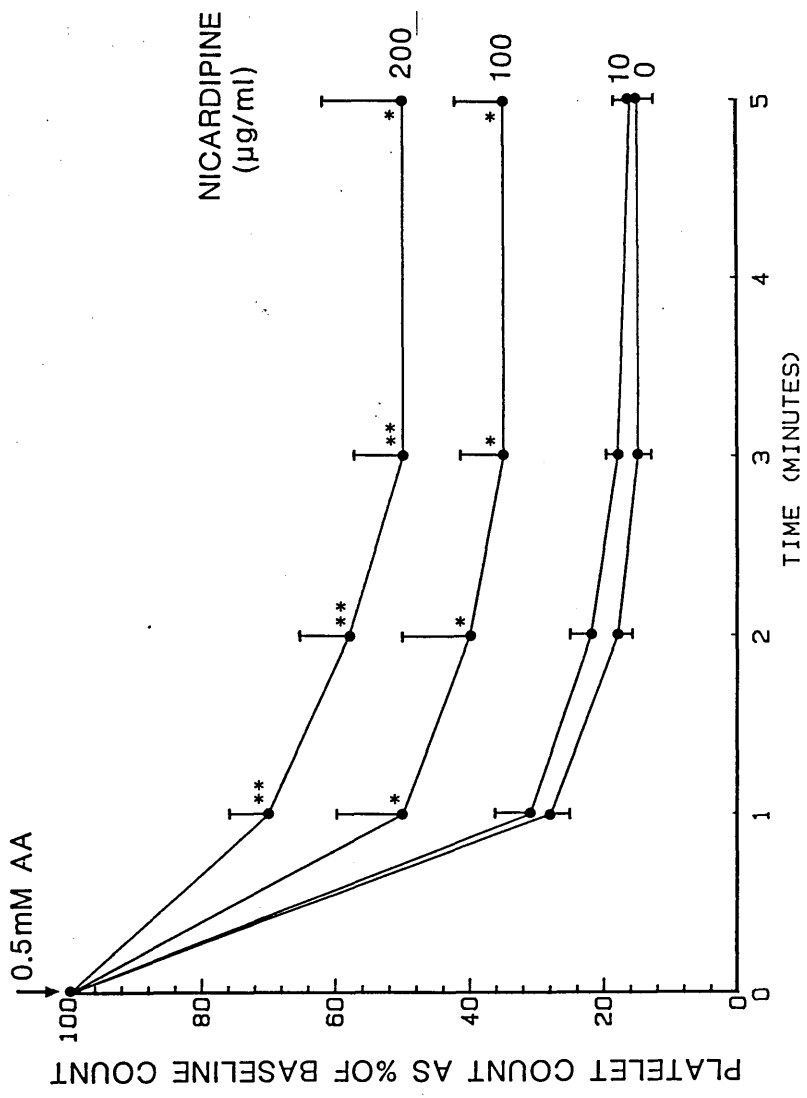


FIGURE 9.3

Effect of nicardipine on aggregation to arachidonic acid (AA).

All points represent the mean \pm SEM of 7 separate experiments using different donors.

* p < 0.05) compared to control

**p < 0.02)

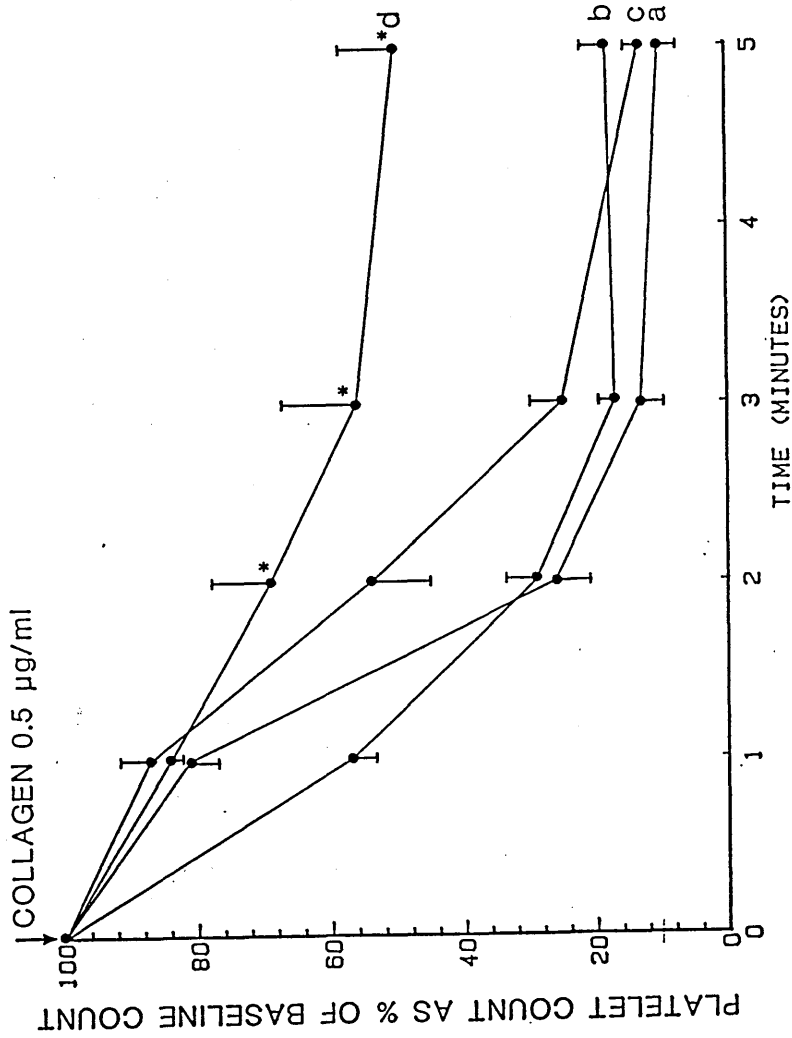


FIGURE 9.5

Synergistic effects of nicardipine and aspirin on platelet aggregation.

All points represent mean \pm SEM of 5 separate experiments using different donors.

*p < 0.05 compared with all other groups

a = control

b = aspirin 5 ug/ml

c = 10 ug/ml nicardipine

d = 5 ug/ml aspirin and 10 ug/ml nicardipine

TABLE 9.1

Effect of Nicardipine on TxB₂ and PGI₂M Production from Whole Blood Clotting

All values given as mean ± SEM (n = 7)

	Control	Nicardipine		
		1 ug/ml	10 ug/ml	100 ug/ml
PGI ₂ M (pg/ml)	562.9 ±55.8	607.1 ±29.7	605.7 ±37.9	757.1* ±27.7
TxB ₂ (ng/ml)	138.3 ±11.5	116.0 ±11.4	116.6 ±10.6	121.7 ± 7.9

* p < 0.05 compared with all other groups

TABLE 9.2

Effect of Nicardipine and Aspirin on PGI₂M Production
from Umbilical Artery Rings

All values given as mean \pm SEM of 5 experiments

	Control	Aspirin (100 ug/ml)	Nicardipine (100 ug/ml)
PGI ₂ M	788.0	540.0*	660.0
(pg/ml)	± 59.5	± 40.0	± 40.0

* p < 0.05 compared with control

(Pool plasma PGI₂M concentration was 6 pg/ml prior to incubation with the artery rings)

Nicardipine had no significant effect on TxB₂ production from whole blood but significantly increased PGI₂M at 100 ug/ml (table 9.1). It had no effect on PGI₂M production from umbilical artery, while 100 ug/ml aspirin significantly inhibited the production of PGI₂M (table 9.2).

9.4 DISCUSSION

These results show that nicardipine can inhibit platelet aggregation in whole blood. Furthermore, a significant effect was noted at lower drug concentrations than previously described in platelet rich plasma experiments although it is difficult to compare studies due to differences in methodology and in the aggregating agents used. This inhibitory effect may be related to the ability of CCBs to reduce the rise in free cytosolic Ca²⁺ which is required for platelet aggregation (Rink et al., 1981; Le Breton et al., 1976).

We have also shown that PGI₂ is synergistic with nicardipine in inhibiting aggregation in whole blood. This supports the findings of Onoda et al., (1984) and Ikeda et al., (1981) who showed that such synergy existed with other CCBs in platelet rich plasma. All the in vitro studies have shown that CCBs can inhibit aggregation only at suprapharmacological concentrations which cannot be obtained in vivo, yet CCBs have been shown to have anti-platelet effects in vivo. Dale et al., (1983) have shown that nifedipine can prolong bleeding time and reduce collagen and ADP induced aggregation in patients with coronary artery disease. This discrepancy suggests that platelets in vivo may be more sensitive than platelets in vitro to CCBs. This might

be explained by the synergy of CCBs with PGI₂ which may occur in the in vivo situation. It is also difficult to relate the concentration of the aggregating agent used in vitro to the concentration which may occur in vivo which is likely to be much lower.

Aspirin is well known to inhibit platelet aggregation (O'Brien, 1968; Evans et al., 1968) and TxB₂ generation (Preston et al., 1981; Smith and Willis, 1971), and has been investigated in the secondary prevention of myocardial infarction and in threatened stroke as previously described (see Chapter 2). More recently a combination of aspirin and dipyridamole have been used with success in pregnancies at high risk of PIH and intra-uterine growth retardation (Beaufils et al., 1985).

Low dose aspirin has been used to try to avoid suppressing prostacyclin production from vascular tissue which may be potentially harmful (De Gaetano, Cerletti and Bertele, 1982; Toivanen et al., 1984). This study has shown that a low concentration of aspirin is synergistic with a low concentration of nicardipine in inhibiting aggregation. Furthermore this study has shown that even at a high drug concentration nicardipine had no effect on the PGI₂ production by vascular tissue in vitro. This synergy may be of value in the prophylaxis of vascular disease, where CCBs are already used in the treatment of angina and hypertension (Braunwald, 1982).

Use of nicardipine concurrently with aspirin may allow even lower doses of aspirin to be used which could still effectively reduce platelet aggregation and TxA_2 production, while sparing vascular PGI_2 production. Reduced aspirin dosage would also reduce gastro-intestinal side-effects.

Such a combination of a vasodilator anti-hypertensive agent with anti-platelet properties and low dose aspirin might also be useful in PIH since it is a disease characterised by vasoconstriction and platelet activation and consumption.

Nicardipine had no effect on TxB_2 production from whole blood clotting, although there was a trend towards lower levels. This is at variance with other studies. Han et al., (1983) showed that both verapamil and nifedipine inhibited TxA_2 production in platelet rich plasma and Dahl et al., (1984) have shown that verapamil inhibits TxB_2 production from whole blood. However, at high drug concentration nicardipine increased PGI_2 production from whole blood. This may be due to diversion of endoperoxides away from TxA_2 production and towards PGI_2 production, but as this only occurred at high drug concentrations it is unlikely to be significant in vivo.

In conclusion, this study has shown nicardipine to be effective in inhibiting aggregation in whole blood. In addition, it is synergistic with both PGI_2 and aspirin. The former effect may explain the antiplatelet effect of CCBs in vivo, while the latter effect may represent a new approach in the preventive treatment of vascular disorders associated with platelet activation.

CHAPTER 10

**A CROSS-SECTIONAL STUDY OF IMMUNOREACTIVE PROSTACYCLIN
AND THROMBOXANE METABOLITES IN NORMAL PREGNANCY
AND THE PUERPERIUM**

10.1 INTRODUCTION

Prostaglandins have long been known to have physiological roles in pregnancy and parturition. Prostacyclin (PGI₂) and thromboxane (TxA₂) which are powerful vasoactive agents, can now be readily assayed, and there is increasing evidence implicating them in the pathophysiology of several disorders of pregnancy. In pregnancy induced hypertension (PIH), several workers have shown a reduction in PGI₂ production from fetal (Remuzzi et al., 1980; Downing et al., 1980; Walsh et al., 1985) and maternal (Bussolino et al., 1980) vascular tissue, and low prostacyclin like activity in amniotic fluid (Bodzenta et al., 1980). In intra-uterine growth retardation (IUGR) PGI₂ production has also been shown to be low (Stuart et al., 1981; Jogee et al., 1983).

Many of the changes seen in PIH and IUGR, such as platelet consumption, vasoconstriction and low renin secretion may result from PGI₂ deficiency, as PGI₂ is a potent vasodilator, platelet inhibitor and renin secretion stimulant (Miyamori et al.., 1979). More recently, PGI₂ production from umbilical artery has been shown to correlate with umbilical artery blood flow (Makila et al., 1983).

TxA₂ is produced from activated platelets and is a PGI₂ antagonist, having platelet aggregatory and vasoconstrictor properties (Hamberg et al., 1975). PGI₂ and TxA₂ oppose each other through regulation of adenylate cyclase in the platelet (Tateson et al., 1977), therefore TxA₂ may also have a role in these disorders. Both these substances are highly unstable and

are usually measured as their stable metabolites. Prostacyclin's major metabolite is 6-keto-PGF_{1α}, and thromboxane is converted to its stable hydration product thromboxane B₂ (TxB₂).

As mentioned previously, measurement of PGI₂ metabolites in peripheral plasma has been, and remains a subject of great controversy. Initial work on 6-keto-PGF_{1α} by radioimmunoassay (RIA) suggested that levels in normal subjects were of the order 70 - 100 pg/ml (Mitchell, 1978), and this was supported by gas-chromatography-mass-spectrometry (GCMS) work which suggested similar levels (Hensby et al., 1979). In 1982, however, Blair et al., published work using negative ion GCMS which showed that the absolute values for 6-keto-PGF_{1α} were below 5 pg/ml, and it was concluded that PGI₂ could not be a circulating hormone in man as previously thought (see chapter 1.4). This, however, does not preclude a role for PGI₂ functioning as a local hormone in the regulation of platelet-vessel wall interaction (Patrono et al., 1984). Changes in levels of its stable metabolites must reflect changes in production, it is still therefore valuable to measure these metabolites to elucidate the role of prostacyclin in disease. RIA has also been discredited by varying 'normal' values obtained by RIA, due to differences in methodology and antibody sensitivity (Vinnikka and Ylikorkala, 1982). While RIA's cannot give absolute values for PGI₂ metabolites, they can still produce accurate comparative values.

Much improved RIA's have been developed recently with sensitivities and normal levels much closer to that obtained by GCMS. RIAs, therefore, can still yield much valuable and

accurate data provided they are properly validated (Salmon, 1983), and have many advantages over GCMS, as they are relatively sensitive, specific and can cope with large numbers of samples. For these reasons RIA is probably the method of choice from routine measurements (Belch et al., 1983; Salmon, 1983).

There is little information on the normal levels of PGI₂ and TxA₂ in pregnancy. Since they may be implicated in disease processes, it is of value to establish the changes in these levels in normal pregnancy. Lewis et al., (1980) published levels of 6-keto-PGF_{1α} and have shown an elevation in late normal pregnancy and the puerperium. Bolton et al., (1981) recorded serial measurements throughout 12 normal pregnancies, and found a peak at 18 - 22 weeks gestation with a subsequent reduction towards term. Other studies have shown no difference in the levels of PGI₂ metabolites throughout normal pregnancy (Mitchell, 1981; Ylikorkala and Viinikka, 1981). All of these studies were performed prior to the work of Blair et al., (1982), and suggested normal ranges of 80 - 500 pg/ml for 6-keto-PGF_{1α}. These studies are still quoted as normal range despite subsequent advances in knowledge and assay techniques. In view of these advances and the conflicting reports of Lewis et al., (1980) and Bolton et al., (1981), the aim of this study was to determine the comparative values of PGI₂ and TxA₂ metabolites throughout normal pregnancy and the puerperium, using a RIA which has been shown to be sensitive, specific and reproducible for PGI₂ metabolites (McLaren et al., 1985).

10.2 PATIENTS AND METHODS

One hundred and fifty five women were studied, 44 normal non-pregnant, 29 women in the first trimester of pregnancy, 31 in the second trimester, 29 in the third trimester, and 21 women on the third day following delivery. All pregnancies were uncomplicated and no subject had taken any aspirin or similar preparations for at least two weeks prior to sampling.

Venous blood was taken without stasis using a 21 gauge butterfly cannula and prepared as described in chapter 4.1.3. Plasma immunoreactive 6-keto-PGF_{1α} (PGI₂M) was measured by RIA as described in chapter 4.1.3. Plasma TxB₂ was also measured by RIA as described in chapter 4.1.4.

Statistical analysis was by the Wilcoxon rank sum test for non-paired data.

10.3 RESULTS

Results for PGI₂M are shown in figure 10.1 and for TxB₂ in figure 10.2. Significantly higher levels of PGI₂M were found in the first trimester ($p < 0.01$) when compared with the non-pregnant group. While there were no significant differences between non-pregnant values and those in the second or third trimesters, a trend towards lower concentrations of PGI₂M on the third post-natal day was noted but this was not statistically significant. Levels of PGI₂M were significantly lower in the second and third trimesters than in the first trimester. There

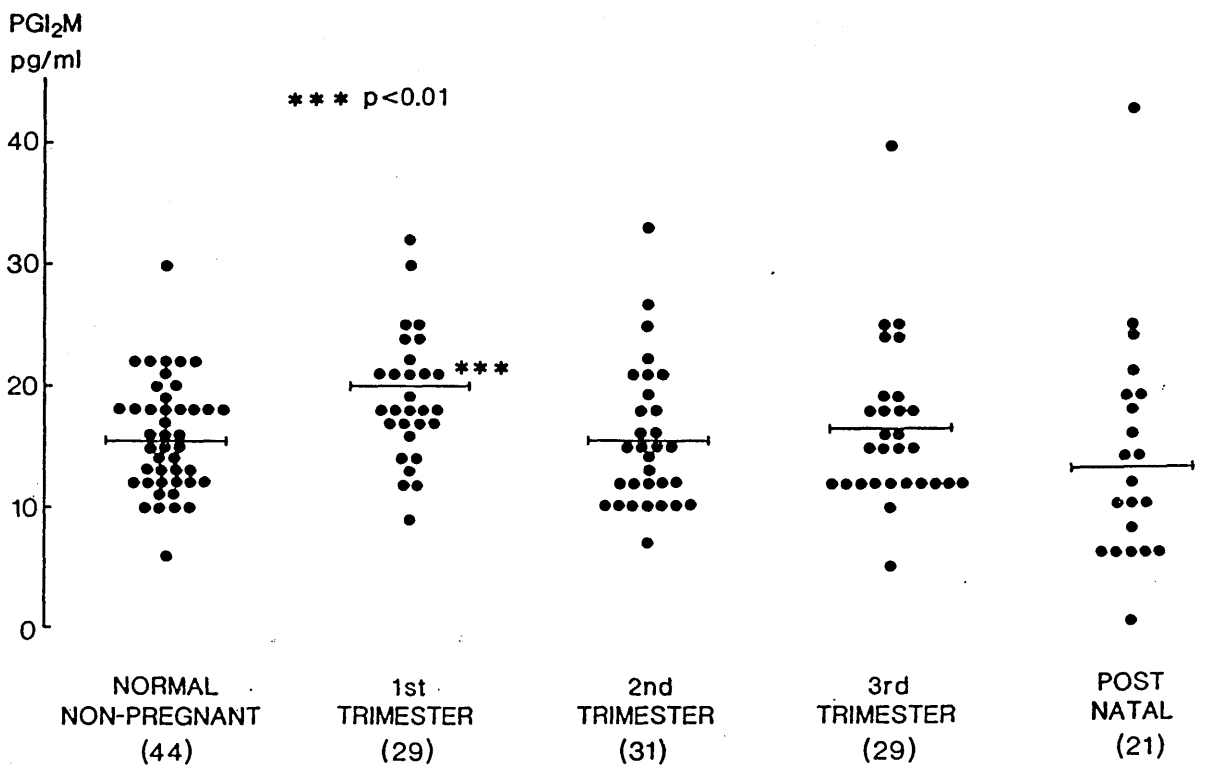


FIGURE 10.1

Plasma concentrations of PGI₂M (pg/ml) in normal pregnancy and the puerperium.

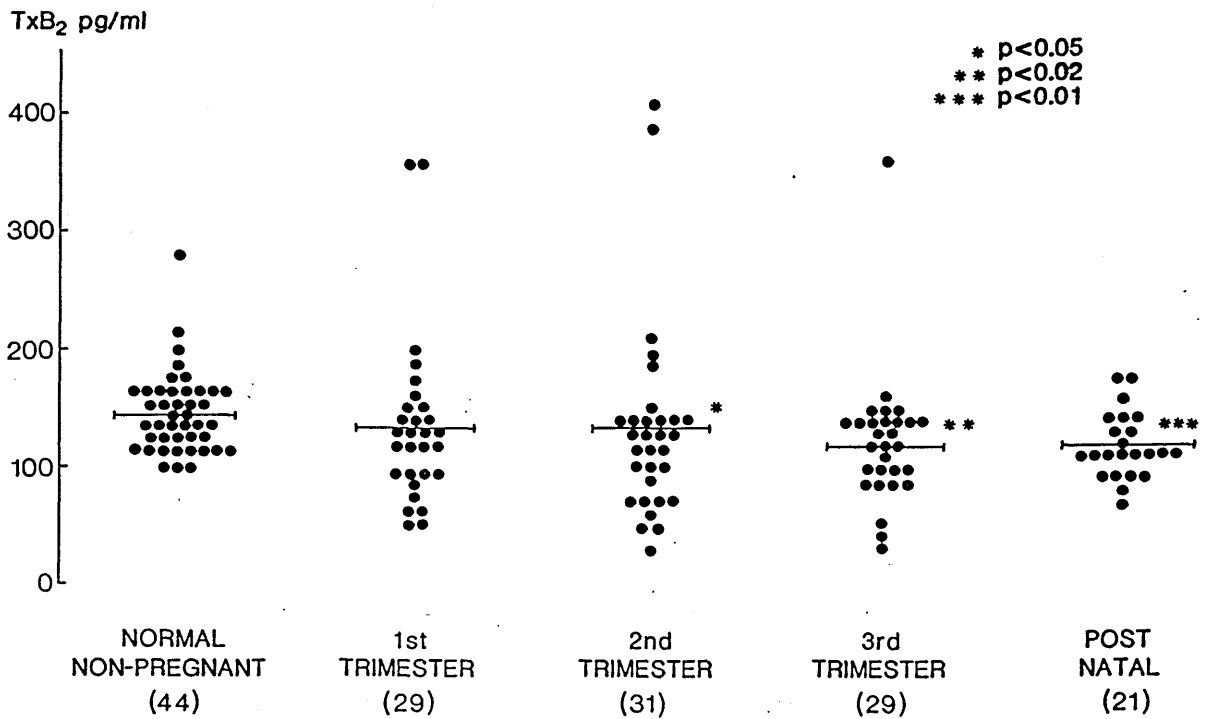


FIGURE 10.2

Plasma concentrations of TxB₂ (pg/ml) in normal pregnancy and the puerperium.

TABLE 10.1

Mean \pm SEM for PGI₂M and TxB₂ in non-pregnant women,
the three trimesters of pregnancy and the puerperium.

	NON PREGNANT	1st TRIMESTER	2nd TRIMESTER	3rd TRIMESTER	POST-NATAL
PGI ₂ M	15.9 \pm 0.68	19.9 \pm 0.96***	15.5 \pm 1.05	16.4 \pm 1.22	13.6 \pm 1.99
TxB ₂	142 \pm 4.9	131 \pm 14.2	133 \pm 14.9*	123 \pm 10.7**	119 \pm 6.3***
n	44	29	31	29	21

*** p < 0.01 compared with non pregnant
 ** p < 0.02 " " " " " " " " " " " "
 * p < 0.05 " " " " " " " " " " " "

p < 0.05 compared with 1st trimester
 " " " " " " " " " " " "
 p < 0.01 " " " " " " " " " " " "
 p < 0.005 " " " " " " " " " " " "

was a progressive reduction in plasma TxB_2 concentrations from the second trimester through the third trimester and into the puerperium when compared to the non-pregnant group.

Table 10.1 gives a fuller statistical analysis.

10.4 DISCUSSION

Despite using larger numbers, this study did not confirm the work of Lewis et al., (1980) who showed a higher concentration of 6-keto-PGF $_{1\alpha}$ in late pregnancy and the puerperium, nor could it confirm the work of Bolton et al., (1981) as peak levels of PGI $_2$ M in this study occurred in the first trimester and not in mid-pregnancy as they reported, although they did not record levels prior to 14 weeks gestation.

Significantly higher levels of PGI $_2$ M were observed in the first trimester as compared to the non-pregnant and second and third trimesters. This early increase may be involved in the physiological vasodilation and insensitivity to angiotensin II (Gant et al., 1973) which are characteristically seen from the early stages of normal pregnancy.

It has recently been shown that trophoblast from early pregnancies has a high capacity to synthesis PGI $_2$, and it has been suggested that colonization of maternal blood vessels by trophoblast is facilitated by PGI $_2$, which may prevent platelet aggregates from halting this process (Rakoczi et al., 1983). This theory might explain our findings of elevated PGI $_2$ M in early pregnancy. Failure of this response may possibly result in

threatened or spontaneous abortion, and may even lead on to IUGR in later pregnancy, a condition where reduced PGI₂ production has previously been recorded (Jogee et al., 1983; Stuart et al., 1981).

Theoretically one might expect high levels of PGI₂ in late pregnancy, due to the potential for increased production from the uterus and feto-placental circulation unit and Goodman et al., (1982) have documented increased urinary PGI₂ metabolites at this stage in pregnancy. However, no increase in PGI₂M was noted in the third trimester in this present study. This may be explained by the findings of Remuzzi et al., (1981b) who found a reduced level of prostacyclin-stimulating factor in late normal pregnancy and this may represent a homeostatic mechanism. Since the capacity to produce PGI₂ is increased, at this stage, less prostacyclin-stimulating factor is required and PGI₂ levels would remain constant, as we have described. Conversely when the ability to produce PGI₂ is low, as in PIH, prostacyclin-stimulating factor rises in an attempt to increase PGI₂. This change has also been noted by Remuzzi et al., (1981b).

In the post-natal group a trend towards lower levels of PGI₂M was observed, which contradicts the work of Lewis et al., (1980), but is in keeping with the findings of Ylikorkala et al., (1981c) who also noted a fall in PGI₂ metabolites postnatally. The sampling in this study was performed only on the third post-natal day, while Lewis et al., sampled between the first and seventh post-natal days. If many of the samples were obtained soon after delivery they may have a high 6-keto-PGF_{1α} level due to vascular

injury. Also, increased production of 6-keto-PGF_{1α} in vitro from sheep cervical tissue taken at parturition has been reported (Ellwood et al., 1981). Since the feto-placental unit, which has enormous capacity to synthesise PGI₂, is removed at birth, some reduction in 6-keto-PGF_{1α} levels post-natally might be expected.

A progressive fall in plasma TxB₂ concentrations through pregnancy and into the puerperium was observed in this study. This may reflect increased platelet stability, or the diversion of TxB₂ precursors into the formation of other prostaglandins, such as PGD₂ or PGE₁. Other possible explanations of this may be related to a change in activity of thromboxane synthetase or decreased substrate availability, but further studies are required to elucidate this. We noted several very high levels of TxB₂ (figure 10.2). These sort of levels are normally associated with traumatic venepuncture or haemolysis, however, we discarded all samples in which either of these problems were apparent. Despite all precautions a minor degree of platelet activation can still occur at venepuncture and this may explain these few outlying levels.

CHAPTER 11

A PROSPECTIVE LONGITUDINAL STUDY OF IMMUNOREACTIVE
PROSTACYCLIN AND THROMBOXANE METABOLITES IN NORMAL
AND HYPERTENSIVE PREGNANCY

11.1 INTRODUCTION

Prostacyclin (PGI₂) is the major prostaglandin produced from arachidonic acid via the cyclo-oxygenase system in vascular tissue (Moncada et al. ., 1977). It is a potent vasodilator (Armstrong et al.., 1978) and inhibitor of platelet aggregation (Moncada et al.., 1976) with a short half-life of approximately three minutes at 37°C (Dusting et al.., 1979), and is thought to function as a local hormone, protecting blood vessels from platelet deposition and subsequent damage. Production of PGI₂ from human vascular tissue can be stimulated in vivo by a variety of minor injuries (Ritter et al. ., 1983). Failure of the vessel to respond in such a manner may therefore allow vasoconstriction, thrombosis and platelet consumption to take place unopposed.

Thromboxane A₂ (TxA₂) is the major cyclo-oxygenase product of arachidonic acid in platelets and is synthesised and released when platelets aggregate (Hamberg et al.., 1975). Its actions are directly opposed to those of PGI₂ as it is a potent vasoconstrictor (Bunting et al.., 1976) and platelet aggregating agent (Hamberg et al.., 1975). It is unstable with a half life of 30 seconds at 37°C. It has been proposed that a balance exists between blood vessel PGI₂ production and platelet TxA₂ production, to maintain vascular integrity (Bunting et al.., 1983).

PGI₂ and TxA₂ have recently been implicated in the pathophysiology of pregnancy induced hypertension (PIH) (see Chapter 3.7). Several workers have shown reduced PGI₂ production from fetal (Remuzzi et al.., 1980; Downing et al.., 1980; Walsh et al.., 1985)

and maternal (Bussolino et al., 1980) vascular tissue. Umbilical cord vessels produce large amounts of PGI₂ (Remuzzi, 1979) and this may be important in the maintenance of flow in the low pressure feto-placental circulation (Tuvemo, 1980). Makila et al., (1983) have recently shown that umbilical artery blood flow is directly related to the vessels ability to produce PGI₂, and shown both to be low in pre-eclampsia. Reduction of PGI₂ production has also been documented in intra-uterine growth retardation (IUGR) (Stuart et al. ., 1981; Jogee et al., 1983). This deficiency in PGI₂ production may be involved in the vasoconstriction and platelet consumption seen in PIH and IUGR, and PGI₂ infusions have been used with some success in the treatment of PIH (Belch et al., 1985; Fidler et al., 1981).

Despite these observations in the abnormal situation, there is little information on maternal plasma PGI₂ in normal pregnancy. In 1980 Lewis et al., published a cross sectional study on 22 pregnancies showing that 6-keto-PGF_{1α}, the stable hydration product of PGI₂ was elevated in late pregnancy and the puerperium as compared with normal non-pregnant and early pregnancy groups. Bolton et al., (1981) in the only prospective longitudinal study of PGI₂ in 12 normal pregnancies reported a peak at 18 - 22 weeks gestation, although they did not sample in the first trimester. Other cross sectional studies have shown no difference (Mitchell et al., 1981; Ylikorkala and Viinika, 1981) in PGI₂ throughout pregnancy. The values given by these studies, using radio-immunoassays (RIA) were between 100 and 500 pg/ml for 6-keto-PGF_{1α}, and were compatible with values for normal non-pregnant subjects obtained by RIA (Mitchell, 1978) and by

gas-chromatography mass-spectrometry (GCMS) (Hensby, 1979). These findings were in keeping with the belief that PGI₂ was a circulating hormone in man (Moncada et al., 1978). In 1982, however, Blair et al., published work using negative ion GCMS which showed that the absolute value for 6-keto-PGF_{1α} in plasma was below 5 pg/ml, and it was concluded that PGI₂ could not be a circulating hormone in man as previously thought. This, however, does not preclude a role for PGI₂ functioning as a local hormone in the regulation of platelet vessel wall interaction (Patrono et al., 1984, see Chapter 4.1.1), as changes in levels of its stable metabolites must reflect changes in production. It is still therefore valuable to measure these metabolites to elucidate the role of prostacyclin both in the normal situation and in disease.

There is little information of TxA₂ in pregnancy (see chapter 3.7). Ylikorkala and Viinikka in 1980 described increased plasma concentrations of TxB₂ in pregnancy as compared with non-pregnant control subjects, but could show no relation to gestation, while Mitchell et al., (1978), showed increased levels at term as compared to "late pregnancy" (28 - 36 weeks gestation).

In view of advances in knowledge and assay techniques for PGI₂ and TxA₂ metabolites and the conflicting reports on these substances in normal pregnancy, the aim of this study was to perform a prospective longitudinal study of PGI₂ and TxA₂ metabolites in normal pregnancy. However, of our initial 14 randomly selected subjects six became hypertensive and we were therefore able to study PGI₂M and TxB₂ prospectively in both normal, and hypertensive pregnancy.

11.2 PATIENTS AND METHODS

11.2.1 Patients

Fourteen randomly selected patients were studied, all were primigravidae and had no history of any hypertensive or medical disorder. No subject had taken any aspirin or similar preparation for a minimum of two weeks prior to each venepuncture. Blood sampling was performed in the first trimester (9 - 12 weeks gestation), the second trimester (17 - 24 weeks gestation) and in the third trimester (32 - 36 weeks gestation). In the patients who became hypertensive further samples were obtained where possible between 36 weeks and term.

Eight patients remained normotensive while 6 developed mild/moderate PIH in the third trimester, defined as a persistent diastolic blood pressure on 90 mm of mercury. Five were treated conservatively, while one required treatment with an anti-hypertensive agent (labetalol). None developed significant platelet consumption or proteinuria.

All patients had vaginal deliveries either spontaneous or induced at term, and all infants were healthy with birth weights above the 10th centile for their gestational age.

The proportion of patients who became hypertensive was unexpected and extraordinarily high. There was no apparent reason for this as all were apparently normal patients at a routine antenatal

clinic. The incidence of PIH in our population is between 5-10%, and the high incidence in the study group appears to be totally coincidental.

11.2.2 Measurement of PGI₂ Metabolite and TxB₂

Venous blood was taken without stasis using a 21 gauge butterfly cannula and prepared as described in chapter 4.1.3. Plasma immunoreactive 6-keto-PGF_{1α} (PGI₂M), the stable metabolite of PGI₂, was measured by radioimmunoassay as described in Chapter 4.1.3. Plasma TxB₂ was also measured by radioimmunoassay as described in Chapter 4.1.4.

All assays were performed "blind" with the operator unaware of either gestation or the presence or absence of hypertension. Unfortunately, 3 specimens had to be discarded due to technical problems at the time of centrifugation, giving incomplete data on 3 of the patients.

11.2.3 Statistical Methods

statistical analysis was by the Wilcoxon paired rank sum test for analysis within group, and by the Wilcoxon rank sum test for non-paired data for analysis between groups. The Spearman rank correlation test was used for correlation of PGI₂M and TxB₂.

11.3 RESULTS

Results are shown in figures 11.1 and 11.2 with a fuller statistical analysis in tables 11.1 and 11.2.

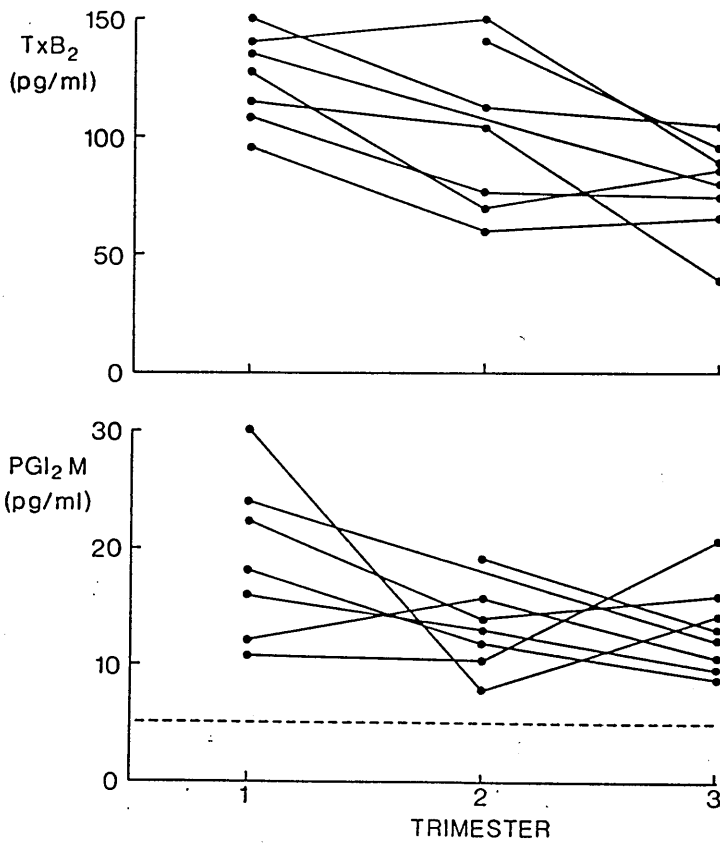


FIGURE 11.1

Plasma PGI₂M and TXB₂ in normal pregnancies (broken line indicates lower limit of detection of assay). Third trimester sample taken between 32 and 36 weeks gestation.

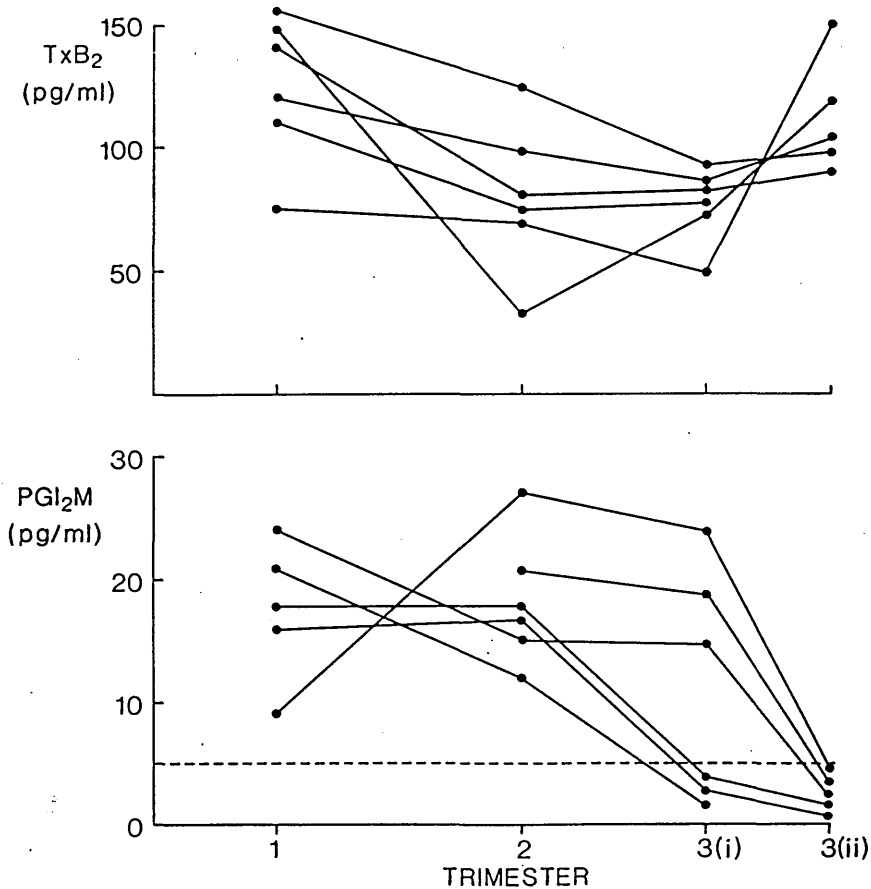


FIGURE 11.2

Plasma PGI₂M and TXB₂ in pregnancies complicated by PIH in the third trimester. Two patients developed PIH in the early third trimester (32-36 weeks gestation) [3(i)] while four developed PIH in the late third trimester (37-40 weeks gestation [3(ii)]). No patient was hypertensive while PGI₂M were recordable. (Broken line indicates lower limit of detection of assay)

TABLE 11.1

Plasma PGI₂M in Normal and Hypertensive Pregnancy (Mean ± SEM)

Trimester	1	2	3(i)	3(ii)
Normal (n = 8)	19.1 ± 2.5	13.1 ± 1.2*	13.3 ± 1.8*	
PIH (n = 6)	17.6 ± 2.5	18.2 ± 2.1	12.2 ± 3.4	<5.0*+

* p < 0.05 compared with 1st trimester of same group

+ p < 0.05 compared with 2nd trimester of same group

3(i) - "early" third trimester (32 - 36 weeks gestation)

3(ii) - "late" third trimester (37 - 40 weeks gestation)

TABLE 11.2

Plasma TxB₂ in Normal and Hypertensive Pregnancy (Mean ± SEM)

Trimester	1	2	3(i)	3(ii)
Normal (n = 8)	125.0 ± 8.8	94.8 ± 13.8*	75.3 ± 5.0**	
PIH (n = 6)	131.5 ± 12.6	82.2 ± 12.6*	74.0 ± 5.0*	113.6 ± 10.4 ⁺⁺

* p < 0.05 compared with 1st trimester of same group

** p < 0.02

⁺⁺ p < 0.05 compared with early 3rd trimester of same group

3(i) - "early" third trimester (32 - 36 weeks gestation)

3(ii) - "late" third trimester (37 - 40 weeks gestation)

In the normal patients PGI₂M were significantly higher in the first than in the second and third trimesters, while TxB₂ levels fell significantly in the second and third trimesters as compared with the first trimester. There was no significant difference for either PGI₂M or TxB₂ between the second and third trimesters of the normal group, although there was a trend towards lower levels of TxB₂ in the third trimester.

In the first and second trimesters, the PIH group was not significantly different from the normal group for either PGI₂M or TxB₂. All hypertensive patients developed unrecordable (<5 pg.ml) levels of PGI₂M in the third trimester when they were hypertensive. In 5 of the 6 PIH patients hypertension had developed prior to the sample showing unrecordable levels, therefore it could not be determined whether undetectable PGI₂M levels preceded the development of hypertension or vice-versa. In one patient, however, a third trimester sample showed unrecordable levels of PGI₂M prior to the development of hypertension, and they remained unrecordable following the development of PIH. No normal patient had unrecordable PGI₂M at any stage.

Owing to the small numbers and differing times of development of PIH it was not possible to compare statistically the third trimester measurements in the PIH patients with the normal group. The late (36 - 40 weeks gestation) third trimester measurement in the PIH group was significantly different from the first and second trimester measurements within the group. There was a significant reduction in TxB₂ in the second and early (32 -36

weeks gestation) third trimester compared to the first trimester in the PIH group and a significant increase in TxB₂ was noted in the late third as compared to the early third trimester. There was no significant difference between normal and PIH groups for TxB₂, and no correlation existed between normal and PIH groups for TxB₂, and no correlation existed between PGI₂M and TxB₂ in either group.

11.4 DISCUSSION

The results from this small prospective longitudinal study show increased concentrations of plasma PGI₂M in the first trimester, and falling levels of plasma TxB₂ in the second and third trimester of normal pregnancy. This confirms our previous cross sectional study of 155 patients comparing PGI₂M and TxB₂ in the third trimesters of pregnancy, the puerperium, and a normal non-pregnant control group, which also showed increased PGI₂M in the first trimester as compared with the second and third trimesters, and also the non-pregnant control group (Greer et al., 1985) (see Chapter 10). The first trimester increase in PGI₂ may be involved in the physiological vasodilation and insensitivity to angiotensin II characteristically seen in early pregnancy (Gant et al., 1973).

Rakoczi et al., (1983) have shown that trophoblast from early pregnancies has a high capacity to produce PGI₂, and suggested that this may facilitate the invasion of maternal vessels by trophoblast, preventing platelet aggregates from halting this process. This theory might explain our findings of elevated PGI₂M in early pregnancy. A failure of such a protective

mechanism might result in spontaneous abortion. This theory may be supported by the findings of Roy et al., (1984) who recently showed that intravesicular fluid from hydatidiform moles had very high levels of PGI₂ metabolites, and this may allow the mole to successfully invade, and escape immobilisation by platelet aggregates.

As the feto-placental unit is largest in the third trimester, one might expect PGI₂M to increase at this time due to the potential for increased PGI₂ production. However, we have found levels to be decreased at this stage compared to the first trimester. This may be explained by the findings of Remuzzi et al. ., (1981b) who found a reduced level of prostacyclin-stimulating factor in late normal pregnancy, and this may be a homeostatic mechanism. Since the capacity to produce PGI₂ is increased at this stage, less prostacyclin-stimulating factor is required and PGI₂ levels would remain constant, as we have found. Conversely, when the ability to produce PGI₂ is low, as in PIH, prostacyclin-stimulating factor may rise in an attempt to increase PGI₂, a finding which was also noted by Remuzzi et al. ., (1981b).

All hypertensive patients were found to have unrecordable levels of PGI₂M. This is consistent with other studies; in 1981 Lewis et al., published a case report showing falling levels of maternal plasma 6-keto-PGF_{1α} in a patient with severe PIH. However, the patient was treated with aspirin over the same time period and it is therefore difficult to know whether this was associated with the disease or was an effect of aspirin acting on vascular tissue to inhibit cyclo-oxygenase. More recently,

significantly lower plasma levels of 6-keto-PGF_{1α} have been found in women with eclampsia or impending eclampsia (Moodley et al. , 1984) than in normal pregnant women, and Yamaguchi and Mori (1985) have also reported lower levels of plasma 6-keto-PGF_{1α} in pregnancies complicated by PIH as compared to normal pregnancies. Goodman et al., (1982) have reported lower urinary metabolites of PGI₂ in six patients with PIH compared to eight normal pregnant control patients and amniotic fluid has also been shown to have lower levels of PGI₂ in PIH (Bodzenta et al., 1980; Ylikorkala et al., 1981a).

The finding of low PGI₂M in the present study is also consistent with the reports of reduced PGI₂ production from both maternal and fetal vascular tissue in pregnancies complicated by PIH (Walsh et al., 1985; Downing et al., 1980; Remuzzi et al., 1980; Bussolino et al., 1980; Makila et al., 1983).

As one patient in the hypertensive group in the present study had unrecordable levels of PGI₂ prior to the development of PIH, this suggests that falling levels of PGI₂M may possibly act as a marker heralding the onset of the disease.

In normal pregnancy women become very insensitive to the pressor effects of exogenous angiotensin II infusions (Chesley, 1966; Talledo et al., 1968; Gant et al., 1973). This insensitivity to angiotensin II can be abolished if women are given inhibitors of prostaglandin synthesis such as indomethacin (Everett et al., 1978a), and enhanced by PGI₂ infusions (Broughton Pipkin et al., 1984). These experiments suggest that in normal pregnancy,

angiotensin II may be balanced by the action of prostacyclin. In PIH this insensitivity to angiotensin II is lost (Chesley, 1966; Talledo et al., 1968; Gant et al., 1973), and this may be due to deficiency of PGI₂ as has been documented in the present study. This increased sensitivity to angiotensin II may also contribute to the pathophysiology of PIH.

A cause and effect relationship for low PGI₂ and PIH cannot be concluded from these studies, however, one could speculate that since PGI₂ is a potent vasodilator and anti-platelet agent, deficiency of this substance may cause the subject to be susceptible to PIH, allowing vasoconstriction and platelet consumption to occur unchallenged, when the disease arises.

Thromboxane A₂ did not seem to be related to PIH, however, a significant increase in the late third trimester of the hypertensive group was noted. It is not possible to comment as to whether this was related to the disease, due to subclinical platelet aggregation, or was a normal finding as we did not have a late third trimester sample in our normal control group. This may, however, be a normal feature, as it would be in agreement with the work of Mitchell et al., (1978) who showed an increase in TxB₂ in the late third trimester.

In conclusion, these results support the hypothesis that PGI₂ deficiency is involved in the pathophysiology of PIH.

CHAPTER 12

A CROSS-SECTIONAL STUDY OF IMMUNOREACTIVE PROSTACYCLIN
AND THROMBOXANE METABOLITES IN PREGNANCY INDUCED
HYPERTENSION, AND THE EFFECT OF LABETALOL TREATMENT ON
THEIR LEVELS AND PLATELET CONSUMPTION

12.1 INTRODUCTION

Pregnancy induced hypertension (PIH) is well known to be associated with activation of the coagulation system (Howie et al., 1971; Howie et al., 1976) and reduced platelet count (Redman et al., 1978; Trudinger, 1976) which have been shown to be related to disease severity (Howie et al., 1976). Platelet life span is known to be reduced in PIH (Rakoczi et al., 1979) and this is thought to be secondary to intravascular platelet aggregation and increased adhesion to damaged vascular endothelium. There is evidence for platelet activation shown by increased β -thromboglobulin, a platelet release product (Douglas et al., 1982; Socol et al., 1985), and decreased intra-platelet serotonin levels (Whigham et al., 1978). Prostacyclin (PGI₂) is the major prostaglandin produced by vascular tissue (Moncada et al., 1977) and is a potent vasodilator and inhibitor of platelet aggregation (Moncada et al., 1976). It has a short half life as it is unstable, and is usually measured as one of its stable metabolites, such as 6-keto-PGF_{1 α} . Over the past few years evidence has accumulated implicating PGI₂ deficiency in the pathophysiology of PIH (see chapter 3.7). Reduced PGI₂ production both from maternal and fetal vascular tissue has been documented in this disease (Remuzzi et al., 1980; Downing et al., 1980; Walsh et al., 1985) and lower levels of amniotic fluid PGI₂ compared to normal pregnancy have also been noted (Bodzenta et al., 1980; Ylikorkala et al., 1981a). More recently reduced plasma levels of PGI₂ metabolites have been reported in eclampsia (Moodley et al., 1984) and in PIH (Yamaguchi and Mori, 1985)

compared to normal pregnancies and lower urinary PGI₂ metabolites have also been reported (Goodman et al., 1982) in PIH.

Thromboxane A₂ (TxA₂) is the major product of arachidonic acid metabolism in platelets and is a potent vasoconstrictor and platelet aggregating agent (Hamberg et al., 1975) which is synthesised and released when platelets are activated. It is highly unstable and is therefore usually measured in plasma as its stable hydration product TxB₂. In view of its properties and the association of platelet consumption with PIH, TxA₂ too may play a role in the pathophysiology of this disease and it has recently been shown that ex vivo platelet TxA₂ production is increased in pregnancies complicated by PIH and intrauterine growth retardation (Wallenburg and Rotmans, 1982), and also that TxA₂ production from placentas of patients with PIH is increased (Walsh, 1985).

Labetalol is an adrenoceptor antagonist which has been used with some success in the treatment of PIH (Walker et al., 1982; Walker et al., 1983; Lardoux et al., 1983; Symonds et al., 1982; Redman, 1982). Some adrenoceptor antagonists including labetalol have inhibitory effects on platelet function and thromboxane A₂ production which have been studied earlier in this thesis (see chapters 5, 7 and 8). Although labetalol inhibits platelet TxA₂ production, vascular prostaglandin production seems to be relatively spared, at least at low drug concentrations (see

chapter 6). This may be beneficial in PIH as further suppression of vascular PGI₂ production in this condition might be detrimental.

The aims of this study were twofold, firstly to perform a cross-sectional study of PGI₂ metabolite (PGI₂M) and TxB₂ in PIH and compare this to normal pregnancy. The second aim was to study the effect of treatment with labetalol on platelet consumption, plasma PGI₂M and TxB₂ in PIH.

12.2 PATIENTS AND METHODS

12.2.1 Cross Sectional Study of PGI₂M and TxB₂ in PIH

Thirty-seven patients with mild/moderate PIH, 6 patients with severe PIH and 40 normal pregnant women in the third trimester were studied, the latter group acting as controls. Mild/moderate PIH was defined as a persistent diastolic blood pressure greater than or equal to 90 mm of mercury after 3 days hospital bed rest in women who had been normotensive in the first trimester. Severe PIH was defined as a persistent diastolic blood pressure of greater than 110 mm of mercury on two occasions at least 8 hours apart, in women who had been normotensive in the first trimester. Proteinuria was not used in the disease classification. The mild/moderate PIH group and the control group were comparable in terms of size, patient age, parity and gestation. The characteristics of the 3 groups are shown in table 12.1. Venous blood was obtained without stasis at the time of diagnosis, for measurement of plasma PGI₂M and TxB₂. No patient had taken any aspirin or any other drug known to interfere with prostaglandin production for at least 2 weeks prior to venepuncture.

TABLE 12.1

	Patient characteristics		
	Normal 3rd Trimester Pregnancy	Mild/Moderate PIH	Severe PIH
n	40	37	6
Primigravid	23 (57.5%)	24 (64.9%)	4 (66.6%)
Parous	17 (42.5%)	13 (35.1%)	2 (33.3%)
Mean (\pm SD) Age (years)	25.2 \pm 5.8	26.1 \pm 5.7	25.3 \pm 5.4
Mean (\pm SD) Gestation at time of first sample (weeks)	35.2 \pm 4.0	33.9 \pm 5.1	29.7 \pm 2.9
Number with significant proteinuria (>0.3 g/24 h)	Nil	3 (8%)	4 (66.6%)
Number with IUGR*	1 (2.5%)	3 (8%)	4 (66.6%)
Live Births	40 (100%)	37 (100%)	6 (100%)

* IUGR: Intra Uterine Growth Retardation

12.2.2 Effect of Labetalol Treatment on PGI₂M and TxB₂

In order to study the effect of treatment with labetalol on plasma PGI₂M and TxB₂, a second blood sample was obtained one week after the initial assessment in 17 of the mild/moderate PIH group who had been randomised to receive either labetalol or bed rest alone, the latter acting as a control group. A second blood sample was also obtained one week after starting treatment in 4 of the severe patients who were all treated with labetalol. Labetalol was given in an initial dose of 200 mg t.i.d. and increased after each 24 hour period if required.

12.2.3 Measurement of PGI₂M and TxB₂

Venous blood was obtained without stasis and prepared as described in chapter 4.1.3. Plasma PGI₂M (immunoreactive 6-keto-PGF_{1α}) and TxB₂ were measured as described in chapter 4.1.3 and 4.1.4 respectively.

All assays were performed "blind" with the operator unaware of the presence or absence of hypertension.

12.2.4 Effect of Labetalol on Platelet Count

One hundred and eight patients with PIH were studied after informed consent had been obtained. No patient had taken any drugs known to affect prostaglandin or thromboxane formation for at least 2 weeks prior to venepuncture. Mild to moderate and severe PIH were defined as above (12.2.1).

Seventy patients with mild/moderate PIH were studied as follows. The patients were divided into primigravid and parous groups prior to randomisation to receive either labetalol or continuation of hospital bed rest, the latter being termed the control group. The proportions of primigravid and parous patients were therefore compatible in both the control and labetalol treated groups. The two groups were also compatible for age and gestation. Mean age was 24.8 ± 5.2 (SD) years in the control group and 26.9 ± 5.9 (SD) years in the labetalol treatment group; mean gestation was 35.1 ± 2.2 (SD) weeks in the control group and 34.9 ± 2.1 (SD) weeks in the treatment group; 37% of the control group and 39% of the labetalol group were parous. Two patients in the control group and 3 patients in the treatment group had significant proteinuria (>0.3 g/24 h). Labetalol was given in an initial dose of 200 mg t.i.d. and increased after each 24 hour period if satisfactory blood pressure control was not obtained.

Thirty-eight patients with severe PIH were studied. All were treated with labetalol, the starting dose was 200 mg t.i.d. and the dose was increased after each 24 hour period if satisfactory control was not obtained. Mean age was 23.1 ± 3.6 (SD) years, mean gestation was 31.6 ± 2.6 (SD) weeks, and 8 of the 38 patients (21 %) were parous, thirty (79%) had significant proteinuria (>0.3 g/24 h).

Venous blood was taken without stasis into edetic acid anti-coagulant for determination of platelet count (Coulter S-plus, Coulter Electronics Ltd., UK) before and one week after entry into the study. The mean of 2 platelet counts was recorded on each occasion.

12.2.5 Statistical Analysis

Statistical analysis was by the Wilcoxon rank sum test for analysis between groups and by the Wilcoxon rank sum test for paired data for analysis within groups. The Spearman rank test was used to assess correlation between PGI₂M and TxB₂.

12.3 RESULTS

The results of the cross-sectional study of PGI₂M and TxB₂ in mild/moderate and severe PIH compared to normal third trimester pregnancy are shown in figures 12.1 and 12.2. As can be seen 19 of the 35 patients with mild/moderate PIH and 5 of the 6 patients with severe PIH had unrecordable levels (<5 pg/ml) of PGI₂M (figure 12.1). No patient in the normal third trimester pregnancy group had unrecordable levels of PGI₂M. The levels of PGI₂M in both the mild/moderate and severe PIH groups were significantly different ($p < 0.001$) from those in the normal group.

There was no significant difference in TxB₂ between any of the 3 groups (figure 12.2) although there was a trend towards higher levels of TxB₂ in the mild/moderate group compared to the normal group.

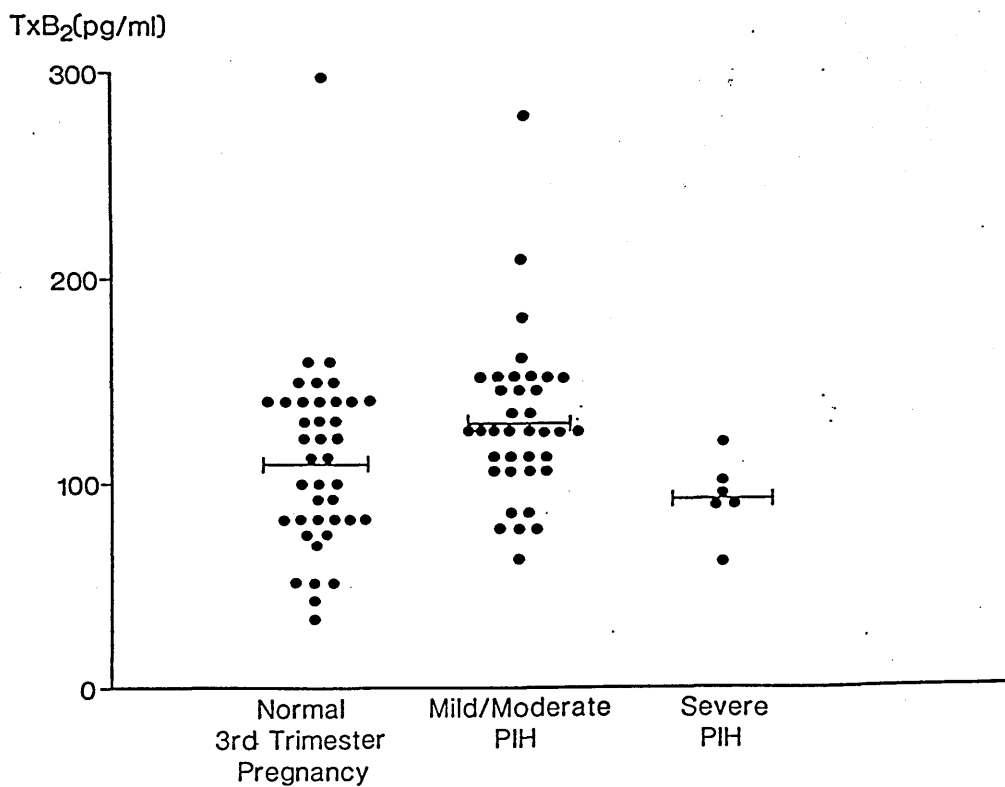


FIGURE 12.2

Plasma TxB₂ in mild/moderate and severe PIH compared to normal third trimester pregnancy. (Horizontal bars indicate the mean value for each group)

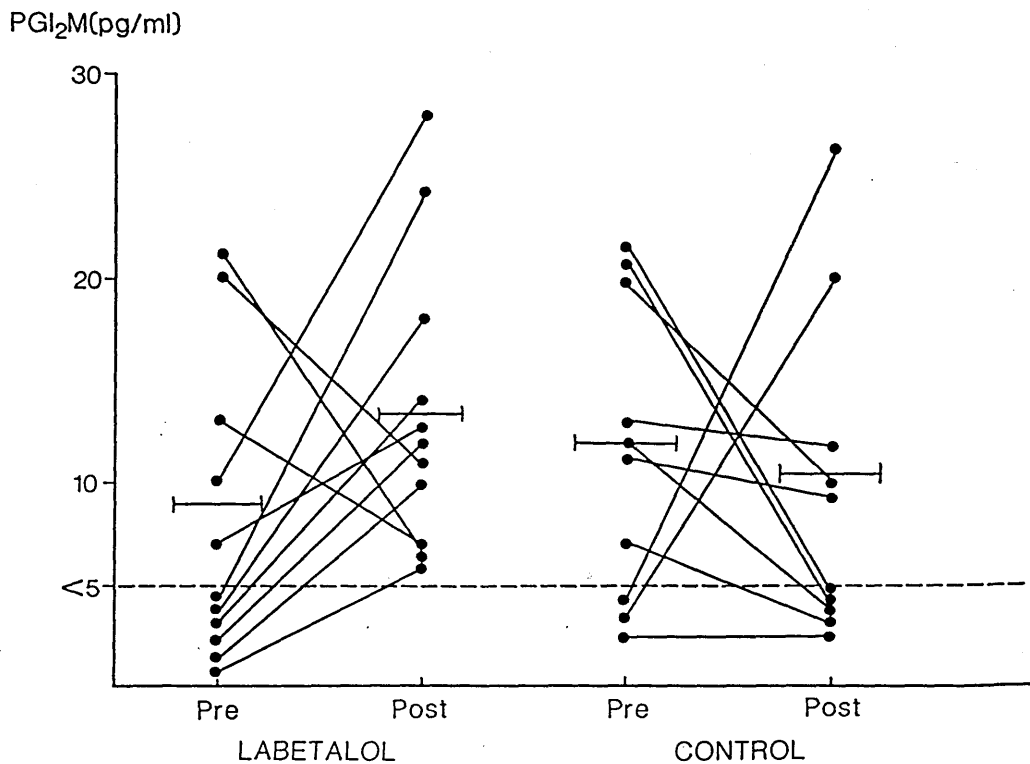


FIGURE 12.3

Effect of labetalol treatment or bed rest alone (control group) on plasma PGI₂M in PIH. The labetalol group consisted of 4 patients with severe PIH and 7 patients with mild/moderate PIH. All patients in the control group had mild/moderate PIH. "Pre" indicates sample taken at diagnosis; "Post" indicates sample taken after 7 days treatment. (Horizontal bars indicate the mean value for each group, and the broken line indicates the lower limit of detection for the assay.)

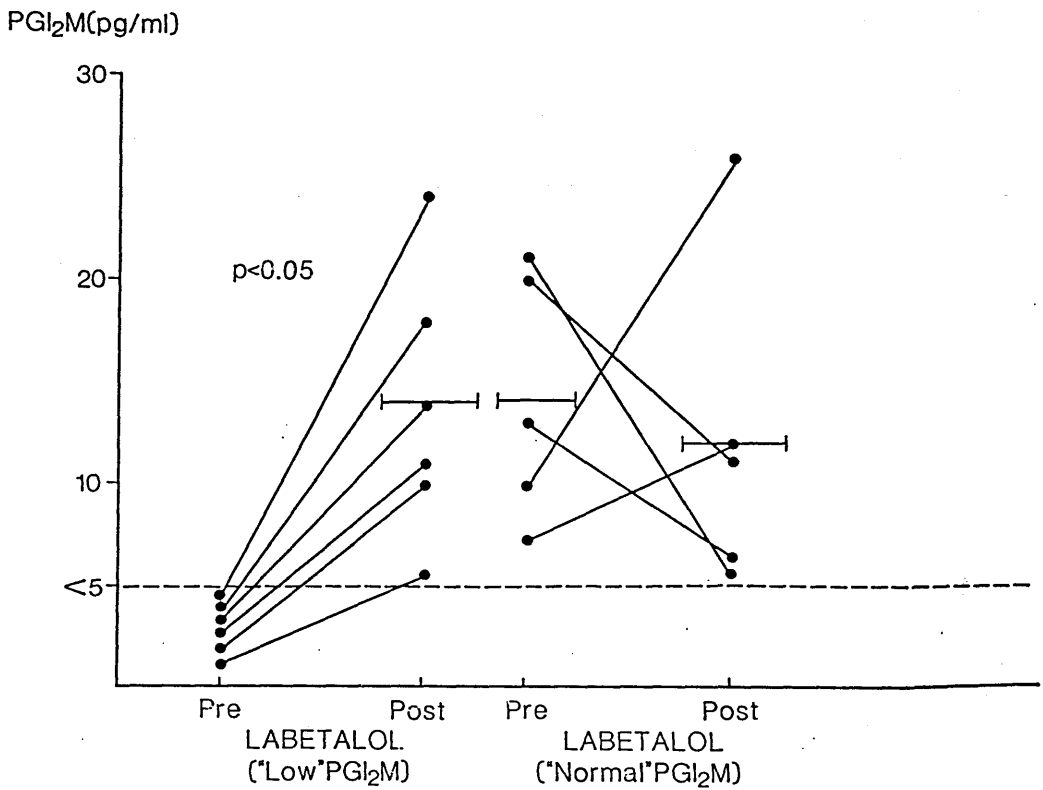


FIGURE 12.4

Effect of labetalol treatment on plasma PGI₂M according to initial level of PGI₂M at diagnosis. "Pre" indicates sample taken at diagnosis; "Post" indicates sample taken following 7 days labetalol therapy. (Horizontal bars indicate the mean values, and the broken line indicates the lower limit of sensitivity for the assay.)

TABLE 12.2

Effect of labetalol treatment on plasma TxB₂ in PIH
(All values are mean ± SEM)

	Control group (bed rest alone)		Labetalol treated group	
	Day 0	Day 7	Day 0	Day 7
Plasma TxB ₂ (pg/ml)	93 ± 11.1	98.3 ± 13.4	116.2 ± 20.0	119.3 ± 24.0
n	10		11	

The effects of labetalol treatment on plasma PGI₂M are shown in figure 12.3. As can be seen, there was no significant overall change in the labetalol treated group, however, following treatment no patient had unrecordable levels of PGI₂M. While in the bed rest control group, although again there was no significant overall change, 1 patient's PGI₂M remained unrecordable and a further 4 developed unrecordable levels, a feature not seen in the labetalol treated group. It should be noted that the labetalol treated group contained 4 patients with severe disease, while the control group consisted entirely of patients with mild/moderate PIH. Three of the 4 patients with severe disease had initially unrecordable levels of PGI₂M which rose following therapy, the other severe patient started with a recordable level and this also increased.

The PGI₂M response to labetalol treatment has also been illustrated according to initial levels of PGI₂M (figure 12.4). All patients who had initially unrecordable PGI₂M levels increased their level of PGI₂M following treatment. While patients with initial levels of PGI₂M above 5 pg/ml (within the normal range) had no pattern of change, although none of them developed unrecordable levels of PGI₂M.

The results for the effect of labetalol treatment on TxB₂ levels are shown in table 12.2. There was no significant change in TxB₂ either in the treatment or in the control group.

No correlation was found between PGI₂M and TxB₂.

TABLE 12.3

Effect of labetalol on platelet count in PIH (mean \pm SD)

	Mild/moderate PIH bed rest control	Mild/moderate PIH labetalol treated	Severe PIH labetalol treated
Day 0 Platelet count ($\times 10^9/1$)	231.8 ± 60.1	209.9 ± 53.1	181.8+ ± 59.4
Day 7 Platelet count ($\times 10^9/1$)	209.0** ± 57.0	214.3 ± 61.9	209.1*** ± 60.1
Mean change in Platelet count ($\times 10^9/1$)	-22.8++ ± 35.0	4.4 ± 35.2	27.3 ± 37.8
n	32	38	38

** p < 0.005) compared to day 0 measurement in same group

*** p < 0.001)

+ p < 0.05 compared to day 0 measurement in mild/moderate groups

++ p < 0.01 compared to change in mild/moderate PIH group treated with labetalol

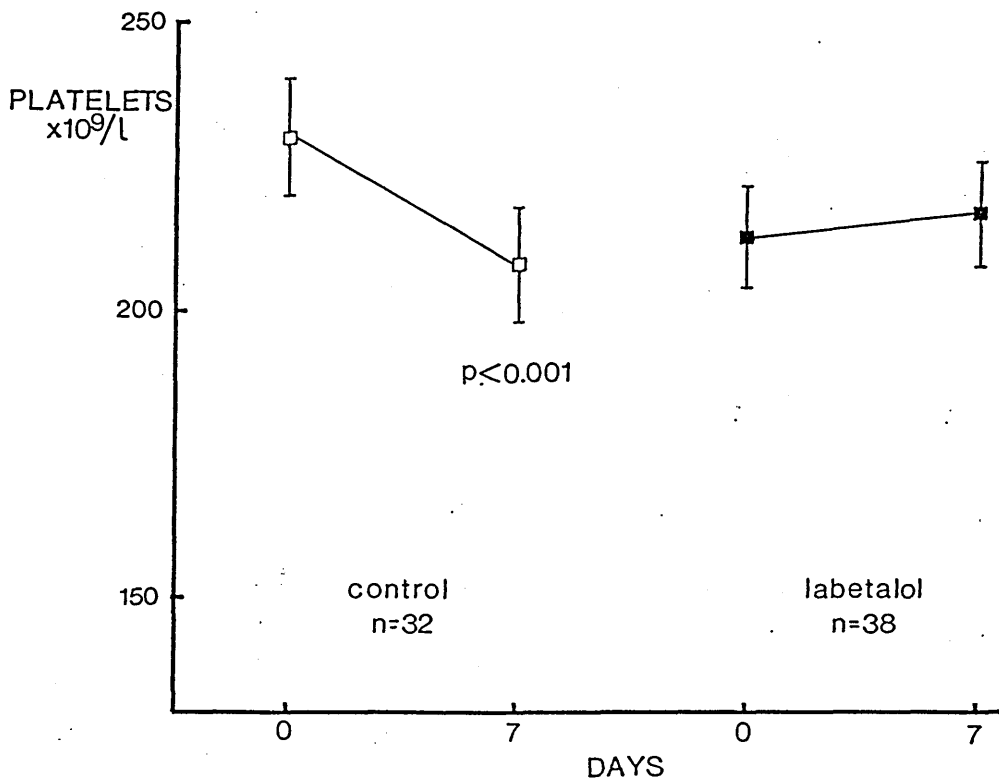


FIGURE 12.5

Change in platelet count following treatment with labetalol or bed rest alone (control) in mild/moderate PIH. (Values shown are mean \pm SEM)

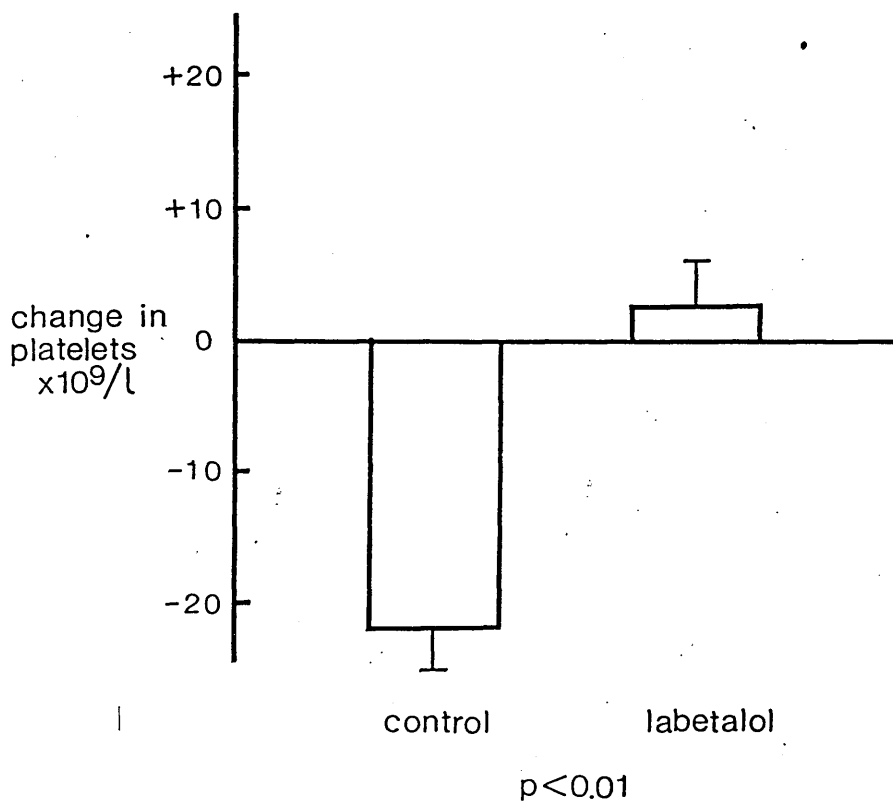


FIGURE 12.6

Change in platelet count in the labetalol treated mild/moderate PIH group compared with change in platelet count in the bed rest control group. (Values shown are mean ± SEM)

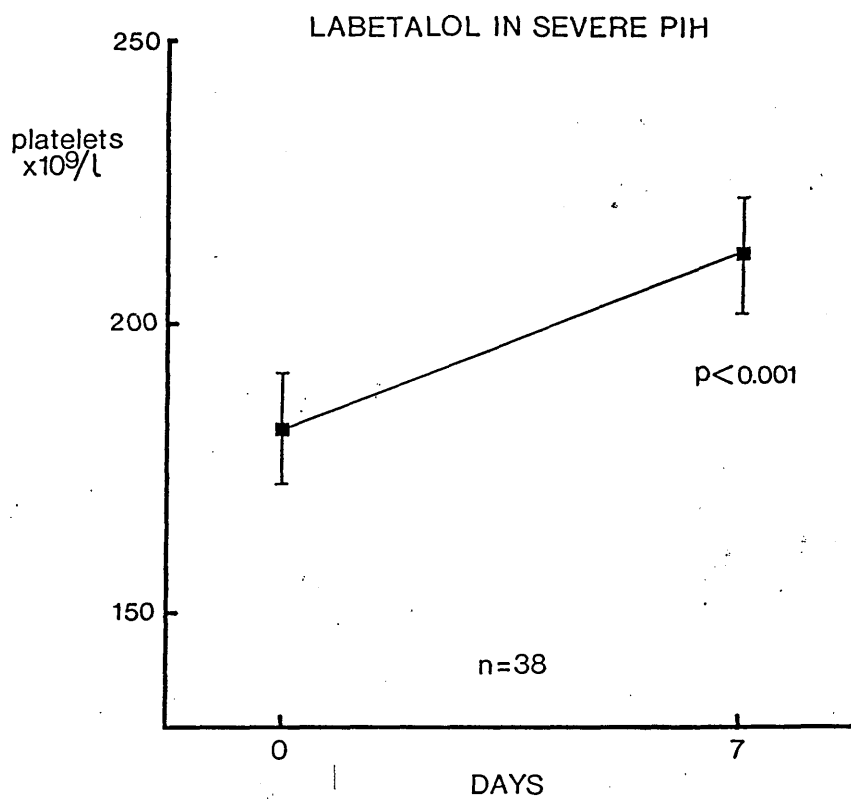


FIGURE 12.7

Change in platelet count over one week in patients with severe PIH treated with labetalol. (Values shown are mean \pm SEM)

Results for the effect of labetalol treatment on platelet count are given in table 12.3 and illustrated in figures 12.5 and 12.6. There was a small but statistically significant fall in platelet count in the mild/moderate control group, while there was no change in platelet count in the labetalol treated mild/moderate group. The magnitude of change in the control group was also significantly different ($p < 0.01$) from the magnitude of change in the labetalol treated mild/moderate group (table 12.3 and figure 12.6). There was no significant difference in the baseline or day 7 platelet counts between these two mild/moderate PIH group.

In the severe PIH group, baseline platelet count was significantly lower than in the mild/moderate groups. Platelet count rose significantly after one week of treatment with labetalol (table 12.3) and was not significantly different from either of the mild/moderate PIH groups on day 7 (table 12.3). This is illustrated in figure 12.7.

12.4 DISCUSSION

This study has shown that low levels of PGI₂M occur in PIH, with over 50% of patients in this study having unrecordable (<5 pg/ml) levels of this metabolite, a feature not seen in any of the normal third trimester control group. This did not seem to be related to disease severity as 19 of 35 patients with mild/moderate disease and 5 of 6 patients with severe disease had unrecordable levels. However, many patients in the mild/moderate group and 1 patient in the severe group had PGI₂M levels within the normal range, suggesting that very low levels of PGI₂M are not always found in PIH.

This finding is consistent with other studies. Yamaguchi and Mori (1985) recently studied 15 women with PIH and found them to have relatively lower levels of plasma 6-keto-PGF_{1α} than normal pregnant women. However, the levels they reported for 6-keto-PGF_{1α} were very high - 318.6 (SE ± 58.0) pg/ml for normal pregnancy and 118.8 (SE ± 16.4) pg/ml for patients with PIH bringing the validity of their assay into question. Moodley et al., (1984) have also reported lower levels of plasma 6-keto-PGF_{1α} in women with eclampsia or impending eclampsia compared to normal pregnancy, but again they reported very high levels of this substance. Not all reports on plasma PGI₂ metabolites in PIH have been consistent as Ylikorkala et al., (1981b) found no change in 22 patients with PIH compared to normal pregnancy, and Strickland et al., (1984) reported higher levels of 6-keto-PGF_{1α} in PIH.

The findings of this present study, however, are also in keeping with the work of Goodman et al., (1982) who studied 6 patients with PIH and found them to have lower levels of urinary PGI₂ metabolites. The reports of reduced amniotic fluid PGI₂ in PIH (Bodzenta et al., 1980; Ylikorkala et al., 1981a) and reduced PGI₂ production from both maternal (Bussolino et al., 1980) and fetal (Walsh et al., 1985; Downing et al., 1980) vascular tissue are also in keeping with the findings of the low PGI₂M in PIH seen in the present study.

In addition, the results of this cross-sectional study confirm the findings of the prospective study of PGI₂M described in chapter 11, which showed that 6 patients who became hypertensive during pregnancy developed unrecordable levels of PGI₂M.

Low levels of PGI₂ may well play a role in the pathophysiology of PIH, as a deficiency of this vasodilator and antiplatelet prostaglandin might allow the vasoconstriction and platelet consumption, which are characteristic of PIH, to go unchallenged.

PGI₂ may also be important in balancing the pressor effects of angiotensin II in normal pregnancy (see chapter 3.7). Normally pregnancy is associated with a relative insensitivity to the effects of exogenous angiotensin II infusion (Chesley, 1966; Talledo et al., 1968) and this insensitivity can be enhanced by PGI₂ infusion (Broughton Pipkin et al., 1984) and reduced by inhibitors of prostaglandin synthesis (Everett et al., 1978a). This suggests that a vasodepressor prostaglandin such as PGI₂ is responsible for this phenomenon. In PIH the insensitivity is lost and patients become very sensitive to the effects of angiotensin II (Gant et al., 1973). The reduced levels of PGI₂ in PIH seen in the present study may explain this sensitivity and this is another mechanism by which PGI₂ deficiency could contribute to the disease process in PIH.

A cause and effect relationship for low PGI₂ and PIH cannot be concluded from this present study, and PGI₂ deficiency is unlikely to be the primary cause of the disease. However, PGI₂

deficiency may occupy a pivotal role in the pathophysiology of PIH, by increasing vascular sensitivity and allowing vasoconstriction and platelet consumption to occur unopposed.

As PGI₂ deficiency may be an important feature in the pathophysiology of PIH, replacement of PGI₂ might be of value in the treatment of this disease. It is now possible to give PGI₂, which is commercially available, and it has been shown to be of benefit in the treatment of peripheral vascular disease, and Raynaud's phenomenon (Belch et al., 1983a; Belch et al., 1983b). There have only been two reports of PGI₂ therapy in PIH (Fidler et al., 1981; Belch et al., 1985) and both studies looked at very severe patients. PGI₂ therapy reduced platelet consumption and helped lower blood pressure, but as both studies were on small numbers of very severe patients it is difficult to know what impact PGI₂ therapy made on outcome of the disease, however, the results on these studies were sufficiently encouraging to warrant further study. The problems of PGI₂ therapy are that the substance is unstable and has to be given by intravenous infusion, in addition the dose is limited by vasodilator side-effects such as flushing, headache and postural hypotension.

An alternative to giving PGI₂ would be to use a drug which might reduce platelet consumption and promote vasodilatation by other means, thereby possibly compensating for the lack of PGI₂. An adrenoceptor antagonist such as labetalol may be such a drug as it has a direct vasodilator action through its α_1 adrenoceptor antagonist properties, and has also been shown to have inhibitory effects on platelet aggregation and TxA₂ production in vitro (see

chapters 5, 7 and 8) while having minimal effects on vascular PGI₂ production (see chapter 6). It has also been shown to be synergistic with PGI₂ in vitro (see chapters 6 and 7) in inhibiting platelet function and this may occur in the in vivo situation.

The results of the present study suggest that labetalol may increase PGI₂M levels if they are initially low. This is an unexpected finding as labetalol probably inhibits the phospholipase enzymes (see chapter 5) which should reduce arachidonic acid availability and subsequent prostaglandin formation. The effect of labetalol and other adrenoceptor antagonists on vascular PGI₂ production were studied in Chapter 6, and no stimulatory effect on PGI₂ production was noted. This suggests that if labetalol does stimulate PGI₂ production in vivo it is not by a direct mechanism and may be secondary to some other aspect of the drug's action in vivo.

This study did not show any increased levels of plasma TxB₂ in PIH, although there was a trend towards higher levels of TxB₂ in the mild/moderate group. This is in agreement with Yamaguchi and Mori (1985) and Mitchell et al., (1978) who studied plasma TxB₂ in PIH and also found no significant increase. It has been shown, however, that placentas from patients with PIH produce increased amounts of TxA₂ (Walsh, 1985) and also that ex vivo platelet TxA₂ production is increased in PIH (Wallenburg and Rotmans, 1982). Since this present study found no difference in peripheral plasma levels of TxB₂, it may be that this increased

production of TxA_2 is apparent only at local situations such as in the placental or renal microvasculature where platelet activation and deposition are known to occur.

In this study platelet count was maintained in the mild/moderate PIH group treated with labetalol and significantly increased in the severe group treated with labetalol. Normally in PIH platelet count falls as gestation progresses (Redman et al., 1978), as seen in the mild/moderate control group. This suggests that labetalol therapy reduces platelet consumption in PIH. Such a reduction in platelet consumption may help maintain utero-placental blood flow, as platelet aggregates are thought to be involved in the thrombotic occlusion of uteroplacental arteries seen in this disease. Platelet activation also occurs within the kidney, damaging the microvasculature and impairing renal function. Labetalol has been shown to have a protective effect on renal function in PIH (Walker et al., 1982) and this may be a result of the platelet protective effects of the drug which have been described in Chapters 5, 7 and 8 of this thesis.

In conclusion, this study has provided further evidence showing that PGI_2 may be reduced in PIH, and this deficiency may contribute to the vascular sensitivity, platelet consumption and vasoconstriction seen in the disease. This study also suggests that labetalol may increase PGI_2 if levels are low, however, the mechanism behind this is obscure and further studies are required to confirm or refute this observation. In addition, this study has shown that labetalol therapy can reduce platelet consumption especially in severe PIH. This effect may be related to the

anti-platelet effects of this drug. Since platelets play an important role in the pathophysiology of PIH, such an effect may be of great value in the treatment of this condition, and may compensate for the reduced PGI₂ production which is also a feature of the disease.

CONCLUSIONS

Pregnancy induced hypertension (PIH) remains a major cause of fetal and maternal mortality and morbidity, both from the effects of the disease itself and also from premature delivery of the fetus. It is a disease characterised by vasoconstriction, platelet activation and consumption, and sensitivity to angiotensin II, although the precise aetiology remains obscure.

In the last 10 years two new products of the arachidonic acid cascade have been identified, prostacyclin (PGI₂) and thromboxane (TxA₂). Prostacyclin is a powerful vasodilator and anti-platelet prostaglandin and is the major product of the arachidonic acid cascade in vascular tissue. Deficiency of such a prostaglandin may result in platelet activation, vasoconstriction and sensitivity to angiotensin II - the very features of PIH. Evidence is accumulating which suggests that PIH is associated with a deficiency of PGI₂ production. Although this is not the primary cause of the disease it may play an important role in the pathophysiology of the condition.

Thromboxane A₂ is a powerful vasoconstrictor and platelet aggregating agent, it is the major product of the arachidonic acid cascade in platelets, and is synthesised and released when platelets aggregate. It has been suggested that normally a balance exists between PGI₂ and TxA₂, and that this balance may be upset in PIH. Increased TxA₂ production from placentas and platelets has been shown to occur in PIH, and this too may contribute to the pathophysiology of the disease.

In recent years adrenoceptor antagonists have been used with some success in the treatment of PIH. The aims of this thesis were firstly to study the effects of a variety of adrenoceptor antagonists on platelet function, TxA₂ and PGI₂ production in vitro and secondly, to study plasma levels of PGI₂ and TxA₂ metabolites in normal pregnancy and PIH, and to assess the effect of treatment with an adrenoceptor antagonist on levels of these substances and platelet consumption in PIH.

The in vitro studies described in this thesis using platelet rich plasma have shown that the adrenoceptor antagonists labetalol, pindolol and propranolol inhibit platelet aggregation and TxA₂ generation. These effects may be due to inhibition of arachidonic acid release from the platelet membrane phospholipids. This property is independent of any α or β adrenoceptor blocking effect as other adrenoceptor antagonists such as atenolol, metoprolol, timolol and prazosin were without effect. The inhibitory action of these drugs on platelet function and TxA₂ production may be related to membrane stabilising activity or lipid solubility, properties which are common to all three effective agents.

Labetalol, pindolol and propranolol were found to have little effect on vascular PGI₂ production, but acted synergistically with prostacyclin to inhibit platelet aggregation in platelet rich plasma.

Recently, it has become possible to study platelet aggregation in whole blood. This may be a more physiological model than traditional platelet rich plasma techniques, as it leaves platelets in their natural milieu with red cells and white cells present, which can themselves influence platelet behaviour by release of ADP and PGI₂ respectively. Using this technique, adrenoceptor antagonists were found to inhibit platelet aggregation and TxA₂ production in whole blood, and were synergistic with PGI₂ in inhibiting platelet aggregation.

Calcium channel blocking agents have recently become established in the treatment of hypertension and ischaemic heart disease, and studies are beginning to emerge on the use of these drugs in the treatment of PIH. The effect of a typical calcium channel blocking agent, nifedipine, on platelet function was therefore studied in vitro. Nifedipine inhibited platelet aggregation in whole blood, and was synergistic with prostacyclin in inhibiting aggregation, but had no effect on TxA₂ production from whole blood or on PGI₂ production from vascular tissue.

Previous in vitro studies have suggested that calcium channel blocking agents and adrenoceptor antagonists can only inhibit platelet function at supra-pharmacological concentrations which cannot be obtained in vivo, yet both types of drug have been shown to possess anti-platelet properties in vivo. This discrepancy suggests that platelets in vivo may be more sensitive than platelets in vitro to the effects of such drugs. This might be explained by the synergy of these agents with PGI₂ which may occur in vivo, enhancing their effects on platelets.

Aspirin has become established as an anti-platelet agent, and has been used with some success in the secondary prevention of myocardial infarction, threatened stroke and more recently in PIH and intra-uterine growth retardation. There is, however, an aspirin dilemma, as although it is a powerful anti-platelet agent, it also inhibits vascular prostacyclin production, which is at least potentially harmful. In an attempt to avoid this problem low dose aspirin has been used as this might selectively inhibit platelet function. The effects of a combination of low dose aspirin and either an adrenoceptor antagonist or calcium channel blocking agent were studied in this thesis. At doses of aspirin and adrenoceptor antagonist or calcium channel blocking agent which on their own had minimal effects, a combination of both drugs resulted in over 50% inhibition of platelet aggregation in whole blood. Such synergy may be useful in the clinical situation allowing low dose aspirin to function as an effective anti-platelet agent.

Whether the anti-platelet properties of the drugs which have been described in this thesis are of clinical value remains to be established. Long term clinical evaluation is required to study the effects of these drugs on PGI₂, TxA₂ and platelet behaviour in vivo, and to determine whether any such properties influence the disease outcome. If found to be effective these drugs would be of value not only in PIH but also in other diseases associated with platelet activation such as ischaemic heart disease.

A recent study has shown that aspirin can improve prognosis following myocardial infarction, especially when combined with an adrenoceptor antagonist. It is tempting to speculate that this cardioprotective effect may be due to the combined inhibitory effects of aspirin and adrenoceptor antagonists on platelet function which have been described in this thesis, but further clinical evaluation of this possibility is required.

Plasma levels of prostacyclin and thromboxane metabolites in normal pregnancy and PIH were also studied in this thesis. In the normal situation plasma prostacyclin metabolites were found to be increased in the first trimester, this may be important in implantation, allowing the trophoblast to escape immobilisation by platelet aggregates as it invades the maternal uterine circulation.

In PIH plasma prostacyclin metabolites (PGI_2M) were found to be reduced. This is in keeping with previous studies, and provides further evidence implicating prostacyclin deficiency in the pathogenesis of this disease. Peripheral plasma levels of TxB_2 - the stable hydration product of TxA_2 - were not significantly different from normal pregnancy levels in PIH. This suggests that if TxA_2 production is increased in PIH it may only be detectable at sites of platelet activation such as the renal and placental microvasculature, and does not occur generally throughout the systemic circulation.

The effects of the adrenoceptor antagonist labetalol on plasma levels of PGI₂M and TxB₂, and platelet consumption in PIH were also studied. When initial plasma levels of PGI₂M were low labetalol therapy seemed to increase levels, but the mechanism behind this is unclear and further studies are required to confirm or refute this observation. Labetalol therapy had no effect on plasma TxB₂ in PIH. However, labetalol therapy significantly reduced platelet consumption especially in severe PIH suggesting that the anti-platelet properties of this drug which were studied in vitro may well occur in vivo. In view of the contribution of platelets to the pathophysiology of PIH, reduction of platelet consumption may be of value in the treatment of this condition.

Future studies are required to examine the effects of adrenoceptor antagonists and calcium channel blocking agents on platelet function and TxA₂ and PGI₂ production in vivo, and to determine whether any beneficial effects will influence the natural course of the disease in patients suffering from PIH. The combination of low dose aspirin and an adrenoceptor antagonist also requires urgent clinical evaluation and may offer an exciting new therapeutic option in the treatment of PIH.

Abitbol, M.M. (1977) Aortic compression and uterine blood flow during pregnancy. Obstetrics and Gynaecology, 50, 562 - 570.

Addonizio, V.P., Fisher, C.A., Strauss, J.F. and Edmunds, L.H. (1982) Inhibition of human platelet function by verapamil. Thrombosis Research, 28, 545 - 556.

Alessandrini, P., Avogaro, P., Bon, B., Patrignani, P. and Patrono, C. (1985) Physiologic variables affecting thromboxane B₂ production in human whole blood. Thrombosis Research, 37, 1 - 8.

Ali, M. and McDonald, J.W.D. (1978) Reversible and irreversible inhibition of platelet cyclo-oxygenase and serotonin release by non-steroidal anti-inflammatory drugs. Thrombosis Research, 13, 1057 - 1065.

Anturane Reinfarction Trial Research Group (1980) Sulphinpyrazone in the prevention of sudden death after myocardial infarction. New England Journal of Medicine, 302, 250 - 256.

Anturan Reinfarction Italian Study Group (1982) Sulfinpyrazone in postmyocardial infarction. Lancet, 1, 237 - 242.

Armstrong, J.M., Lattimer, N., Moncada, S. and Vane, J.R. (1978) Comparison of the vasodepressor effects of prostacyclin and 6-oxo-PGF_{1α} with those of prostaglandin E₂ in rats and rabbits. British Journal of Pharmacology, 62, 125 - 130.

Ashford, T.P. (1969) The role of endothelium in the resolution of a thrombus. In Thrombosis, ed. Sherry, S., Brinkhouse, K., Genton, E. and Stengle, J.M. p 447, Washington D.C.:National Academy of Sciences.

Aspirin Myocardial Infarction Study Research Group (1980) The aspirin myocardial infarction study:final results. Circulation, 62 (Suppl.V), 79 - 84.

Baenziger, N.L., Becherer, P.R. and Majerus, P.W. (1980) Characterization of prostacyclin synthesis in cultured human arterial smooth muscle cells, venous endothelial cells and skin fibroblasts. Cell, 16, 967 - 974.

Baird, D. (1977) Epidemiological aspects of hypertensive pregnancy. Clinics in Obstetrics and Gynaecology, 4, 531 - 548.

Bakhle, Y.S. (1983) Synthesis and catabolism of cyclo-oxygenase products. British Medical Bulletin, 39, 214 - 218.

Bangham, A.D. (1961) A correlation between surface charge and coagulant action of phospholipids. Nature, 192, 1197 - 1198.

Baumgartner, H.R. (1973) The role of blood flow in platelet adhesion, fibrin deposition, and formation of mural thrombi. Microvascular Research, 5, 167 - 179.

Baumgartner, H.R., Tschopp, T.B. and Meyer, D. (1980) Shear rate dependent inhibition of platelet adhesion and aggregation on collagenous surfaces by antibodies to human factor VIII/von Willebrand factor. British Journal of Haematology, 44, 127 -139.

Beattie, T.J., Murphy, A.V., Willoughby, M.L. and Belch, J.J.F. (1981) Prostacyclin infusion in haemolytic-uraemic syndrome of children. British Medical Journal, 283, 470.

Beaufils, M., Uzan, S., Donsimoni, R. and Colau, J.C. (1985) Prevention of pre-eclampsia by early anti-platelet therapy. Lancet, 1, 840 - 842.

Belch, J.J.F., Newman, P., Drury, J.K., Capell, H., Leiberman, P., James, W.B., Fobes, C.D. and Prentice, C.R.M. (1981) Successful treatment of Raynaud's syndrome with prostacyclin. Thrombosis and Haemostasis, 45, 255 - 256.

Belch, J.J.F., McKay, A., McArdle, B., Leiberman, P., Pollock, J.G., Lowe, G.D.O., Forbes, C.D. and Prentice, C.R.M. (1983a) Epoprostenol (prostacyclin) and severe arterial disease. A double blind trial. Lancet, 1, 315 - 317.

Belch, J.J.F., Newman, P., Drury, J.K., McKenzie, F., Capell, H., Leiberman, P., Forbes, C.D. and Prentice, C.R.M. (1983b) Intermittent poprostenol (prostacyclin) infusion in patients with Raynaud's syndrome. A double blind controlled trial. Lancet, 1, 313 - 315.

Belch, J.J.F., Greer, I.A., McLaren, M., Walker, J., Forbes, C.D. (1983c) Measurement of prostacyclin metabolites. Lancet, ii, 1504.

Belch, J.J.F., Thorburn, J., Greer, I.A., Sarfo, S. and Prentice, C.R.M. (1985) Intravenous prostacyclin in the management of pregnancies complicated by severe hypertension. Clinical and Experimental Hypertension Part B - Hypertension in Pregnancy, B4, 75 - 86.

Bennett, J.S. and Vilaire, G. (1979) Exposure of platelet fibrinogen receptors by ADP and epinephrine. Journal of Clinical Investigation, 64, 1393 - 1401.

Bergstrom, S., Danielsson, H. and Samuelsson, B. (1964) The enzymatic formation of prostaglandin E₂ from arachidonic acid. Biochemica et Biophysica Acta, 90, 207 - 210.

Bergstrom, S. (1967) Isolation, structure and action of the prostaglandins. In Prostaglandins, Nobel Symposium 2, ed. Bergstrom, S. and Samuelsson, B. pp 21 - 30. New York: Interscience Publishers.

Bertele, V., Cerletti, C., Schieppati, A., diMinno, G. and DeGaetano, G. (1981) Inhibition of thromboxane synthetase does not necessarily prevent platelet aggregation. Lancet, 1, 1057 -1058.

Blackwell, G.J., Flower, R.J., Russell-Smith, N., Salmon, J.A., Thorogood, P.B., Vane, J.R. (1978) Prostacyclin is produced in whole blood. British Journal of Pharmacology, 64, 436P.

Blackwell, G.J. and Flower, R.J. (1983) Inhibition of phospholipase. British Medical Bulletin, 39, 260 - 264.

Blair, I.A., Barrow, S.E., Waddell, K.A., Lewis, P.J. and Dollery, C.T. (1982) Prostacyclin is not a circulating hormone in man. Prostaglandins, 23, 579 - 589.

Blajchman, M.A., Senyi, A.F., Hirsh, J., Surya, Y., Buchanan, M. and Mustard, J.F. (1979) Shortening of the bleeding time in rabbits by hydrocortisone caused by inhibition of prostacyclin generation by the vessel wall. Journal of Clinical Investigation, 63, 1026 - 1035.

Bodzenta, A., Thomson, J.M. and Poller, L. (1980) Prostacyclin activity in amniotic fluid in pre-eclampsia. Lancet, 2, 650.

Bolton, P.J., Jogee, M., Myatt, L. and Elder, M.G. (1981) Maternal plasma 6-oxo-prostaglandin $F_{1\alpha}$ levels throughout pregnancy: a longitudinal study. British Journal of Obstetrics and Gynaecology, 88, 1101 - 1103.

Bonnar, J., McNicol, G.P. and Douglas, A.S. (1970) Coagulation and fibrinolytic mechanisms during and after normal childbirth. British Medical Journal, ii, 200 - 203.

Bonnar, J., McNicol, G.P. and Douglas, A.S. (1971) Coagulation and fibrinolytic systems in pre-eclampsia. British Medical Journal, 2, 12 - 16.

Bonnar, J. and Shepherd, B.L. (1981) Coagulation activation and local vascular changes in pre-eclampsia. In Kidney and Pregnancy Contributions to Nephrology 25. ed. Brod, J. pp 98 -107. Basel:Karger.

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

Born, G.V.R. (1970) Observations on the change in shape of blood platelets brought about by adenosine diphosphate. Journal of Physiology, 209, 487 - 511.

Born, G.V.R. and Cross, M.J. (1963) The aggregation of blood platelets. Journal of Physiology, 168, 178 - 195.

Born, G.V.R. and Hume, M. (1967) Effects of the numbers and sizes of platelet aggregates on the optical density of plasma. Nature, 215, 1027 - 1029.

Born, G.V.R. and Wehmeier, A. (1979) Inhibition of platelet thrombus formation by chlorpromazine acting to diminish haemolysis. Nature, 282, 212 - 213.

- Brandt, R., Seppala, E., Nowak, J. and Vapaatalo, H. (1984) Effect of propranolol, practolol and atenolol on human platelet thromboxane formation and plasma levels of prostaglandins 6-keto-F_{1α} and E₂. Prostaglandins, Leukotrienes and Medicine, 16, 191 - 203.
- Braunwald, E. (1982) Mechanism of action of calcium-channel-blocking agents. New England Journal of Medicine, 307, 1618 -1627.
- Briel, R.C., Kieback, D.G. and Lippert, T.H. (1984) Platelet sensitivity to a prostacyclin analogue in normal and pathological pregnancy. Prostaglandins, Leukotrienes and Medicine, 13, 335 -340.
- Broughton Pipkin, F., Hunter, J.C., Turner, S.R. and O'Brien, P.M.S. (1982) Prostaglandin E₂ attenuates the pressor response to angiotensin II in pregnant subjects but not in nonpregnant subjects. American Journal of Obstetrics and Gynecology, 142, 168 - 176.
- Broughton Pipkin, F., Morrison, R., O'Brien, P.M.S. (1984) Effects of prostacyclin on the pressor response to angiotensin II in human pregnancy. European Journal of Clinical Investigation, 14, 3.
- Brown, J.J., Davies, D.L., Doak, P.B., Lever, A.F., Robertson, J.I.S. and Trust, P. (1965) Plasma renin concentration in hypertensive disease of pregnancy. Lancet, 2, 1219.
- Bunting, S., Gryglewski, R., Moncada, S. and Vane, J.R. (1976) Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. Prostaglandins, 12, 897 - 913.
- Bunting, S., Moncada, S. and Vane, J.R. (1976) The effects of prostaglandin endoperoxides and thromboxane A₂ on strips of rabbit coeliac artery and other smooth muscle preparations. British Journal of Pharmacology, 57, 462.
- Bunting, S., Moncada, S. and Vane, J.R. (1983) The prostacyclin-thromboxane balance: pathophysiological and therapeutic implications. British Medical Bulletin, 39, 271 -276.
- Burch, J.W., Baenziger, N., Stanford, N. and Majerus, P.W. (1978) Sensitivity of fatty acid cyclo-oxygenase from human aorta to acetylation by aspirin. Proceedings of the National Academy of Sciences of the U.S.A., 75, 5181 - 5184.
- Bussolino, F., Benedetto, C., Massobrio, M. and Camussi, G. (1980) Maternal vascular prostacyclin activity in pre-eclampsia. Lancet, 2, 702.
- Butler, N.R. and Bonham, D.G. (1963) Perinatal Mortality. pp 86 - 100, Edinburgh: E. and S. Livingstone.

Butler, N.R. and Alberman, E.D. (1969) Perinatal problems: Second Report of the 1958 British Perinatal Mortality Survey. Edinburgh: E. and S. Livingstone.

Campbell, W.B., Johnson, A.R., Callahan, K.S. and Graham, R.M. (1981a) Anti-platelet activity of beta-adrenergic antagonists: Inhibition of thromboxane synthesis and platelet aggregation in patients receiving long term propranolol treatment. Lancet, 2, 1382 - 1384.

Campbell, W.B., Callahan, K.S., Johnson, A.R. and Hirsch, P.D. (1981b) Mechanism of the anti-platelet activity of beta adrenergic antagonists. Progress in Clinical Pharmacology, 3, 117 - 126.

Canadian Co-operative Study Group (1978) A randomised trial of aspirin and sulphindiazole in threatened stroke. New England Journal of Medicine, 299, 53 - 59.

Carreras, L.O., Defreyn, G., van Houtte, E., Vermeylen, J. and van Assche, A. (1981) Prostacyclin and pre-eclampsia. Lancet, 1, 442.

Cavanagh, D., Rao, P.S., Tsai, C.C. and O'Connor, T.C. (1977) Experimental toxemia in the pregnant primate. American Journal of Obstetrics and Gynecology, 128, 75 - 85.

Cazenave, J.-P., Packham, M., Kinlough-Rathbone, R.L. and Mustard, J.F. (1978) Platelet adherence to the vessel wall and to collagen-coated surfaces. In Thrombosis: Animal and Clinical Models, ed. Day, H.J., Molony, A., Nishizawa, E.E. and Rynbrandt, R.H. pp 31 - 49. New York: Plenum Press.

Chamberlain, G. Philipp, E., Howlett, B. and Masters, K. (1978) British Births 1970, pp 80 - 107. London: E. and S. Livingstone.

Chamberlain, G.V.P., Lewis, P.J. de Swiet, M. and Bulpitt, C.J. (1978) How obstetricians manage hypertension in pregnancy. British Medical Journal, 1, 626 - 629.

Chesebro, J.H., Clements, I.P., Fuster, V. Elveback, L.R., Smith, H.C., Bardsleg, W.T., Frye, R.L., Holmes, D.R., Vlietstra, R.E., Pluth, J.R., Wallace, R.R., Pugh, F.J., Orszulak, T.A., Piehler, J.M., Schaff, H.V. and Danielson, G.K. (1982) A platelet-inhibitor-drug trial in coronary-artery bypass operations: benefit of peri-operative dipyridamole and aspirin therapy on early postoperative vein-graft patency. New England Journal of Medicine, 307, 73 - 78.

Chesley, L.C. (1966) Vascular reactivity in normal and toxemic pregnancy. Clinical Obstetrics and Gynecology, 9, 871 - 881.

Chesley, L.C. (1972) Plasma and red cell volumes during pregnancy. American Journal of Obstetrics and Gynecology, 112, 440 - 450.

Chesley, L.C. (1978) Hypertensive Disorders. New York: Appleton-Century-Crofts.

Chesley, L.C. and Williams, L.O. (1945) Renal glomerular and tubular functions in relation to the hyperuricaemia of pre-eclampsia and eclampsia. American Journal of Obstetrics and Gynecology, 50, 367 - 375.

Chesley, L.C., Annitto, J.E. and Jarvis, D.G. (1947) A study of the interaction of pregnancy and hypertensive disease. American Journal of Obstetrics and Gynecology, 53, 851 - 863.

Chesley, L.C., Valenti, C. and Rein, H. (1958) Excretion of sodium loads by non-pregnant and pregnant normal, hypertensive and pre-eclamptic women. Metabolism, 7, 575 - 588.

Chesley, L.C., Annitto, J.E. and Cosgrove, R.A. (1968) The familial factor in toxemia of pregnancy. Obstetrics and Gynaecology, 32, 303 - 311.

Christ-Hazelhof, F., Nugteren, D.H. (1981) Prostacyclin is not a circulating hormone. Prostaglandins, 22, 739 - 746.

Cockburn, J., Moar, V.A., Ounsted, M. and Redman, C.W.G. (1982) Final report of study on hypertension during pregnancy: the effects of specific treatment on the growth and development of the children. Lancet, i, 647 - 649.

Cohen, P. and McCombs, H.L. (1967) Platelets and atherogenesis. I. Augmentation of cholesterol atherogenesis in the rabbit by a phlebotomy programme designed to induce thrombocytosis. British Journal of Experimental Pathology, 48, 346 - 356.

Coker, S.J. and Parratt, J.R. (1983) Effects of dazoxiben on arrhythmias and ventricular fibrillation induced by coronary artery occlusion and reperfusion in anaesthetised greyhounds. British Journal of Clinical Pharmacology, (Supplement 15), 87S - 96S.

Coker, S.J., Parratt, J.R., Ledingham, M.C.A. and Zeitlin, I.J. (1981) Thromboxane and prostacyclin release from ischaemic myocardium in relation to arrhythmias. Nature, 291, 323 - 324.

Coller, B.S. (1984) Disorders of platelets. In Disorders of Hemostasis. ed. Ratnoff, O.D. and Forbes, C.D. pp 73 - 176. New York:Grune and Stratton.

Condie, R.G. and Ogston, D. (1976) Sequential studies on components of the haemostatic mechanism in pregnancy with particular reference to the development of pre-eclampsia. British Journal of Obstetrics and Gynaecology, 83, 938 - 942.

Coronary Drug Project Research Group (1980) Aspirin in coronary heart disease. - Circulation, 62 (Supplement V), 59 - 62.

Crawford, M.A. (1983) Background to essential fatty acids and their prostanoid derivatives. British Medical Bulletin, 39, 210 - 213.

Cross, M.J. (1964) Effect of fibrinogen on the aggregation of platelets by adenosine diphosphate. Thrombosis et Diathesis Haemorrhagica, 12, 524 - 527.

Crowley, J.G. and Pierce, R.A. (1981) The affinity of platelets for the subendothelium. The American Surgeon, 47, 529 - 532.

Cucuianu, M.P., Nishizawa, E.E. and Mustard, J.F. (1971) Effect of pyrimido-pyrimidine compounds on platelet function. Journal of Laboratory and Clinical Medicine, 77, 958 - 974.

Dachany-Prigent, J., Dufourcq, J., Lussan, C. and Boisseau, M. (1979) Propranolol, chlorpromazine and platelet membrane; a fluorescence study of the drug membrane interaction. Thrombosis Research, 14, 15 - 22.

Dahl, M.L. and Votila, P. (1984) Thromboxane formation during blood clotting is decreased by verapamil. Prostaglandins, Leukotrienes and Medicine, 13, 217 - 218.

Dale, J., Landmark, K.M. and Myhre, E. (1983) The effects of nifedipine, a calcium antagonist, on platelet function. American Heart Journal, 105, 103 - 105.

Davie, E.W. and Ratnoff, O.D. (1964) Waterfall sequence for intrinsic blood clotting. Science, 145, 1310.

Davies, A.M. (1971) Geographical Epidemiology of the Pregnancy Toxemias. Springfield: Charles C. Thomas.

Davies, M.H., Wilkinson, S.P., Hanid, M.A., Portmann, B., Brudenell, J.M., Newton, J.R. and Williams, R. (1980) Acute liver disease with encephalopathy and renal failure in late pregnancy and the early puerperium: a study of fourteen patients. British Journal of Obstetrics and Gynaecology, 87, 1005 - 1014.

Day, H.J., Young, E.B.S. and Helfrich, M.B.S. (1980) An evaluation of a whole blood platelet counter. American Journal of Clinical Pathology, 73, 588 - 593.

Deckmyn, H., Proesmans, W. and Vermynen, J. (1983) Prostacyclin production in whole blood from children: Impairment in the haemolytic uraemic syndrome and excessive formation in chronic renal failure. Thrombosis Research, 30, 13 - 18.

Deckmyn, H., Gresele, P., Arnout, J., Todisco, A. and Vermynen, J. (1984) Prolonging prostacyclin production by nafazotrom or dipyridamole. Lancet, 2, 410 - 411.

van Dorp, D.A., Beerthuis, R.K., Nugteren, D.H. and Vonkeman, H. (1964) The biosynthesis of prostaglandins. Biochimica et Biophysica Acta, 90, 207 - 210.

Douglas, J.T., Shah, M., Lowe, G.D.O., Belch, J.J.F., Forbes, C.D. and Prentice, C.R.M. (1982) Plasma fibrinopeptide A and beta-thromboglobulin in pre-eclampsia and pregnancy hypertension. Thrombosis and Haemostasis, 47, 54 - 55.

Downing, I., Shepherd, G.L. and Lewis, P.J. (1980) Reduced prostacyclin production in pre-eclampsia. Lancet, 2, 1374.

Durao, V., Prata, M.M. and Goncalves, L.M.P. (1977) Modification of anti-hypertensive effect of B-adrenoreceptor blocking agents by inhibition of endogenous prostaglandin synthesis. Lancet, 1005 - 1007.

Dusting, G.J., Moncada, S. and Vane, J.R. (1978) Disappearance of prostacyclin (PGI₂) in the circulation of the dog. British Journal of Pharmacology, 62, 414 - 415P.

Dyerberg, J., Bang, H.O., Stoffersen, E., Moncada, S. and Vane, J.R. (1978) Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. Lancet, 2, 117 - 119.

Dyerberg, J. and Bang, H.O. (1979) Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet, 2, 433 - 435.

Edgar, W., McKillop, C., Howie, P.W. and Prentice, C.R.M. (1977) Composition of soluble fibrin complexes in pre-eclampsia. Thrombosis Research, 10, 567 - 574.

Ehrman, M.L. and Jaffe, E.A. (1980) Prostacyclin inhibits the development in human platelets of ADP and arachidonic acid induced shape change and procoagulant activity. Prostaglandins, 20, 1103 - 1116.

Eika, C. (1972) The platelet aggregating effects of eight commercial heparins. Scandinavian Journal of Haematology, 9, 480 - 482.

Eldor, A., Falcone, D.J., Hajjar, D.P., Minick, C.M. and Weksler, B.B. (1981) Recovery of prostacyclin production by de-endothelialized rabbit aorta. Critical role of neointimal smooth muscle cells. Journal of Clinical Investigation, 67, 735 - 741.

Elwood, P.C. and Sweetnam, P.M. (1980) Aspirin and secondary mortality after myocardial infarction. Circulation, 62 (Supplement V), 53 - 58.

Ellwood, D.A., Anderson, A.B.M., Mitchell, M.D., Murphy, G. and Turnbull, A.C. (1981) Prostanoids, collagenase and cervical softening in the sheep. In The Cervix in Pregnancy and Labour, ed. Ellwood, D.A. and Anderson, A.B.M. pp 57 - 73. Edinburgh:Churchill Livingstone.

von Euler, U.S. (1936) On the specific vasodilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). Journal of Physiology, 88, 213 - 234.

Evans, G., Packham, M.A., Nishizawa, E.E., Mustard, J.F. and Murphy, E.A. (1968) The effect of acetylsalicylic acid on platelet function. Journal of Experimental Medicine, 128, 877 - 894.

Everett, R.B., Worley, R.J., MacDonald, P.C. and Gant, N.F. (1978a) Oral administration of theophylline to modify pressor responsiveness to angiotensin II in women with pregnancy-induced hypertension. American Journal of Obstetrics and Gynecology, 132, 359 - 362.

Everett, R.B., Worley, R.J., MacDonald, P.C. and Gant, N.F. (1978b) Modification of vascular responsiveness to angiotensin II in pregnant women by intravenously infused 5 α -dihydroprogesterone. American Journal of Obstetrics and Gynecology, 131, 352 - 357.

Feely, J., de Vane, P.J. and MacLean, D. (1983) Beta-blockers and sympathomimetics. British Medical Journal, 286, 1043 -1046.

Feinman, R.D. and Detwiler, T.C. (1974) Platelet secretion induced by divalent cation ionophores. Nature, 249, 172 - 173.

Feinstein, M.B. (1978) The role of calcium in blood platelet-function. In Calcium in drug action. ed. Weiss, G. pp 197 - 239. New York:Plenum.

Felding, C.F. (1969) Obstetric aspects in women with histories of renal disease. Acta Obstetrica et Gynecologica Scandinavia, 48 (Supplement 2), 1 - 43.

Fidler, J., Ellis, C., Bennett, M.J., de Swiet, M. and Lewis, P.J. (1981) Prostacyclin and pre-eclamptic toxemia. In Clinical Pharmacology of Prostacyclin, ed. Lewis, P.J. and O'Grady, J. pp 141 - 144. New York:Raven Press.

Firkin, B.G. (1984) The Platelet and Its Disorders. Lancaster:M.T.P. Press.

Fitzgerald, G.A., Oates, J.A., Hawiger, J., Maas, R.L., Roberts, L.J., Lawson, J.A. and Brash, A.R. (1983) Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. Journal of Clinical Investigation, 71, 676 - 688.

Forster, W. (1980) Effect of various agents on prostaglandin biosynthesis and the anti-aggregatory effect. Acta Medica Scandinavica, (Supplement) 642, 35 - 46.

Fox, S.C., Burgess-Wilson, M., Heptinstal, S. and Mitchell, J.R.A. (1982) Platelet aggregation in whole blood determined using the Ultra Flo 100 platelet counter. Thrombosis and Haemostasis, 48, 327 - 329.

Friedman, R.J., Stemerma, M.B., Wenz, B., Moore, S., Gaudie, J., Gent, M., Tiell, M.L. and Spaet, T.M. (1977) The effect of thrombocytopaenia on experimental arteriosclerotic lesion formation in rabbits: smooth muscle cell proliferation and re-endothelialization. Journal of Clinical Investigation, 60, 1191 - 1201.

Frishman, W.H., Weksler, B., Christodoulou, J.F., Smithen, C. and Killip, T. (1974) Reversal of abnormal platelet aggregability and change in exercise tolerance in patients with angina pectoris following oral propranolol. Circulation, 50, 887 - 898.

Frishman, W.H., Christodoulou, J., Weksler, B., Smithen, C., Killip, T.E., Scheidt, S. (1978) Abrupt propranolol withdrawal in angina pectoris; effects on platelet aggregation and exercise tolerance. American Heart Journal, 95, 169 - 179.

Frishman, W. (1979) Clinical pharmacology of the new beta-adrenergic blocking drugs. Part I pharmacodynamic and pharmacokinetic properties. American Heart Journal, 97, 663 -670.

Frojmovic, M.M. and Panjwani, R. (1976) Geometry of normal mammalian platelets by quantitative microscopic studies. Biophysical Journal, 16, 1071 - 1089.

Fuster, V. (1981) Role of platelets in the development of atherosclerotic disease and possible interference with platelet inhibitor drugs. Scandinavian Journal of Haematology, Supplement 38, 1 - 33.

De Gaetano, G., Cerletti, C. and Bertele, V. (1982) Pharmacology of anti-platelet drugs and clinical trials on thrombosis prevention: a difficult link. Lancet, 2, 974 - 977.

Gallery, E.D.M., Hunyor, S.N. and Gyory, A.Z. (1979a) Plasma volume contraction: a significant factor in both pregnancy-associated hypertension (pre-eclampsia) and chronic hypertension in pregnancy. Quarterly Journal of Medicine, 48, 593 - 602.

Gallery, E.D.M., Saunders, D.M., Hunyor, S.N. and Gyory, A.Z. (1979b) Randomised comparison of methyldopa and oxprenolol for treatment of hypertension in pregnancy. British Medical Journal, 1, 1591 - 1594.

Gallery, E.D.M., Ross, M.R. and Gyory, A.Z. (1985) Anti-hypertensive treatment in pregnancy: analysis of different responses to oxprenolol and methyldopa. British Medical Journal, 291, 563 - 566.

Gant, N.F., Daley, G.L., Chand, S., Whalley, P.J. and MacDonald, P.C. (1973) a study of angiotensin II pressor response throughout primigravid pregnancy. Journal of Clinical Investigation, 52, 2682 - 2689.

Gardner, F. (1972) Platelet kinetics and lifespan. Clinics in Haematology, 1, 307 - 324.

Gear, A.R.L. and Lambrecht, J.K. (1981) Reduction in single platelets during primary and secondary aggregation. Thrombosis and Haemostasis, 45, 298.

Gerrard, J.M., Butler, A.M. and White, J.G. (1977) Calcium release from a platelet calcium-sequestering membrane fraction by arachidonic acid and its prevention by aspirin. Prostaglandins, 15, 703.

Giles, C. and Inglis, T.C.M. (1981) Thrombocytopenia and macrothrombocytosis in gestational hypertension. British Journal of Obstetrics and Gynaecology, 88, 1115 - 1119.

Gold, E.W. and Edgar, P.R. (1978) The effect of physiological levels of non-esterified fatty acids on the radioimmunoassay of prostaglandins. Prostaglandins, 16, 945 - 952.

Goldblatt, M.W. (1935) Properties of human seminal plasma. Journal of Physiology, 84, 208 - 218.

Goldby, F.S. and Bellin, L.J. (1972) Relationship between arterial pressure and the permeability of arterioles to carbon particles in acute hypertension in the rat. Cardiovascular Research, 6, 384 - 390.

Goldsmith, H.L. (1971) Red cell motions and wall interactions in tube flow. Federation Proceedings, 30, 1578 - 1588.

Goodman, R.P., Killam, A.P., Brash, A.R. and Branch, R.A. (1982) Prostacyclin production during pregnancy: Comparison of production during normal pregnancy and pregnancy complicated by hypertension. American Journal of Obstetrics and Gynecology, 142, 817 - 822.

Gorman, R.R. (1979) Modulation of human platelet function by prostacyclin and thromboxane A₂. Federation Proceedings, 38, 83 - 88.

Gorman, R.R., Bunting, S. and Miller, O.V. (1977) Modulation of human platelet adenylate cyclase by prostacyclin (PGX). Prostaglandins, 13, 377 - 388.

Govan, A.D.T. (1954) Renal changes in eclampsia. Journal of Pathology and Bacteriology, 67, 311 - 322.

Granstrom, E. and Kindahl, H. (1978) Radioimmunoassay of prostaglandins and thromboxane. In Advances in Prostaglandin and Thromboxane Research, 5, 119 - 210.

Greaves, M. and Preston, F.E. (1982) Plasma 6-keto-PGF_{1α}: fact or fiction Thrombosis Research, 26, 145 - 157.

Green, D., Rossi, E.C. and Haring, O. (1982) The B-blocker heart attack trial: studies of platelets and factor VIII. Thrombosis Research, 28, 261 - 267.

Greenberg, J., Packham, M.A., Cazenave, J.P., Reimers, H-J. and Mustard, J.F. (1975) Effects on platelet function of removal of platelet sialic acid by neuramidase. Laboratory Investigation, 32, 476 - 484.

Greer, I.A., Walker, J.J., McLaren, M., Bonduelle, M., Cameron, A.D., Calder, A.A., Forbes, C.D. (1985) Immunoreactive prostacyclin and thromboxane metabolites in normal pregnancy and the puerperium. British Journal of Obstetrics and Gynaecology, 92, 581 - 585.

Halushka, P.V., Dollery, C.T. and MacDermot, J. (1983) Thromboxane and prostacyclin in disease: a review. Quarterly Journal of Medicine, 208, 461 - 470.

Hamberg, M., Svensson, J., Wakabayashi, T. and Samuelsson, B. (1974) Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proceedings of the National Academy of Sciences (USA), 71, 345 - 349.

Hamberg, M., Svensson, J. and Samuelsson, B. (1975) Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proceedings of the National Academy of Sciences (USA), 72, 2994 - 2998.

Han, P. and Ardlie, N.G. (1974) The influence of pH, temperature and calcium on platelet aggregation: Maintenance of environmental pH and platelet function for in-vitro studies in plasma stored at 37°C. British Journal of Haematology, 26, 373 - 389.

Han, P., Boatwright, C. and Ardlie, N.G. (1983) Effect of the calcium-entry blocking agent nifedipine on activation of human platelets and comparison with verapamil. Thrombosis and Haemostasis, 50, 513 - 517.

Harker, L.A. and Slichter, S. (1972) Platelet and fibrinogen consumption in man. New England Journal of Medicine, 287, 999 -1005.

Harrison, M.J.G. and Mitchell, J.R.A. (1966) Influence of red blood cells on platelet adhesiveness. Lancet, 2, 1163 - 1164.

Hathaway, D.R., Eaton, C.R. and Adelstein, R.S. (1980) Regulation of human platelet myosin kinase by calcium-calmodulin and cyclic AMP. In The Regulation of Coagulation, ed. Mann, K.G. and Taylor, F.B. Jr. pp 271 - 276. New York:Elsevier/North Holland.

Hawiger, J., Parkinson, S. and Timmons, S. (1980) Prostacyclin inhibits mobilization of fibrinogen binding sites on human ADP and thrombin treated platelets. Nature, 283, 195 - 197.

Heinroth, I., Block, H.U., Ponicke, R. and Forster, W. (1983) Influence of propranolol, pindolol, practolol and talinolol on arachidonic acid (AA) and U-46619-induced aggregation and on AA-induced thromboxane A₂ formation in platelets. Prostaglandins, Leukotrienes and Medicine, 12, 189 - 206.

Henriksson, P., Edhag, O. and Wennmalm, A. (1985) Limitation of myocardial infarction with prostacyclin: a double-blind study. In Prostacyclin Clinical Trials, ed. Gryglewski, R.J., Szczeklik, A. and McGiff, J.C. pp 31 - 42, New York:Raven Press.

Hensby, C.N., Fitzgerald, G.A., Friedman, L.A., Lewis, P.J. and Dollery, C.T. (1979) Measurement of 6-oxo-PGF_{1α} in human plasma using gas chromatography-mass spectrometry. Prostaglandins, 18, 731 - 736.

Heyns, A. due P., Van der Berg, D.J., Potgieter, G.M. and Retsef, F.P. (1974) The inhibition of platelet aggregation by an aorta intima extract. Thrombosis et Diathesis Haemorrhagica, 33, 354 - 360.

Higgs, E.A., Moncada, S., Vane, J.R., Caen, J.P., Michel, H. and Tobelem, G. (1978) Effects of prostacyclin (PGI₂) on platelet adhesion to rabbit arterial subendothelium. Prostaglandins, 16, 17 - 22.

Hirsh, P.D., Hillis, L.D., Campbell, W.B., Firth, B.G. and Willerson, J.T. (1981) Release of prostaglandins into the coronary circulation in patients with ischaemic heart disease. New England Journal of Medicine, 304, 685 - 691.

Hjalmarson, A., Elmfeldt, D., Herlitz, J. Holmberg, S., Halek, I., Nyberg, G., Ryden, L., Swedberg, K., Vedin, A., Wilhelmsen, L. and Wilkelmsson, C. (1981) Effect on mortality of metoprolol in acute myocardial infarction. Lancet, 2, 823 - 826.

Hong, S.-C.L. and Levine, L. (1976) Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. Proceedings of the National Academy of Sciences (USA), 73, 1730 - 1734.

Hopkins, N.K. and Gorman, R.R. (1981) Prostacyclin stimulation of human umbilical vein endothelial cell adenosine 3,5, cyclic monophosphate levels. Journal of Clinical Investigation, 68, 540 - 546.

Howie, P.W. (1977) The haemostatic mechanisms of pre-eclampsia. Clinics in Obstetrics and Gynecology, 4, 595 - 609.

Howie, P.W., Prentice, C.R.M. and McNicol, G.P. (1971) Coagulation, fibrinolysis and platelet function in pre-eclampsia, essential hypertension and placental insufficiency. Journal of Obstetrics and Gynaecology of the British Commonwealth, 78, 992 - 1003.

Howie, P.W., Begg, C.B., Purdie, D.W. and Prentice, C.R.M. (1976) Use of coagulation tests to predict the clinical progress of pre-eclampsia. Lancet, 2, 323 - 325.

Ikeda, Y., Kikuchi, M., Toyama, K., Watanabe, K. and Ando, Y. (1981) Inhibition of human platelet functions by verapamil. Thrombosis and Haemostasis, 45, 158 - 161.

Jaffe, E.A. and Weksler, B.B. (1979) Recovery of endothelial cell prostacyclin production after inhibition by low doses of aspirin. Journal of Clinical Investigation, 63, 532 - 535.

Jogee, M., Myatt, L. and Elder, M.G. (1983) Decreased prostacyclin production by placental cells in culture from pregnancies complicated by fetal growth retardation. British Journal of Obstetrics and Gynaecology, 90, 247 - 250.

Johansson, B., Strandgaard, S. and Lassen, N.A. (1974) On the pathogenesis of hypertensive encephalopathy. Circulation Research, 34 (Suppl.1), 167 - 71.

Johnson, A.R. (1980) Human pulmonary endothelial cells in culture. Activities of cells from arteries and cells from veins. Journal of Clinical Investigation, 65, 841 - 850.

Johnson, R.A., Morton, D.R., Kinner, J.H., Gorman, R.R., McGuire, J.R., Sun, F.F., Whittaker, N., Bunting, S., Salmon, J.A., Moncada, S. and Vane, J.R. (1976) The chemical structure of prostaglandin X (prostacyclin). Prostaglandins, 12, 915 - 928.

Jorgensen, L., Packham, M.A., Roswell, H.C. and Mustard, J.F. (1972) Deposition of formed elements of blood on the intima and signs of intimal injury in the aorta of rabbit, pig and man. Laboratory Investigation, 27, 341 - 350.

Karpatkin, S. (1969) Heterogeneity of human platelets II. Functional evidence suggestive of young and old platelets. Journal of Clinical Investigation, 48, 1083 - 1087.

Karpatkin, S. and Charmatz, A. (1969) Heterogeneity of human platelets I. Metabolic and kinetic evidence suggestive of young and old platelets. Journal of Clinical Investigation, 48, 1073 - 1082.

Karpatkin, S. (1978) Heterogeneity of human platelets VI. Correlation of platelet function with platelet volume. Blood, 51, 307 - 316.

Kaser-Glanzmann, R., Gerber, E. and Luscher, E.F. (1979) Regulation of the intracellular calcium level in human blood platelets: cyclic adenosine 3',5'-monophosphate dependent phosphorylation of a 22 000 dalton component in isolated Ca^{2+} -accumulating vesicles. Biochemica et Biophysica Acta, 558, 344 - 347.

Killam, A., Dillard, S., Patton, R. and Pederson, P. (1975) Pregnancy-induced hypertension complicated by acute liver disease and disseminated intravascular coagulation. American Journal of Obstetrics and Gynecology, 123, 823 - 828.

Kindahl, H., Granstrom, E. (1980) Limitations in measurement of prostaglandins and thromboxanes. In Prostaglandins, Prostacyclin and Thromboxane Measurement, ed. Boeynaems, J.M., Herman, A.G. pp. 1 - 16, The Hague:Martinus Nijhoff.

Kinlough-Rathbone, R.L., Groves, H.M., Cazenave, J.P., Richardson, M. and Mustard, J.F. (1978) Effect of dipyridamole and aspirin on platelet adherence to damaged rabbit aortas in vitro and in vivo. Federation Proceedings, 37, 260.

- Koh, H., Inoue, A., Mashimo, N., Numano, F. and Maezawa, H. (1980) A radioimmunoassay of thromboxane B₂ with thromboxane B₂-¹²⁵I-tyramide and its application to the study on the thromboxane B₂ formation during platelet aggregation. Thrombosis Research, 17, 403 - 413.
- Koullapis, E.N., Nicolaides, K.H., Collins, W.P., Rodeck, C.H. and Campbell, S. (1982) Plasma prostanoids in pregnancy-induced hypertension. British Journal of Obstetrics and Gynaecology, 89, 617 - 621.
- Kulkarni, P.S., Roberts, R. and Needleman, P. (1976) Paradoxical endogenous synthesis of a coronary dilating substance from arachidonate. Prostaglandins, 12, 337 - 353.
- Kurzrok, R. and Lieb, C.C. (1930) Biochemical studies on human semen: II. The action of semen on the human uterus. Proceedings of the Society for Experimental Biology and Medicine, 28, 268 -272.
- Lardoux, H., Gerard, J., Blazquez, G. and Flouvat, B. (1983) Which beta-blocker in pregnancy induced hypertension. Lancet, 2, 1194.
- Leather, H.M., Baker, P., Humphreys, D.M. and Chadd, M.A. (1968) A controlled trial of hypertensive agents in hypertension in pregnancy. Lancet, 2, 488.
- Le Breton, G.C., Dinerstein, R.J., Roth, L.J. and Feinberg, H. (1976) Direct evidence for intracellular divalent cation redistribution associated with platelet shape-change. Biochemical and Biophysical Research Communications, 72, 362 -370.
- Lees, M.M., Scott, D.B., Kerr, M.G. and Taylor, S.H. (1967a) The circulatory effects of recumbent postural change in late pregnancy. Clinical Science, 32, 453 - 65.
- Lees, M.M., Taylor, S.H., Scott, D.B. and Kerr, M.G. (1967b) A study of cardiac output at rest throughout pregnancy. Journal of Obstetrics and Gynaecology of the British Commonwealth, 74, 319 - 27.
- Levin, R., Jaffe, E.A., Weksler, B.B. and Tack-Goldman, K. (1981) Nitroglycerin stimulates the synthesis of prostacyclin by cultured human endothelial cells. Journal of Clinical Investigation, 67, 762 - 769.
- Lewis, P.J. (1982) The role of prostacyclin in pre-eclampsia. British Journal of Hospital Medicine, 28, 393 - 395.
- Lewis, P.J., Boylan, P., Friedman, L.A., Hensby, C.N. and Downing, I. (1980) Prostacyclin in pregnancy. British Medical Journal, 280, 1581 - 1582.
- Lewis, P.J., Shepherd, G.L., Ritter, J., Chan, S.M.T., Bolton, P.J., Jogee, M., Myatt, L. and Elder, M.G. (1981) Prostacyclin and pre-eclampsia. Lancet, 1, 559.

Lewy, R.I., Wrener, L., Smith, J.B., Walinsky, P., Silver, M.J. and Saia, J. (1979) Comparison of plasma concentrations of thromboxane B₂ in Prinzmetal's variant angina and classical angina pectoris. Clinical Cardiology, 2, 404 - 406.

Lin, C., Lindheimer, M.D., River, B.A. and Mowat, A.H. (1982) Fetal outcome in hypertensive disorders of pregnancy. American Journal of Obstetrics and Gynecology, 142, 255 - 260.

Lippton, H.L., Horwitz, P.M., McNamara, D.B., Landry, A.Z. and Kadowitz, P.J. (1984) Influence of verapamil and diltiazem on aggregatory responses in cat and rabbit platelet rich plasma. Prostaglandins, Leukotrienes and Medicine, 16, 121 - 130.

Lubbe, W.F. (1984) Hypertension in pregnancy pathophysiology and management. Drugs, 28, 170 - 188.

Lubbe, W.F. and Hodge J.V. (1981) Combined α - and β -adrenoceptor-antagonism with prazosin and oxprenolol in control of severe hypertension in pregnancy. New Zealand Medical Journal, 94, 169 - 72.

Lumley, P. and Humphrey, P.P.A. (1981) A method for quantitating platelet aggregation and analysing drug-receptor interactions on platelets in whole blood. Journal of Pharmacological Methods, 6, 153 - 166.

MacDermot, J., Barnes, P.J., Waddell, K.A., Dollery, C.T. and Blair, I.A. (1981) Prostacyclin binding to guinea pig pulmonary receptors. European Journal of Pharmacology, 75, 127 - 130.

MacGillivray, I. (1958) Some observations on the incidence of pre-eclampsia. Journal of Obstetrics and Gynaecology of the British Commonwealth, 65, 536 - 9.

MacGillivray, I. (1961) Hypertension in pregnancy and its consequences. Journal of Obstetrics and Gynaecology of the British Commonwealth, 68, 557 - 69.

MacGillivray, I. (1981) Aetiology of pre-eclampsia. British Journal of Hospital Medicine, 26, 110 - 119.

MacGillivray, I., Rose, G.A. and Rowe, B. (1969) Blood pressure survey in pregnancy. Clinical Science, 37, 395 - 407.

McIlwaine, G.M., Dunn, F., Howat, R.C.L., Smalls, M., Wylie, M.M. and Macnaughton, M.C.M. (1984) Perinatal Mortality Survey Scotland 1977 - 1981, Glasgow:University of Glasgow.

MacIntyre, D.E., Pearson, J.D. and Gordon, J.L. (1978) Localisation and stimulation of prostacyclin production by vascular cells. Nature, 271, 549 - 551.

McKay, D.G., Merrill, S.J., Weiner, A.E., Hertig, A.T. and Reid, D.E. (1953) The pathologic anatomy of eclampsia, bilateral renal cortical necrosis, pituitary necrosis and the acute fetal complications and its possible relationship to the generalized Schwartzman phenomenon. American Journal of Obstetrics and Gynecology, 66, 507 - 539.

McKillop, C., Howie, P.W., Forbes, C.D. and Prentice, C.R.M. (1976) Soluble fibrinogen/fibrin complexes in pre-eclampsia. Lancet, 1, 56 - 58.

McLaren, M., Belch, J.J.F., Forbes, C.D. and Prentice, C.R.M. (1985) Development of a radioimmunoassay for the measurement of prostacyclin metabolites in unextracted plasma. Thrombosis Research, 37, 177 - 183.

Maclean, P.R., Paterson, W.G., Smart, G.E., Petrie, J.J.B., Robson, J.S. and Thomson, P. (1972) Proteinuria in toxemia and abruptio placentae. Journal of Obstetrics and Gynaecology of the British Commonwealth, 79, 321 - 6.

Majerus, P.W., Miletich, J.P. and Kane, W.H. (1980) The formation of thrombin on the platelet surface. In The Regulation of Coagulation, ed. Mann, K.G. and Taylor, F.B. Jr., pp. 237 - 243. New York:Elsevier/North Holland.

Makarainen, L. and Ylikorkala, O. (1984) Amniotic fluid 6-keto-Prostaglandin F_{1α} and thromboxane B₂ during labour. American Journal of Obstetrics and Gynecology, 150, 765 - 768.

Makila, U.M., Jouppila, P., Kirkinen, P., Viinikka, L. and Ylikorkala, O. (1983) Relation between umbilical prostacyclin production and blood flow in the fetus. Lancet, 1, 728 - 729.

Makila, U.M., Viinikka, L. and Ylikorkala, O. (1984) Evidence that prostacyclin deficiency is a specific feature in pre-eclampsia. American Journal of Obstetrics and Gynecology, 148, 772 - 774.

Manucci, P.M. and Sharp, A.A. (1967) Platelet volume and shape in relation to aggregation and adhesion. British Journal of Haematology, 13, 604.

Marcus, A.J. (1976) The role of lipids in platelet function: with particular reference to the arachidonic acid pathway. Journal of Lipid Research, 9, 793 - 826.

Marcus, A.J., Weksler, B.B. and Jaffe, E.A. (1978) Enzymatic conversion of prostaglandin endoperoxide H₂ and arachidonic acid to prostacyclin by cultured human endothelial cells. Journal of Biological Chemistry, 253 - 7138 - 7141.

Marcus, A.J., Weksler, B.B., Jaffe, E.A. and Broekman, M.J. (1980) Synthesis of prostacyclin from platelet-derived endoperoxides by cultured human endothelial cells. Journal of Clinical Investigation, 66, 979 - 986.

Mathews, D.D. (1977) A randomised controlled trial of bed rest and sedation or normal activity and non-sedation in the management of non-albuminuric hypertension in late pregnancy. British Journal of Obstetrics and Gynaecology, 84, 108 - 114.

Mathews, D.D., Agarwal, V. and Shuttleworth, T.P. (1982) A randomised controlled trial of complete bed rest versus ambulation in the management of proteinuric hypertension during pregnancy. British Journal of Obstetrics and Gynaecology, 89, 128 - 131.

Mehta, J. (1983) Platelets and prostaglandins in coronary artery disease: Rationale for use of platelet suppressive drugs. Journal of the American Medical Association, 249, 2818 - 2823.

Mehta, J. and Mehta, P. (1982) Effect of propranolol therapy on platelet release and prostaglandin generation in patients with coronary artery disease. Circulation, 66, 1294 - 1299.

Mehta, J., Mehta, P. and Pepine, C.J. (1978) Platelet aggregation in aortic and coronary venous blood in patients with and without coronary disease. III Role of tachycardia stress and propranolol. Circulation, 58, 881 - 886.

Mehta, J., Mehta, P. and Ostrowski, N. (1983) Influence of propranolol and 4-hydroxypropranolol on platelet aggregation and thromboxane A_2 generation. Clinical Pharmacology and Therapeutics, 34, 559 - 564.

Mehta, J., Mehta, P., Ostrowski, N. and Crews, F. (1983) Effects of verapamil on platelet aggregation, ATP release and thromboxane generation. Thrombosis Research, 30, 469 - 475.

Mehta, J. and Mehta, P. (1984) Effect of propranolol therapy on platelet release and prostaglandin generation in patients with coronary heart disease. Circulation, 66, 1294 - 1299.

Mehta, P. and Mehta, J. (1980) Platelet function studies in coronary heart disease: decreased platelet sensitivity to PGI_2 in patients with myocardial infarction. Thrombosis Research, 18, 273 - 277.

Minkes, M., Stanford, N., Chi, M.M.Y., Roth, G.J., Raz, A., Needleman, P. and Majerus, P.W. (1977) Cyclic adenosine 3',5'-monophosphate inhibits the availability of arachidonate to prostaglandin synthetase in human platelet suspensions. Journal of Clinical Investigation, 59, 449.

Mitchell, M.D. (1978) A sensitive radioimmunoassay for 6-keto- $PGF_{1\alpha}$: Preliminary observations on circulating concentrations. Prostaglandins and Medicine, 1, 13 - 21.

Mitchell, M.D. (1981) Prostacyclin during human pregnancy and parturition. In Clinical Pharmacology of Prostacyclin, ed. Lewis, P.J. and O'Grady, J. pp.121 - 129, New York:Raven Press.

Mitchell, M.D., Bibby, J.G., Hicks, B.R., Redman, C.W.G., Anderson, A.B.M. and Turnbull, A.C. (1978) Thromboxane B₂ and human parturition: Concentration in the plasma and production in vitro. Journal of Endocrinology, 78, 435 - 441.

Mitchell, M.D., Keirse, M.J.N.C., Brunt, J.D., Anderson, A.B.M. and Turnbull, A.C. (1979) Concentrations of the prostacyclin metabolite 6-keto-prostaglandin F_{1α} in amniotic fluid during late pregnancy and labour. British Journal of Obstetrics and Gynaecology, 86, 350 - 353.

Miyamori, I., Fitzgerald, G.A., Brown, M.J. and Lewis, P.J. (1979) Prostacyclin stimulates the renin angiotensin aldosterone system in man. Journal of Clinical Endocrinology and Metabolism, 49, 493 - 494.

Moncada, S., Gryglewski, R.J., Bunting, S. and Vane, J.R. (1976) An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature, 263, 663 - 665.

Moncada, S., Herman, A.G., Higgs, E.A., Vane, J.R. (1977) Differential formation of prostacyclin (PGX or PGI₂) by layers of the arterial wall. An explanation for the antithrombotic properties of vascular endothelium. Thrombosis Research, 11, 323 - 344.

Moncada, S. and Korbust, R. (1978) Dipyridamole and other phosphodiesterase inhibitors act as antithrombotic agents by potentiating endogenous prostacyclin. Lancet, 1, 1286 - 1289.

Moncada, S., Korbust, R., Bunting, S. and Vane, J.R. (1978) Prostacyclin is a circulating hormone. Nature, 273, 767 - 768.

Moodley, J., Norman, R.J. and Reddi, K. (1984) Central venous concentrations of immunoreactive prostaglandins E, F and 6-keto-prostaglandin F_{1α} in eclampsia. British Medical Journal, 288, 1487 - 1489.

Morrison, A.R., Thornton, F., Blumberg, A. and Vaughan, E.D. (1981) Thromboxane A₂ is the major arachidonic acid metabolite of human cortical hydronephrotic kidney. Prostaglandins, 21, 471 - 481.

Murphy, E.A. and Mustard, J.F. (1962) Coagulation tests and platelet economy in atherosclerotic and control subjects. Circulation, 25, 114 - 125.

Mustard, J.F. and Packham, M.A. (1978) Platelets, thrombosis and drugs. In Cardiovascular Drugs, Vol. 3: Antithrombotic Drugs, ed. Avery, G.S., pp. 1 - 83, New York: ADIS Press.

Myatt, L. and Elder, M.G. (1977) Inhibition of platelet aggregation by a placental substance with prostacyclin-like activity. Nature, 268, 159 - 160.

Nachmias, V.T., Kavalier, J. and Jacobowitz, S. (1985) Reversible association of myosin with the platelet cytoskeleton. Nature, 313, 70 - 72.

Needleman, P., Raz, A., Ferrendelli, J.A. and Minkes, M. (1977) Application of imidazole as a selective inhibitor of thromboxane synthetase in human platelets. Proceedings of the National Academy of Sciences of the U.S.A., 74, 1716 - 1720.

Nichols, A.R. and Bosmann, H.B. (1979) Platelet aggregation: newly quantified using non empirical parameters. Thrombosis and Haemostasis, 42, 679 - 693.

Niewiarowski, S. (1981) Platelet release reaction and secreted platelet proteins. In Haemostasis and Thrombosis, ed. Bloom, A.L. and Thomas, D.P. pp. 73 - 83, Edinburgh:Churchill Livingstone.

Northover, B.J. (1977) Indomethacin - a calcium antagonist. General Pharmacology, 8, 293 - 296.

Norwegian Multicentre Study Group (1981) Timolol induced reduction in mortality and reinfarction in patients surviving acute myocardial infarction. New England Journal of Medicine, 304, 801 - 807.

Nurden, A.T. and Caen, J.P. (1979) The different glycoprotein abnormalities in thrombasthenic and Bernard-Soulier platelets. Seminars in Haematology, 16, 234 - 250.

O'Brien, J.R. (1962) Platelet aggregation II. Some results from a new method of study. Journal of Clinical Pathology, 15, 452 - 455.

O'Brien, J.R. and Heywood, J.B. (1966) Effect of aggregating agents and their inhibitors on the mean platelet shape, Journal of Clinical Pathology, 19, 148 - 153.

O'Brien, J.R. (1968) Effects of salicylates on human platelets. Lancet, 1, 779 - 83.

Ogletree, M.L., Lefer, A.M., Smith, J.B. and Nicolaou, K.C. (1979) Studies on the protective effect of prostacyclin in acute myocardial ischemia. European Journal of Pharmacology, 56, 95 - 103.

Onoda, J.M., Sloane, B.F. and Honn, K.V. (1984) Antithrombotic effects of calcium channel blockers: synergism with prostacyclin and thromboxane synthase inhibitors. Thrombosis Research, 34, 367 - 378.

Pace-Asciak, C. and Wolfe, L.S. (1971) A novel prostaglandin derivative formed from arachidonic acid by rat stomach homogenates. Biochemistry, 10, 3657 - 3669.

Packham, M.A., Kinlough-Rathbone, R.L. and Mustard, J.F. (1976) Aggregation and agglutination. In Platelet Function Testing, ed. Day, H.J., Holmsen, H. and Zucker, M.B. pp. 66 - 92, Washington:Department of Health, Education and Welfare.

Page, E.W. and Christianson, R. (1976) The impact of mean arterial pressure in the middle trimester upon the outcome of pregnancy. American Journal of Obstetrics and Gynecology, 125, 740 - 6.

Parks, W.M., Hoak, J.C. and Czervionke, R.L. (1981) Comparative effect of ibuprofen on endothelial and platelet prostaglandin synthesis. Journal of Pharmacology and Experimental Therapy, 219, 415 - 419.

Patrignani, P., Filabozzi, P. and Patrono, C. (1982) Selective cumulative inhibition of platelet thromboxane production by low dose aspirin in healthy subjects. Journal of Clinical Investigation, 69, 1366 - 1372.

Patrono, C., Ciabattoni, G., Pinca, E., Pugliese, F., Castrucci, G., de Salvo, A., Satta, M.A. and Peskar, B.A. (1980) Low dose aspirin and inhibition of thromboxane B₂ production in healthy subjects. Thrombosis Research, 17, 317 - 327.

Patrono, C., Pugliese, F., Ciabattoni, G., Patrignani, P., Maseri, A., Chierchia, S., Peskar, B.A., Cinotti, G.A., Simonetti, B.M. and Pierucci, A. (1982) Evidence for a direct stimulatory effect of prostacyclin on renin release in man. Journal of Clinical Investigation, 69, 231 - 239.

Patrono, C., Preston, F.E. and Vermylen, J. (1984) Platelet and vascular arachidonic acid metabolites: can they help detect a tendency towards thrombosis British Journal of Haematology, 57, 209 - 212.

Persantine - Aspirin Reinfarction Study Research Group (1980) Persantine and aspirin in coronary heart disease. Circulation, 62, 449 - 461.

Persantine - Aspirin Reinfarction Study II Research Group (1985) Persantine - aspirin reinfarction study part II secondary coronary prevention with persantine and aspirin. Circulation (in press).

Phillips, D.R. (1980) An evaluation of membrane glycoproteins in platelet adhesion and aggregation. In Progress in Haemostasis and Thrombosis, ed. Spaet, T.H. pp. 81 - 109, New York:Grune and Stratton.

Piper, P.J. (1983) Pharmacology of leukotrienes. British Medical Bulletin, 39, 255 - 259.

Piper, P.J. and Vane, J.R. (1969) Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. Nature, 223, 29 - 35.

Pollak, V.E. and Nettles, J.B. (1960) The kidney in toxemia of pregnancy: a clinical and pathologic study based on renal biopsies. Medicine, 39, 469 - 526.

Preston, F.E., Greaves, M. and Jackson, C.A. (1989) Cumulative inhibitory effect of daily 40 mg aspirin on prostacyclin synthesis. Lancet, 1, 1211 - 1212.

Preston, F.E., Whipps, S., Jackson, C.A., French, A.J., Wyld, P.J. and Stoddard, C.J. (1981) Inhibition of prostacyclin and platelet thromboxane A₂ after low dose aspirin. New England Journal of Medicine, 304, 76 - 79.

Rakoczi, I., Tallian, F., Bagdany, S. and Gati, I. (1979) Platelet life-span in normal pregnancy and pre-eclampsia as determined by a non-radioisotope technique. Thrombosis Research, 15, 553 - 556.

Rakoczi, I., Tihanyi, K., Falkay, G., Rozsa, I., Demeter, J. and Gati, I. (1983) Prostacyclin production in trophoblast. In Prostacyclin in Pregnancy, ed. Lewis, P.J., Moncada, S. and O'Grady, J. pp. 15 - 24, New York:Raven Press.

Ramwell, P.W., Foegh, M., Loeb, R. and Leovey, E.M.K. (1980) Synthesis and metabolism of prostaglandins, prostacyclin and thromboxanes: The arachidonic acid cascade. Seminars in Perinatology, 4, 3 - 13.

Randall, M.J., Parry, M.J., Hawkeswood, E., Cross, P.E. and Dickinson, R.P. (1981) UK-37,248, a novel, selective thromboxane synthetase inhibitor with anti-aggregatory and antithrombotic activity. Thrombosis Research, 23, 145 - 162.

Redd, J., Mosey, L.M. and Langford, H.G. (1968) Effect of posture upon sodium excretion in pre-eclampsia. American Journal of Obstetrics and Gynecology, 100, 343 - 7.

Redman, C.W.G. (1980) Immunological aspects of eclampsia and pre-eclampsia. In Immunological Aspects of Reproduction and Fertility Control, ed. Hearn, J.P., pp. 83 - 104, Lancaster:MTP Press.

Redman, C.W.G. (1982) A controlled trial of the treatment of hypertension in pregnancy: labetalol compared with methyldopa. In The Investigation of Labetalol in the Management of Hypertension in Pregnancy, ed. Riley, A. and Symonds, E.M. pp. 101 - 110, Excerpta Medica, International Congress Series 591.

Redman, C.W.G., Beilin, L.J. and Bonnar, J. (1976a) Variability of blood pressure in normal and abnormal pregnancy. In Hypertension in Pregnancy, ed. Lindheimer, M.D., Katz, A.I. and Zuspan, F.P., pp. 53 - 60, New York:John Wiley.

Redman, C.W.G., Beilin, L.J. and Bonnar, J. (1976b) Renal function in pre-eclampsia. Journal of Clinical Pathology, 10 (Suppl. Royal College of Pathologists) 91 - 4.

Redman, C.W.G., Beilin, L.J., Bonnar, J. and Ounsted, M.K. (1976c) Fetal outcome in trial of antihypertensive treatment in pregnancy. Lancet, 2, 753 - 6.

Redman, C.W.G., Allington, M.J., Bolton, F.G. and Stirrat, G.M. (1977a) Plasma β -thromboglobulin in pre-eclampsia. Lancet, 2, 248.

Redman, C.W.G., Beilin, L.J. and Bonnar, J. (1977b) Treatment of hypertension in pregnancy with methyldopa: blood pressure control and side effects. British Journal of Obstetrics and Gynaecology, 84, 419 - 26.

Redman, C.W.G., Denson, K.W.E., Beilin, L.J., Bolton, F.G. and Stirrat, G.M. (1977c) Factor VIII consumption in pre-eclampsia. Lancet, 2, 1249 - 52.

Redman, C.W.G., Bonnar, J. and Beilin, L.J. (1978) Early platelet consumption in pre-eclampsia. British Medical Journal, 1, 467 -9.

Report on Confidential Enquiries into Maternal Deaths in England and Wales 1973 - 1975 (1979), pp. 21 - 29, London, HMSO.

Report on Confidential Enquiries into Maternal Deaths in England and Wales 1976 - 1978 (1982), pp. 19 - 25, London, HMSO.

Remuzzi, G., Misiani, R., Muratore, D., Marchesi, D., Livio, M., Scheppati, A., Mecca, G., de Gaetano, G. and Donati, M.B. (1979) Prostacyclin and human foetal circulation. Prostaglandins, 18, 341 - 348.

Remuzzi, G., Marchesi, D., Zoja, C., Muratore, D., Mecca, G., Misiani, R., Rossi, E., Barbato, M., Capetta, P., Donati, M.B. and de Gaetano, G. (1980) Reduced umbilical and placental vascular prostacyclin in severe pre-eclampsia. Prostaglandins, 20, 105 - 110.

Remuzzi, G., Mecca, G., Livio, M., de Gaetano, G., Donati, M.B., Pearson, J.D. and Gordon, J.L. (1980) Prostacyclin generation by cultured endothelial cells in haemolytic uraemic syndrome. Lancet, ii, 656 - 657.

Remuzzi, G., Livio, M., Mecca, G., de Gaetano, G. and Donati, M.B. (1981a) Prostacyclin and kidney disease: focus on uremia and thrombotic microangiopathy. In Clinical Pharmacology of Prostacyclin, ed. Lewis, P.J. and O'Grady, J. pp. 63 - 71, New York:Raven Press.

Remuzzi, G., Zoja, C., Marchesi, D., Schieppati, A., Mecca, G., Misiani, R., Donati, M.B. and de Gaetano, G. (1981b) Plasmatic regulation of vascular prostacyclin in pregnancy. British Medical Journal, 282, 512 - 514.

Reynolds, B., Butters, L., Evans, J., Adams, T. and Rubin, P.C. (1984) First year of life after the use of atenolol in pregnancy associated hypertension. Archives of Diseases of Children, 59, 1061 - 1063.

Rink, R.J., Smith, S.W. and Tsien, R.Y. (1981) Intracellular free calcium in platelet shape change and aggregation. Journal of Physiology, 324, 53 - 54P.

- Ritter, J.M., Ongari, M.A., Barrow, S.E., Orchard, M.A., Blair, I.A. and Lewis, P.J. (1982) Prostanoid synthesis by human umbilical artery. Prostaglandins, 24, 881 - 886.
- Ritter, J.M., Barrow, S.E., Blair, I.A. and Dollery, C.T. (1983) Release of prostacyclin in vivo and its role in man. Lancet, 1, 317 - 319.
- Roberts, L.J., Sweetman, B.J., Payne, N.A. and Oates, J.A. (1977) Metabolism of thromboxane B₂ in man. Journal of Biological Chemistry, 252, 7415 - 7417.
- Robertson, E.G. (1971) the natural history of oedema during pregnancy. Journal of Obstetrics and Gynaecology of the British Commonwealth, 78, 520 - 529.
- Robertson, R.M., Robertson, D., Roberts, L.J., Maas, R.L., Fitzgerald, G.A., Friesinger, G.C. and Oates, J.A. (1981) Thromboxane A₂ in vasotonic angina pectoria. New England Journal of Medicine, 304, 998 - 1003.
- Rosenkranz, B., Fischer, C., Weimer, K.E. and Frohlich, J.C. (1980) Metabolism of prostacyclin and 6-keto-PGF_{1α} in man. Journal of Biological Chemistry, 255, 10194 - 10198.
- Rosenkranz, B. and Frohlich, J.C. (1985) Plasma concentrations and anti-platelet effects after low dose acetylsalicylic acid. Prostaglandins, Leukotrienes and Medicine, 19, 289 - 300.
- Ross, R. and Glomset, J.A. (1976) The pathogenesis of atherosclerosis. New England Journal of Medicine, 295, 369 - 377.
- Ross, R., Glomset, J., Kariya, B. and Harker, L. (1974) A platelet dependant serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proceedings of the National Academy of Sciences of the USA, 71, 1207 - 1210.
- Rossi, E.C., Louis, G. (1977) Kinetic parameters of platelet aggregation as an expression of platelet responsiveness. Thrombosis and Haemostasis, 37, 283 - 290.
- Roth, G.J., Stanford, N. and Majerus, P.W. (1975) Acetylation of prostaglandin synthase by aspirin. Proceedings of the National Academy of Sciences of the USA, 72, 3073 - 3076.
- Roy, A.C., Karim, S.M.M., Kottegoda, S.R., Ratnam, S.S. (1984) Thromboxane A₂ and prostacyclin levels in molar pregnancy. British Journal of Obstetrics and Gynaecology, 91, 908 - 912.
- Rubegni, M., Provvedi, D. and Bellini, P.G. (1974) Beta blocking agents and platelet aggregation. Journal of the American Medical Association, 228, 462.
- Rubin, P.C. (1981) Beta-blockers in pregnancy. New England Journal of Medicine, 305, 1323 - 1326.

Rubin, P.C., Butters, L., Clark, D.M., Reynolds, B., Sumner, D.J., Steedman, D., Low, R.A. and Reid, J.L. (1983) Placebo controlled trial of atenolol in treatment of pregnancy associated hypertension. Lancet, 1, 431 - 434.

Saito, H. (1984) Normal hemostatic mechanisms. In Disorders of Hemostasis, ed. Ratnoff, O.D. and Forbes, C.D. pp. 23 - 42, New York:Grune and Stratton.

Salmon, J.A. (1983) Measurement of elcosanoids by bioassay and radioimmunoassay. British Medical Bulletin, 39, 227 - 231.

Salmon, J.A., Smith, D.R., Flower, R.J., Moncada, S. and Vane, J.R. (1978) Further studies on the enzymatic conversion of prostaglandin endoperoxide into prostacyclin by porcine aorta microsomes. Biochimica et Biophysica Acta, 523, 250 - 262.

Salzman, E.W. (1963) Measurement of platelet adhesiveness. A simple in vitro technique demonstrating an abnormality in von Willebrand's disease. Journal of Laboratory and Clinical Medicine, 62, 724 - 735.

Salzman, E.W., MacIntyre, D.E., Steer, M.L. and Gordon, J.L. (1978) Effect on platelet activity of inhibition of adenylate cyclase. Thrombosis Research, 13, 1089 - 1101.

Saniabadi, A.R., Lowe, G.D.O., Forbes, C.D., Prentice, C.R.M. and Barbenel, J.C. (1983a) Platelet aggregation studies in whole human blood. Thrombosis Research, 30, 625 - 632.

Saniabadi, A.R., Lowe, G.D.O., Belch, J.J.F., Forbes, C.D., Prentice, C.R.M. and Barbenel, J.C. (1983b) The novel effect of a new prostacyclin analogue ZK36374 on the aggregation of human platelets in whole blood. Thrombosis and Haemostasis, 50, 718 - 721.

Saniabadi, A.R., Lowe, G.D.O., Barbenel, J.C. and Forbes, C.D. (1984a) A comparison of spontaneous platelet aggregation in whole blood with platelet rich plasma: Additional evidence for the role of ADP. Thrombosis and Haemostasis, 51, 115 - 118.

Saniabadi, A.R., Lowe, G.D.O., Belch, J.J.F., Barbenel, J.C. and Forbes, C.D. (1984b) Effect of prostacyclin (epoprostenol) on the aggregation of human platelets in whole blood in vitro. Haemostasis, 14, 487 - 494.

Schick, P.K. (1979) The role of platelet membrane lipids in platelet hemostatic activities. Seminars in Hematology, 16, 221 - 233.

Schmorl, G. (1893) Pathologische - Anatomische Untersuchungen uber Puerperaleklampsie. Leipzig:F.C.W. Vogel.

Senior, J.B., Fahim, I., Sullivan, F.M. and Robson, J.M. (1963) Possible role of 5-hydroxytryptamine in toxemia of pregnancy. Lancet, ii, 553 - 554.

Sheehan, H.L. and Lynch, J.B. (1973) Pathology of Toxaemia of Pregnancy, pp. 328 - 330, London:Churchill-Livingstone.

Shepherd, G.L., Lewis, P.J., Blair, I.A., de Mey, C. and McDermot, J. (1983) Epoprostenol (prostacyclin) binding and activation of adenylate cyclase in platelets of diabetic and control subjects. British Journal of Clinical Pharmacology, 15, 77 - 81.

Shukla, P.K., Sharma, D. and Mandal, R.K. (1978) Serum lactate dehydrogenase in detecting liver damage associated with pre-eclampsia. British Journal of Obstetrics and Gynaecology, 85, 40 - 42.

Siegl, A.M., Smith, J.B., Silver, M.J., Nicolaou, K.C. and Ahern, D. (1979) Selective binding site of prostacyclin on platelets. Journal of Clinical Investigation, 63, 215 - 220.

Siess, W., Roth, P. and Weber, P.C. (1981) Stimulated platelet aggregation, thromboxane B₂ formation and platelet sensitivity to prostacyclin - a critical evaluation. Thrombosis and Haemostasis, 45, 204 - 207.

Siess, W. and Dray, F. (1982) Very low levels of 6-keto-Prostaglandin F_{1α} in human plasma. Journal of Laboratory and Clinical Medicine, 99, 388 - 398.

Siess, W., Roth, P., Scherer, B., Kurzmann, I., Bohlig, B. and Weber, P.C. (1980) Platelet-membrane fatty acids, platelet aggregation and thromboxane formation during a mackerel diet. Lancet, 1, 441 - 444.

Sinzinger, H., Feigl, W. and Silberbauer, K. (1979) Prostacyclin generation in atherosclerotic arteries. Lancet, 2, 469.

Sloan, A.W. (1951) The normal platelet count in man. Journal of Clinical Pathology, 4, 37.

Small, M., Douglas, J.T., Aherne, G.W., Orr, M., Lowe, G.D.O., Forbes, C.D. and Prentice, C.R.M. (1982) Effects of the non-selective beta-adrenoceptor blocking agent, carteolol, on platelet function, blood coagulation and viscosity. Thrombosis Research, 25, 351 - 360.

Smith, K., Browne, J.C.Mc., Shackman, R. and Wrong, O.M. (1968) Renal failure of obstetric origin. British Medical Bulletin, 24, 49 - 58.

Smith, J.B. and Willis, A.L. (1971) Aspirin selectively inhibits prostaglandin production in human platelets. Nature, 231, 235 - 237.

Smith, R.W. (1970) Cardiovascular alterations in toxemia. American Journal of Obstetrics and Gynecology, 107, 979 - 83.

Socol, M.L., Weiner, C.P., Louis, G., Rehnberg, K. and Rossi, E.C. (1985) Platelet activation in pre-eclampsia. American Journal of Obstetrics and Gynecology, 151, 494 - 497.

Steele, P.P., Weily, H.S., Davies, H. and Genton, E. (1973) Platelet function in coronary artery diseases. Circulation, 48, 1194 - 1200.

Strauss, R.G., Keefer, J.R., Burke, T. and Civetta, J.M. (1980) Hemodynamic monitoring of cardiogenic pulmonary edema complicating toxemia of pregnancy. Obstetrics and Gynaecology, 55, 170 - 4.

Stirling, Y., Woolf, L., North, W.R.S., Seghatchian, M.J. and Meade, T.W. (1984) Haemostasis in normal pregnancy. Thrombosis and Haemostasis, 52, 176 - 182.

Strickland, D.M., Cox, K., McCubbin, J.H., Whalley, P.J., MacDonald, P.C. and Mitchell, M.D. (1984) Plasma prostaglandins during the intravenous infusion of angiotensin II in pregnant women. American Journal of Obstetrics and Gynecology, 150, 952 - 955.

Stuart, M.J., Clark, D.A., Sunderji, S.G., Allen, J.B., Yambo, T., Elrad, H. and Slott, J.H. (1981) Decreased prostacyclin production: a characteristic of chronic placental insufficiency syndromes. Lancet, 1, 1126 - 1128.

Symonds, E.M., Lamming, G.D., Jadoul, F. and Broughton Pipkin, F. (1982) Clinical and biochemical aspects of the use of labetalol in the treatment of hypertension in pregnancy: comparison with methyl dopa. In The Investigation of Labetalol in the Management of Hypertension in Pregnancy, ed. Riley, A. and Symonds, E.M. pp. 62 - 76, Amsterdam: Excerpta Medica.

Szczeklik, A., Nizankowski, J., Szczeklik, J., Tabeau, J. and Krolkowski, W. (1985) Prostacyclin therapy in subgroups of patients with spontaneous angina. In Prostacyclin Clinical Trials, ed. Gryglewski, R.J., Szczeklik, A., and McGiff, J.C. pp. 43 - 54, New York: Raven Press.

Talledo, O.E., Chesley, L.C. and Zuspan, F.D. (1968) Renin, angiotensin system in normal and toxemic pregnancies. III. Differential sensitivity to angiotensin II and norepinephrine in toxemia of pregnancy. Obstetrics and Gynaecology, 100, 218 - 221.

Tateson, J.E., Moncada, S. and Vane, J.R. (1977) Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. Prostaglandins, 13, 389 - 399.

Terragno, N.A., McGiff, J.C. and Smigel, M. (1978) Pattern of prostaglandin production in bovine fetal and maternal vasculature. Prostaglandins, 16, 847 - 856.

Thomson, A.M., Hytten, R.E. and Billewicz, W.Z. (1967) The epidemiology of edema during pregnancy. Journal of Obstetrics and Gynaecology of the British Commonwealth, 74, 1 - 10.

Thorngren, M. and Gustafson, A. (1981) Effects of 11-week increase in dietary eicosapentaenoic acid on bleeding time, lipids, and platelet aggregation. Lancet, 2, 1190 - 1193.

Tobelem, G., Levy-Toledano, S., Bredoux, R., Michel, H., Nurden, A., Caen, J.P. and Degos, L. (1976) New approach to determination of specific functions of platelet membrane sites. Nature, 263, 427 - 429.

Toivanen, J., Ylikorkala, O. and Viinikka, L. (1984) One milligramme of acetylsalicylic acid daily inhibits platelet thromboxane A₂ production. Thrombosis Research, 35, 681 -687.

Trowbridge, E.A., Martin, J.F. and Slater, D.N. (1982) Evidence for a theory of physical fragmentation of megakaryocytes, implying that all platelets are produced in the pulmonary circulation. Thrombosis Research, 28, 461 - 475.

Trudinger, B.J. (1976) Platelets and intra-uterine growth retardation in pre-eclampsia. British Journal of Obstetrics and Gynaecology, 83, 284 - 286.

Ts'ao, C.H., Lo, R. and Raymond, J. (1976) Critical importance of citrate - blood ratio in platelet aggregation studies. American Journal of Clinical Pathology, 65, 518 - 522.

Turitto, V.T. and Baumgartner, H.R. (1975) Platelet interaction with subendothelium in a perfusion system: Physical role of red blood cells. Microvascular Research, 9, 335 - 344.

Turitto, V.T. and Baumgartner, H.R. (1979) Platelet interaction with subendothelium in flowing rabbit blood: effect of blood shear rate. Microvascular Research, 17, 38 - 54.

Tuvemo, T. (1980) Role of prostaglandins, prostacyclin and thromboxanes in the control of the umbilical - placental circulation. Seminars in Perinatology, 4, 91 - 95.

Tyler, H.M., Saxton, C.A.P.D. and Parry, M.J. (1981) Administration to man of UK-37,248-01, a selective inhibitor of thromboxane synthetase. Lancet, 1, 629 - 632.

Ueland, K., Novy, M.J., Peterson, E.N. and Metcalfe, J. (1969) Maternal cardiovascular dynamics. IV. The influence of gestational age on the maternal cardiovascular response to posture and exercise. American Journal of Obstetrics and Gynecology, 104, 856 - 64.

Ubatuba, F.B., Moncada, S. and Vane, J.R. (1979) The effects of prostacyclin on platelet behaviour thrombus formation in vivo and bleeding time. Thrombosis et Diathesis Haemorrhagica, 41, 425 -434.

Vanderhoek, J.Y. and Feinstein, M.B. (1979) Local anesthetics, chlorpromazine and propranolol inhibit stimulus-activation of phospholipase A₂ in human platelets. Molecular Pharmacology, 16, 171 - 180.

Vane, J.R. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature, 231, 232 -235.

Vargaftig, B.B., Chignard, M. and Benveniste, J. (1981) Present concepts on the mechanisms of platelet aggregation. Biochemical Pharmacology, 30, 263 - 271.

Vargaftig, B.B. and Zirinus, P. (1973) Platelet aggregation induced by arachidonic acid is accompanied by release of potential inflammatory mediators distinct from PGE₂ and PGF₂. Nature (New Biology), 244, 114 - 116.

Vassali, P., Simon, G. and Rouiller, C. (1963) Production of ultra-structural glomerular lesions resembling those of toxemia of pregnancy by thromboplastin infusion in rabbits. Nature, 199, 1105 - 1106.

Vermynen, J., Chamone, D.A.F. and Verstraete, M. (1979) Stimulation of prostacyclin release from vessel wall by BAY g 6575, an antithrombotic compound. Lancet, 1, 518 - 520.

Vermynen, J., Carreras, L.O., Van Schaeren, J., Defreyn, G., Machin, S.J. and Verstraete, M. (1981) Thromboxane synthetase inhibition as antithrombotic strategy. Lancet, 1, 1073 - 1075.

Viinikka, L., Ylikorkala, O. (1982) Different results of plasma PGI₂ measurements utilising two antisera with apparently similar specificity. Prostaglandins, Leukotrienes and Medicine, 9, 1 - 7.

Walker, J.J., Crooks, A., Erwin, L. and Calder, A.A. (1982) Labetalol in pregnancy-induced hypertension: fetal and maternal effects. In The Investigation of Labetalol in the Management of Hypertension in Pregnancy, ed. Riley, A. and Symonds, E.M. pp. 148 - 160, Amsterdam:Excerpta Medica.

Walker, J.J., Greer, I.A., Calder, A.A. (1983) Treatment of acute pregnancy related hypertension: labetalol and hydralazine compared. Postgraduate Medical Journal, 59 (Supplement 3), 168 - 170.

Wallenburg, H.C.S. and Rotmans, N. (1982) Enhanced reactivity of the platelet thromboxane pathway in normotensive and hypertensive pregnancies with insufficient fetal growth. American Journal of Obstetrics and Gynecology, 144, 523 - 528.

Wallenburg, H.C.S. and van Kessel, P.H. (1978) Platelet lifespan in normal pregnancy as determined by a nonradioisotopic technique. British Journal of Obstetrics and Gynaecology, 85, 33 - 36.

Walsh, P.N. (1972) The effects of collagen and kaolin on the intrinsic coagulant activity of platelets: evidence for an alternative pathway in intrinsic coagulation not requiring factor XII. British Journal of Haematology, 22, 393 - 405.

Walsh, P.N. (1972) The role of platelets in the contact phase of blood coagulation. British Journal of Haematology, 22, 237 - 254.

Walsh, P.N., Pareti, F.I. and Corbett, J.J. (1977) Platelet coagulant activities and serum lipids in transient cerebral ischemia. New England Journal of Medicine, 295, 854 - 858.

Walsh, S.W. (1985) Pre-eclampsia: an imbalance in placental prostacyclin and thromboxane production. American Journal of Obstetrics and Gynecology, 152, 335 - 340.

Walsh, S.W., Behr, M.J. and Allen, N.H. (1985) Placental prostacyclin production in normal and toxemic pregnancies. American Journal of Obstetrics and Gynecology, 151, 110 - 115.

Walters, B.N.J. and Redman, C.W.G. (1982) Control of severe hypertension in pregnancy with nifedipine. In Proceedings of 3rd Congress of the International Society of Hypertension in Pregnancy, Dallas. p. 196.

Warso, M.A. and Lands, W.E.M. (1983) Lipid peroxidation, prostacyclin and thromboxane. British Medical Bulletin, 39, 277 - 280.

Weiss, H.J., Aledort, L.M. and Kochwa, S. (1968) The effect of salicylates on the hemostatic properties of platelets in man. Journal of Clinical Investigation, 47, 2169 - 2180.

Weksler, B.B. (1982) Prostacyclin. In Progress in Haemostasis, ed. Spaet, T.M. Volume 6, pp. 113 - 138, New York:Grune and Stratton.

Weksler, B.B., Gillick, M. and Pink, J. (1977) Effect of propranolol on platelet function. Blood, 49, 185 - 196.

Weksler, B.B., Marcus, A.J. and Jaffe, E.A. (1977) Synthesis of prostaglandin I₂ by cultured human and bovine endothelial cells. Proceedings of the National Academy of Sciences of the USA, 74, 3922 - 3926.

Weksler, B.B., Ley, C.W. and Jaffe, E.A. (1978) Stimulation of endothelial cell prostacyclin by thrombin, trypsin, and the ionophore A23187. Journal of Clinical Investigation, 62, 923 - 930.

Weksler, B.B., Pett, S.B., Alonso, D., Richter, R.C., Stelzer, P., Subramanian, V., Tack-Goldman, K. and Gay, W.A. (1983) Differential inhibition by aspirin of vascular and platelet prostaglandin synthesis in atherosclerotic patients. New England Journal of Medicine, 308, 800 - 805.

Whigham, K.A.E., Howie, P.W., Drummond, A.H. and Prentice, C.R.M. (1978) Abnormal platelet function in pre-eclampsia. British Journal of Obstetrics and Gynaecology, 85, 28 - 32.

White, J.G. and Gerrard, J.M. (1976) Ultrastructural features of abnormal blood platelets. A review. American Journal of Pathology, 83, 589 - 632.

- White, J.G., Clawson, C.C. and Gerrard, J.M. (1981) Platelet ultrastructure. In Haemostasis and Thrombosis, ed. Bloom, A.L. and Thomas, D.P. pp. 22 - 49, Edinburgh:Churchill-Livingstone.
- Whittle, B.J.R. and Moncada, S. (1983) Pharmacological interactions between prostacyclin and thromboxanes. British Medical Bulletin, 39, 232 - 238.
- Whittle, B.J.R., Moncada, S. and Vane, J.R. (1978) Formation of prostacyclin by the gastric mucosa and its actions on gastric function. Prostaglandins, 15, 704 - 705.
- Winocour, P.D., Kinlough-Rathbone, R.L. and Mustard, J.F. (1979) Effect of the phospholipase A₂ inhibitor mepacrine on aggregation of washed rabbit platelets. Federation Proceedings, 38, 1271.
- Wong, P.Y.K. and Cheung, W.Y. (1979) Calmodulin stimulates human platelet phospholipase A₂. Biochemical and Biophysical Research Communications, 90, 473 - 480.
- Wood, A.J., Bolli, P. and Simpson, F.O. (1976) Prazosin in normal subjects: plasma levels, blood pressure and heart rate. British Journal of Clinical Pharmacology, 3, 199 - 201.
- Woods, P.B. and Robinson, M.L. (1981) An investigation of the comparative liposolubilities of B-adrenoceptor blocking agents. Journal of Pharmacy and Pharmacology, 33, 172 - 173.
- Yamaguchi, M. and Mori, N. (1985) 6-keto-Prostaglandin F_{1α}, thromboxane B₂ and 13,14-dihydro-15-keto prostaglandin F concentrations of normotensive and pre-eclamptic patients during pregnancy, delivery and the postpartum period. American Journal of Obstetrics and Gynecology, 151, 121 - 127.
- Ylikorkala, O. and Viinikka, L. (1980) Thromboxane A₂ in pregnancy and puerperium. British Medical Journal, 281, 1601 -1602.
- Ylikorkala, O. and Viinikka, L. (1981) Maternal plasma levels of 6-keto-Prostaglandin F_{1α} during pregnancy and puerperium. Prostaglandins and Medicine, 7, 95 - 99.
- Ylikorkala, O., Makila, U.M. and Viinikka, L. (1981a) Amniotic fluid prostacyclin and thromboxane in normal pre-eclamptic and some other complicated pregnancies. American Journal of Obstetrics and Gynecology, 141, 487 - 490.
- Ylikorkala, O., Kirkinen, P. and Viinikka, L. (1981b) Maternal plasma prostacyclin concentration in pre-eclampsia and other pregnancy complications. British Journal of Obstetrics and Gynaecology, 88, 968 - 972.
- Ylikorkala, O., Makarainen, L. and Viinikka, L. (1981c) Prostacyclin production increases during human parturition. British Journal of Obstetrics and Gynaecology, 88, 513 - 516.

Zahavi, J. and Zahavi, M. (1985) Enhanced platelet release reaction shortened platelet survival time and increased platelet aggregation and plasma thromboxane B₂ in chronic obstructive arterial disease. Thrombosis and Haemostasis, 53, 105 - 109.

Zeek, P.M. and Assali, N.S. (1950) Vascular changes with eclamptogenic toxemia of pregnancy. American Journal of Clinical Pathology, 20, 1099 - 1109.

Zucker, M.B. (1975) Effect of heparin on platelet function. Thrombosis et Diathesis Haemorrhagica, 33, 63 - 65.

Zucker, M.B. and Borrelli, J. (1962) Platelet clumping produced by connective tissue suspensions and by collagen. Proceedings of the Society for Experimental Biology and Medicine, 109, 779.

