ASPECTS OF PROSTACYCLIN
IN EXPERIMENTAL HYPERTENSION

by

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– ABSTRACT –

A new prostaglandin - prostaglandin X (later renamed prostacyclin or prostaglandin I_2 (PGI_2)), was discovered by Moncada, Gryglewski, Bunting and Vane in 1976. This unstable substance was shown to be produced by vascular tissue and to be a vasodilator and the most potent endogenous inhibitor of platelet aggregation known. Because of its properties, it appeared that a lack of it may be related to the development and/or maintenance of hypertension, a disorder featuring vasoconstriction and an increased tendency to arterial thrombosis. The present studies aimed to investigate this possibility using a rat model.

A bioassay for prostacyclin was first perfected. This consisted of a modification of the method used by Moncada, Higgs and Vane (1977): PGI_2 released by rat aortic strips, during incubation in tris buffer, was measured by assessing the ability of the incubate to inhibit adenosine diphosphate induced aggregation of human platelets, as compared to the inhibitory effect of standard prostacyclin sodium salt. The specificity of the assay for the detection of PGI_2 was tested.

The ability of hypertensive rat aorta to release prostacyclin was investigated in two studies. The first compared aortas of Wistar rats of the New Zealand genetically hypertensive strain (GH) with those of matched normotensive Wistar controls. In the second study, hypertension was induced by wrapping the right kidney with surgical silk and removing the contralateral kidney. Ten weeks later, aortic generation of prostacyclin by these animals was compared with that of matched sham controls which had received identical surgical manipulation but for the application of silk to the right kidney. Contrary to expectation, in both forms of hypertension, aortas of the

rats with elevated pressure produced consistently more prostacyclin than those of matched controls.

In order to discover more about the relationship between elevated pressure and elevated PGI_2 production, the effect of pressure reduction with hypotensive agents on the ability of GH rat aortas to produce prostacyclin, was investigated. After pressure had been controlled within normal range for one week (achieved by oral administration of furosemide, dihydralazine and reserpine for one month), aortic PGI_2 was reduced in comparison with matched GH controls. However, the reduction was not consistent and statistical significance was not reached. Because it was subsequently reported by other workers, that some of the hypotensive agents which had been employed may effect prostaglandin levels per se, no conclusions could be drawn from this study as to any possible direct relationships between pressure and aortic prostacyclin generating capacity. A further means of reducing elevated pressure (which had no inherent effect on prostaglandin levels) was thus sought.

A mechanical method was eventually selected, application of a silver clip to the aortas of GH rats, just below the diaphragm, producing an immediate reduction in pressure distal to the constriction. Eighteen hours later, PGI_2 production by these distal aortas was compared with those of matched sham GH controls and was found to be consistently reduced. These results indicate that the ability to produce PGI_2 may be influenced by prior local pressure changes and that the increased capacity of hypertensive rat aortas to generate prostacyclin may be related to the increased mechanical transmural stress consequent on elevated pressure.

Since haemostatic balance must be influenced not only by vascular

PGI_2 generation but also by platelet sensitivity to PGI_2, the response of GH platelets to the anti-aggregatory effect of prostacyclin was also investigated. As it had been shown by Sinzinger, Silberbauer, Horsch and Gall (1981) that intra-arterial infusion of PGI_2 in humans decreased platelet sensitivity to the substance, the possibility existed that platelet sensitivity in hypertension might be reduced. This hypothesis was, however, invalidated as the sensitivity of GH platelets to the anti-aggregatory effect of PGI_2 was almost identical to that of normotensive controls.

The shortcomings of the methodology and the possible importance of these findings in the hypertensive animal are discussed. The idea that elevated PGI_2 in hypertension may play a protective role both with respect to platelet aggregation and in attenuating further pressure rises is considered. It is finally suggested that it will be possible to draw more accurate conclusions as to the meaning of the increased PGI_2 generation in hypertension (both in relation to vascular tone and platelet function) only when details of production of, and sensitivity to, thromboxane A_2 are known. Thromboxane A_2 (TXA_2) is a vasoconstrictor and promotor of aggregation (Hamberg, Svensson and Samuelson, 1975) and it may be that, despite elevated vascular PGI_2 generation, the TXA_2/PGI_2 balance is still tipped in favour of vasoconstriction and platelet aggregation in hypertension.

CHAPTER I

INTRODUCTION

– ACKNOWLEDGEMENTS –

Recognition of the fact that complications attending an elevated blood pressure constitute one of the most frequent causes of a decreased life expectancy (Grollman, 1980) has inspired a tremendous interest in hypertension. The scientific medical literature is replete with conflicting elaborate theories as to its pathogenesis.

In 1976 Moncada, Gryglewski, Bunting and Vane discovered a new derivative of arachidonic acid - prostaglandin X, later renamed prostacyclin or prostaglandin I_2 (Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada and Vane, 1976 and Nomenclature Announcement, 1977).

The site of production and the properties of this prostaglandin immediately stimulated interest in its possible involvement in hypertension.

Prostacyclin was found to be locally generated in blood vessel walls and was able to relax vascular smooth muscle (Bunting, Gryglewski, Moncada and Vane, 1976) suggesting that it might play a part in the control of vascular tone. This may be of particular relevance in situations of sustained elevation of arterial pressure which are associated in most cases with increased peripheral vascular resistance due to decreased diameter i.e. vasoconstriction of resistance vessels. Cardiac output is generally normal (Pickering, 1974).

While the many vasoregulatory mechanisms which might be deranged in hypertension have received much attention, reports of their involvement are conflicting.

Prostacyclin has a second notable property - it is a potent

inhibitor of platelet aggregation (Moncada, Gryglewski, Bunting and Vane, 1976a). This too may have a bearing on hypertension, since it has been suggested that a major determinant of the thrombotic and atherosclerotic complications of hypertensive vascular disease, may be the increased tendency to adhere and aggregate which has been reported for platelets of hypertensive patients (Lentini and Bologna, 1974).

The discovery of this exciting new vasodilatory, anti-aggregatory prostaglandin created a new dimension in the knowledge of cardiovascular function and pathology. An investigation into the role of prostacyclin in hypertension, using a rat model, is the subject of this study.

CHAPTER 2

LITERATURE REVIEW

2.1. <u>PROSTAGLANDINS - AN INTRODUCTION</u>

In 1930, two American gynaecologists noted that strips of human uterus relaxed or contracted when exposed to human semen (Kurzrok and Lieb, 1930). A few years later, Goldblatt in England and von Euler in Sweden independently reported vasodepressor and smooth muscle contracting activity in seminal fluid and accessory reproductive glands. Von Euler identified the active material as a lipid soluble acid which he named "prostaglandin" (Goldblatt, 1935 and von Euler, 1936). More than 20 years passed before technical advances allowed the isolation, in pure form, of some prostaglandins and the elucidation of their structure (Figure 1) as 20-carbon unsaturated carboxylic acids with a cyclopentane ring (Bergström and Sjövall, 1960, and Samuelsson, 1963). Now, in 1981, few substances command more widespread interest in biological circles than the prostaglandins and related compounds. They are among the most prevalent of autocoids, being widely distributed throughout the animal kingdom in tissues and body fluids, and are being seen to be involved in an ever increasing number of fundamental responses of cells and organs.

Two independent groups (Bergström, Danielsson, and Samuelsson, 1964 and van Dorp, Beerthuis, Nugteren and Vonkeman, 1964) demonstrated that prostaglandins are biosynthesised from polyunsaturated fatty acids.

These acids include (Figure 2) eicosatrienoic acid (dihomo-gamma-linolenic acid), eicosatetraenoic acid (arachi-
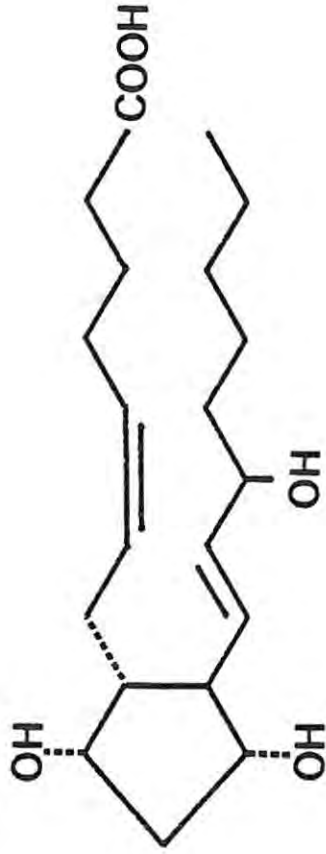
FIG.1: THE STRUCTURE OF PGF$_{2\alpha}$

donic acid) and eicosapentaenoic acid, which give rise to the mono - (1 series), bis - (2 series) and trienoic - (3 series) prostaglandins respectively. Each series carries the relevant subscript 1, 2 or 3 after the abreviation of prostaglandin PG, e.g. PG_2 series. The number indicates the number of double bonds in the side chain. Prostaglandins are further distinguished by substitutions on the cyclopentane ring and are designated accordingly by capital letters, e.g. see Figure 1. Members of the F series are further designated α or β depending upon the spatial arrangement of the OH group at position 9.

The unsaturated fatty acid precursors are normally not found free in any important quantity but are esterified as a component of phospholipids or other complex lipids. The first step in prostaglandin synthesis is therefore the release of the precursors from the bound esterified form in tissue stores by acylhydrolases such as phospholipase A_2.

Arachidonic acid, which can be obtained directly from the diet or by anabolic desaturation and chain elongation from dietry linoleic acid, is the most common fatty acid precursor of prostaglandins in membrane phospholipids (Moncada and Vane, 1979a).

After release from phospholipids, it is metabolised by two types of enzymes:

The lipoxygenases peroxidize arachidonic acid at different carbon atoms 5 and 12 to form unstable hydroperoxides (e.g. 5-HPETE and 12-HPETE) which then break down to stable hydroxyacids, e.g. 12-hydroxyarachidonic acid (HETE) or are further transformed to other products such as the recently
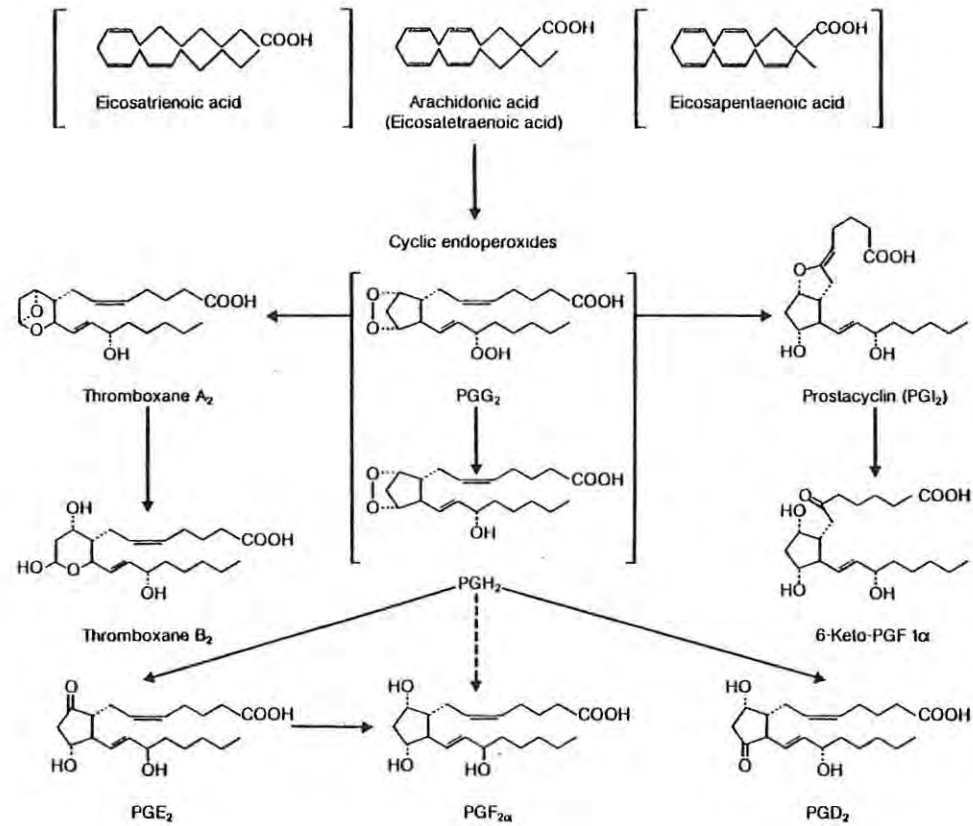
FIG.2: DIAGRAM SHOWING THE PROSTAGLANDIN PRECURSORS AND SOME IMPORTANT METABOLITES OF ARACHIDONIC ACID.

(Adapted from McGiff 1979)

described leukotrienes. (This pathway is reviewed by Moncada, Flower and Vane, 1980).

The second group of enzymes, the <u>cyclo-oxygenase</u> system, metabolises arachidonic acid to another range of products some of which are shown in Figure 2. The first step consists of an oxygenation and a cyclisation to form the cyclic endoperoxide derivatives PGG_2 and H_2. These unstable substances break down enzymatically or nonenzymatically to the more stable substances PGE_2, $PGF_{2\alpha}$ and PGD_2 and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) as well as to malondialdehyde (MDA). The prostaglandin endoperoxides are also transformed enzymatically into two other products, namely prostacyclin (PGI_2) and the chemically distinct thromboxane $A_2(TXA_2)$. These two unstable substances break down nonenzymatically to the more stable 6-keto-$PGF_{1\alpha}$ and TXB_2 respectively.

The final product formed from arachidonic acid varies with the type of tissue, the physical state of the animal and the presence of injury and disease.

Until recently it was believed that PGE_2 and $PGF_{2\alpha}$ were the most important prostaglandins. However, the discoveries of the last decade have caused a radical shift in emphasis towards the newer prostaglandins and the thromboxanes.

2.2.   <u>ISOLATION OF THE ENDOPEROXIDES AND DISCOVERY OF THROMBOXANES.</u>

Although an endoperoxide was proposed as an intermediate in the biosynthesis of prostaglandins by Samuelsson in 1965, it was only during the early 1970's that the unstable endoperoxides

were isolated (Hamberg and Samuelsson, 1973, Nugteren and Hazelhof, 1973 and Hamberg, Svensson, Wakabayashi and Samuelsson, 1974). The endoperoxides had some effects that could not be attributed to conversion into the known stable prostaglandins: they induced rapid aggregation when added to a suspension of washed human platelets (Hamberg, Svensson, Wakabayashi and Samuelsson, 1974) and were potent stimulators of the isolated rabbit aortic strip (Hamberg and Samuelsson, 1973 and Hamberg, Svensson, Wakabayashi and Samuelsson, 1974). This latter property led Samuelsson's group to compare the endoperoxides with the so-called rabbit aorta contracting substance (RCS) discovered by Piper and Vane in 1969. However, such factors as the difference in half lives between the endoperoxides and RCS, led them to look for additional substances. They reported (Hamberg, Svensson and Samuelsson, 1975) that PGG_2 was converted by platelets to an unstable, non-prostaglandin substance which they named thromboxane A_2 (TXA_2). TXA_2 degraded to the relatively inert substance, thromboxane B_2 (TXB_2). Thromboxane A_2 was more potent than the parent endoperoxide in inducing platelet aggregation (Hamberg, Svensson and Samuelsson, 1975) and in constricting rabbit aorta (Needleman, Moncada, Bunting, Vane, Hamberg and Samuelsson, 1976 and Bunting, Moncada and Vane, 1976). In fact, it has been concluded that the activity of RCS is mainly due to thromboxane A_2 (Hamberg, Svensson and Samuelsson, 1975 and Bunting, Moncada and Vane, 1976).

2.3.   DISCOVERY OF PROSTACYCLIN.

Moncada, Gryglewski, Bunting and Vane were interested in the
possibility that thromboxane A_2 synthesised in the
vasculature might explain the vasoconstriction which
immediately follows the cutting of a small vessel. They
accordingly searched the vessel wall for the thromboxane
synthetase which had been recently identified in platelets
(Needleman, Moncada, Bunting, Vane, Hamberg and Samuelsson,
1976).

This work led to the discovery of one of the most exciting
substances in the prostaglandin family. In 1976 they reported
that microsomes prepared from rabbit and pig aortas transformed
endoperoxides, not to thromboxanes, but to an unstable,
hitherto unknown substance not recognisable by their standard
bioassay tissues. They called the substance protaglandin X
(Moncada, Gryglewski, Bunting and Vane, 1976a and b and
Gryglewski, Bunting, Moncada, Flower and Vane, 1976).
Investigators of the Upjohn and Wellcome groups later
identified the chemical structure of prostaglandin X as
9-deoxy-6,9α-epoxy-Δ^5-$PGF_{1\alpha}$ and proposed the trivial name
prostacyclin for the substance (Johnson, Morton, Kinner,
Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada and
Vane, 1976). Finally, in acknowledgement of the alphabetical
progression of its predecessors, it was rechristened
prostaglandin I_2 (Nomenclature Announcement, 1977).

2.4.   STABILITY OF PROSTACYCLIN.

Prostacyclin is unstable in aqueous solutions, at neutral pH. Gryglewski, Bunting, Moncada, Flower and Vane (1976) reported the disappearance of its activity within 15 seconds on boiling or within 10 minutes at 37°C. Alkaline pH increases the stability (Cho and Allen, 1978 and Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada and Vane, 1976), and recent evidence suggests an extended stability in plasma or blood, possibly associated with binding to albumin (Vane, 1981).

Prostacyclin hydrolyses in aqueous solutions (Cho and Allen, 1978) to 6-keto-PGF$_{1\alpha}$ which was first described by Pace-Asciak in 1976. In fact, Pace-Asciak and co-author Wolfe postulated the structure of prostacyclin as a metabolite of arachidonic acid in rat stomach as early as 1971.

2.5.   BIOSYNTHESIS AND METABOLISM OF PROSTACYCLIN.

In addition to microsomes of rabbit aorta, pig aorta and mesenteric arteries, and rat stomach fundus all of which were shown to transform endoperoxides predominantly to prostacyclin, microsomal fractions from other tissues, e.g. rat stomach corpus and liver, rabbit lung, spleen, kidney and heart and ram seminal vesicles, generated much smaller amounts of prostacyclin together with other prostaglandin products (Moncada, Gryglewski, Bunting and Vane, 1976b and Gryglewski, Bunting, Moncada, Flower and Vane, 1976). Bunting,

Gryglewski, Moncada and Vane (1976) also reported that prostacyclin was produced by fresh chopped arterial tissue, either from exogenous arachidonic acid or endoperoxides, or from endogenous substrate. Early in 1977, Moncada, Higgs and Vane reported the generation of prostacyclin by human arterial and venous tissue. Thus, besides the fact that prostacyclin has been demonstrated to be the main prostaglandin generated by vascular tissue (Gryglewski, Bunting, Moncada, Flower and Vane, 1976, Moncada, Gryglewski, Bunting and Vane, 1976b and Johnson et al. 1976), it has also been shown to be formed by many vascular tissues from several species. Further examples include:- bovine coronary arteries (Raz, Isakson, Minkes and Needleman, 1977), foetal arteries (Powel and Solomon, 1977), the ductus arteriosus of the lamb (Pace-Asciak and Rangaraj, 1977) and bovine ductus arteriosus (Terragno, Terragno, McGiff and Rodriguez, 1977).

Prostacyclin is also the major prostaglandin released from isolated perfused rabbit and rat hearts (Isakson, Raz, Denny, Pure and Needleman, 1977 and de Deckere, Nugteren and ten Hoor, 1977).

The lung is of particular interest in relation to prostacyclin biosynthesis and metabolism. Unlike other prostaglandins such as E_2 and $F_{2\alpha}$, prostacyclin is not inactivated in vivo by passage through the pulmonary circulation (Armstrong, Lattimer, Moncada and Vane, 1978, Dusting, Moncada and Vane, 1978a and b, Pace-Asciak, Carrara and Nicolaou, 1978, Pace-Asciak, Rosenthal and Domazet, 1979 and Waldman, Alter, Kot, Rose and Ramwell, 1978). On the contrary the pulmonary circulation constantly

releases small amounts of prostacyclin into passing blood (Gryglewski, Korbut and Ocetkiewiez, 1978). Dusting, Moncada and Vane (1978b) have reported that, though the activity of prostacyclin does not disappear after passage through the lungs, it is inactivated in the hind quarters and the liver. The concentration of prostacyclin is thus higher in arterial than in venous blood (Moncada, Korbet, Bunting and Vane, 1978 and Hensby, Barnes, Dollery and Dargie, 1979). The lack of uptake of prostacyclin by the pulmonary vascular bed has prompted the suggestion that it may function as a circulating hormone (Gryglewski, Korbut and Ocetkiewicz, 1978, Moncada, Korbut, Bunting and Vane, 1978, Pace-Asciak and Carrara, 1978 and Gryglewski, 1979). Other work has, however, provided evidence against this possibility (Smith, Ogletree, Lefer and Nicolaou, 1978, Christ-Hazelhof and Nugteren, 1980, Steer, MacIntyre, Levine and Salzman, 1980, Haslam and McClenaghan, 1981 and Pace-Asciak, Carrara, Levine and Nicolaou, 1981). Quilley, McGiff, Lee, Sun and Wong (1980) suggest that 6-keto-PGE$_1$ may be one of the products of either prostacyclin or 6-keto-PGF$_{1\alpha}$ metabolism in the liver and that, since it is not inactive, it may account for some of the prolonged effects occasionally seen with prostacyclin. Though it contracts bovine coronary arterial strips, it is equipotent to PGI$_2$ in its ability to decrease mesenteric, renal and systemic vascular resistance and inhibit responses to pressor stimuli (Pontecorvo, Myers, Lippton and Kadowitz, 1981). However, its exact potency as an anti-aggregant is debated (Miller, Aiken, Shebuski and Gorman, 1980) and further research will be required to determine its relative importance as a potentially naturally occurring antithrombotic and vasodepressive molecule.

2.6.   BIOLOGICAL PROPERTIES OF PROSTACYCLIN.

Prostacyclin has two main properties:- (i) it is a potent
inhibitor of platelet aggregation and (ii) it relaxes vascular
smooth muscle. In terms of both activities it is opposite to
thromboxane A_2.

2.6.1.   INHIBITION OF PLATELET AGGREGATION.

The potent inhibitory effect of prostacyclin on in vitro
platelet aggregation was soon noted and it was shown to be some
30 times more potent in this respect than PGE_1 (Moncada,
Gryglewski, Bunting and Vane, 1976a and Gryglewski, Bunting,
Moncada, Flower and Vane, 1976). It inhibited in vitro
aggregation induced by a number of aggregants, e.g. arachidonic
acid, adenosine diphosphate (ADP), adrenaline, 5-hydroxy-
tryptamine (5HT), PGG_2, PGH_2 and collagen (Moncada,
Gryglewski, Bunting and Vane, 1976b). PGI_2 also inhibited
platelet aggregation in vivo: applied locally it inhibited
thrombus formation due to ADP in the microcirculation of the
hamster cheek pouch (Higgs, Higgs, Moncada and Vane, 1978) and
when given systemically, prevented electrically induced
thrombus formation in the carotid artery of the rabbit
(Ubatuba, Moncada and Vane, 1979). Prostacyclin was also able
to disaggregate platelets in vitro (Moncada, Gryglewski,
Bunting and Vane, 1976b) and in vivo (Ubatuba, Moncada and
Vane, 1979).

The ability of the vascular wall to actively prevent aggrega-

tion has been postulated before (Saba and Mason, 1974). For example, the presence of an ADP-ase in the vessel wall has led to the suggestion that this enzyme, by breaking down ADP, limits platelet aggregation (Heyns, van den Berg, Potgieter and Retief, 1974 and Lieberman, Lewis and Peters, 1977). While the Wellcome team have confirmed the presence of an ADP-ase (Bunting, Moncada and Vane, 1977), there is now much evidence that the anti-aggregatory activity of the wall is related mainly to the release of prostacyclin (Bunting, Moncada and Vane, 1977 and Bunting, Moncada, Reed, Salmon and Vane, 1978). Though vascular tissue utilises both arachidonic acid and endoperoxides as precursors in prostacyclin metabolism, it is far more effective in using endoperoxides (Bunting, Gryglewski, Moncada and Vane, 1976). Though disputed by some authors, there is considerable evidence (Moncada and Vane, 1979b) that the vessel wall can form prostacyclin not only from its own endogenous precursors, but also from endoperoxides released by platelets. Accordingly, a biological relationship between platelet and vessel has been suggested, such that platelets adhering to the vessel wall release endoperoxides which are then utilised by the vessel to produce prostacyclin which, in turn, prevents formation of platelet aggregates.

It is apparent that the prostaglandin endoperoxides are pivotal in arachidonic acid metabolism as they are precursors of substances with opposed biological properties. Prostacyclin inhibits platelet aggregation by stimulating adenyl cyclase thus leading to an increase in cyclic adenosine 3,5-mono-

phosphate (cAMP) levels in platelets (Gorman, Bunting and Miller, 1977 and Tateson, Moncada and Vane, 1977), and thromboxane A_2 reduces cAMP formation in platelets (Miller, Johnson and Gorman, 1977) and produces platelet aggregation. Owing to these opposite effects, it has been proposed (Moncada, Gryglewski, Bunting and Vane, 1976a) that a balance between the thromboxane generating system in platelets and the prostacyclin generating system in the vascular endothelium, regulates platelet aggregation. This proposal has been supported by Gorman, Bunting and Miller (1977) who constructed the postulated mechanism of platelet homeostasis illustrated in Figure 3. A further relationship between the two subtances is the ability of PGI_2 to prevent platelet TXA_2 production (Lefer, Ogletree, Smith, Silver, Nicoloau, Barnette and Gasic, 1978 and Dembinska-Kiec, Rücker and Schönhöffer, 1979).

While prostacyclin may provide an explanation for the resistance of the healthy vessel wall to the deposition of platelet aggregates, whether or not injury to the wall leads to thrombus formation, depends to a considerable extent on the amount of vascular damage. Prostacyclin inhibits platelet aggregation (platelet-platelet interaction) at lower concentrations than those needed to inhibit adhesion (platelet-collagen interaction) (Higgs, Moncada, Vane, Caen, Michel and Tobelem, 1978), suggesting that prostacyclin allows platelets to stick to vascular tissue and interact with it, while at the same time limiting thrombus formation (Higgs, Higgs, Moncada and Vane, 1978). Vascular damage leads to adhesion but not necessarily to thrombus formation. When the injury is minor, platelet thrombi are formed which break away
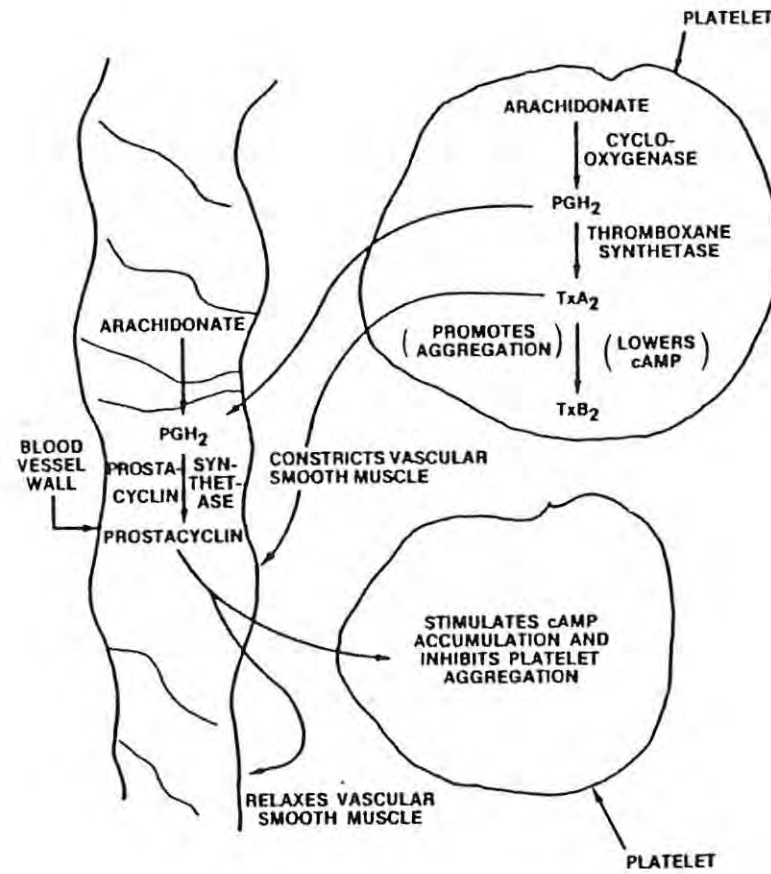
FIG.3: MODEL OF HUMAN PLATELET HOMEOSTASIS
PROPOSED BY GORMAN, BUNTING & MILLER
(1977)

from the vessel wall and are washed away by the circulation. For the development of thrombosis severe damage or physical detachment of the endothelium must occur (Moncada and Vane, 1979a). These observations are consistent with the distribution of prostacyclin synthetase, the concentration of which progressively decreases from the intima to the adventitia. These two opposing factors result in an anti-aggregatory endothelial lining and thrombogenic outer layer of the vascular wall (Moncada, Herman, Higgs and Vane, 1977).

2.6.2.   RELAXATION OF VASCULAR SMOOTH MUSCLE.

There are a few vascular strips, e.g. porcine coronary arteries (Dusting, Moncada and Vane, 1977a) and some strips of rat venous tissue (Levy, 1978) which are weakly contracted by prostacyclin in vitro. In human umbilical artery, prostacyclin has been shown to induce relaxation at low concentrations and contraction at higher concentrations (Pomerantz, Sintos and Ramwell, 1978). However, it relaxes most vascular tissues in vitro e.g. rabbit coeliac and mesenteric arteries (Bunting, Gryglewski, Moncada and Vane, 1976), bovine coronary arteries (Dusting, Moncada and Vane, 1977b and Needleman, Bronson, Wyche, Sivakoff and Nicolaou, 1978), cat coronary arteries (Lefer, Ogletree, Smith, Silver, Nicolaou, Barnette and Gasic, 1978 and Ogletree, Smith and Lefer, 1978), human and baboon cerebral arteries (Boullin, Bunting, Blaso, Hunt and Moncada, 1979), and lamb ductus arteriosus (Coceani, Bishai, Bodach, White and Olley, 1979). Sintetos, Bolger and Ramwell (1979) have reported that while prostacyclin did not relax canine

renal artery or vein strips with basal resting tone, it induced relaxation in contracted preparations.

In vivo, it causes vasodilation and hypotension. Passage of arachidonic acid across various circulations of the anaesthetised dog, e.g. hind quarter and pulmonary circulations, results in the production of prostacyclin-like material and a concomitant vasodilatation (Mullane, Moncada and Vane, 1979). Langendorff perfused hearts of rabbits, rats and guinea pigs convert arachidonic acid principally to prostacyclin and the dilation of coronary arteries observed is due to this metabolite (de Deckere, Nugteren and ten Hoor, 1977, Needleman, Bronson, Wyche, Sivakoff and Nicolaou, 1978 and Schrör, Moncada, Ubatuba and Vane, 1978).

Direct application of prostacyclin produces vasodilation in several vascular beds : for example, local injection into the coronary circulation increased coronary blood flow in anaesthetised open-chested dogs (Armstrong, Chapple, Dusting, Hughes, Moncada and Vane, 1977 and Dusting, Chapple, Hughes, Moncada and Vane, 1978). In the same model prostacyclin applied epicardially to the left ventricle also caused marked and prolonged coronary vasodilation (Dusting, Chapple, Hughes, Moncada and Vane, 1978). Prostacyclin also causes vasodilation in the mesenteric and hind limb circulation in the dog (Dusting, Moncada and Vane, 1978c), on the precapillary side of the microcirculation of the hamster cheek pouch (Higgs, Cardinal, Moncada and Vane, 1979), in the pulmonary vascular bed of the foetal lamb (Leffler and Hessler 1979) and locally, in a portion of the epicerebral network when applied topically

to the cerebral cortex of the dog (Branan, Poulsen, Wolfe, Dila, Hodge and Feindel, 1979).

In the dog, prostacyclin infused intrarenally increased renal blood flow and urinary excretion of sodium and potassium (Bolger, Eisner, Ramwell, Slotkoff and Corey, 1978 and Hill and Moncada, 1979). Bolger et al. gathered data to suggest that the net effect of prostacyclin on renin release, is the summation of its stimulatory action by means of haemodynamic changes and/or direct stimulation of the juxtaglomerular apparatus, versus a dampening effect produced by altered sodium transport at the macula densa. There is increasing evidence, reviewed by Moncada and Vane (1980a), that prostacyclin mediates the release of renin from the renal cortex.

The vasodepressor effect of prostacyclin produces hypotension when administered to anaesthetised rats and rabbits (Armstrong, Lattimer, Moncada and Vane, 1978), the anaesthetised cat (Lefer, Ogletree, Smith, Silver, Nicolaou, Barnette and Gasic, 1978), the anaesthetised dog (Armstrong, Chapple, Dusting, Hughes, Moncada and Vane, 1977 and Fitzpatrick, Alter, Corey, Ramwell, Rose and Kot, 1978) and anaesthetised rhesus monkeys (Fletcher and Ramwell, 1979).

The vasodilator effects of prostacyclin caused a lowering of diastolic blood pressure when infused in conscious man. The heart rate was also increased (O'Grady, Warrington, Moti, Bunting, Flower, Fowle, Higgs and Moncada, 1979 and Szczeklik and Gryglewski, 1979). At doses higher than those required to produce tachycardia and change in diastolic pressure,

prostacyclin produced bradycardia and a distinct fall in both systolic and diastolic pressure (Szczeklik, Gryglewski, Nizankowski, Musial, Pieton and Mruk, 1978). This effect is probably mediated by a vagal reflex, for in dogs, prostacyclin produces a bradycardia which is abolished by vagotomy or reversed by atropine (Chapple, Dusting, Hughes and Vane, 1978 and 1980). These authors also report that the hypotensive effects of prostacyclin were reduced by the vagotomy to a greater extent than by atropine, suggesting that the hypotension has at least two components: direct relaxion of vascular smooth muscle and reflex non-cholinergic vasodilation.

2.7. POSSIBLE IMPORTANCE OF PROSTACYCLIN AND A PROSTACYCLIN THROMBOXANE BALANCE.

2.7.1. THERAPEUTIC POTENTIAL OF PROSTACYCLIN.

Because of its biological properties, there has been considerable interest in the possible usefulness of prostacyclin or chemical analogues as "hormone replacement therapy" in various pathological conditions. It has been suggested that these properties may be potentially useful in such situations as: unstable angina (Borer, 1980), acute myocardial ischaemia (Lefer, Ogletree, Smith, Silver, Nicolaou, Barnette and Gasic, 1978), deep vein thrombosis, different types of shock, disseminated intravascular coagulation and organ transplantation (Moncada and Vane, 1980b).

Infusions of prostacyclin have been used with a degree of success in patients with advanced peripheral arterial disease of the lower extremities (Szczeklik, Nizankowski, Skawinski, Szczeklik, Głuszko and Gryglewski, 1979 and Szczeklik, Gryglewski, Nizankowski, Skawinski, Głuszko and Korbut, 1980) and have been shown to have beneficial effect when used in several extracorporeal systems in experimental animals. Preliminary indications are that in patients receiving prostacyclin during cardiopulmonary bypass, platelet number and function is better preserved and blood loss decreased in the post-operative period (O'Grady, 1980).

Obviously, the most direct approach to antithrombotic therapy in general will be a method directed at increasing platelet cAMP, i.e. prostacyclin or an analogue (with or without a phosphodiesterase inhibitor), or a substance which stimulates endogenous prostacyclin production. This is so because increasing platelet cAMP inhibits most forms of aggregation, whether or not they are dependent on arachidonic acid metabolic products (Moncada and Vane, 1980a). However, other attempts at thrombosis prevention, which relate to manipulation of the proposed thromboxane/prostacyclin haemostatic mechanism, have received widespread attention.

2.7.2.   THROMBOXANE SYNTHETASE INHIBITION.

Selective inhibition of thromboxane synthetase is one approach to the development of antithrombotic agents. Such inhibition

might result, not only in preventing formation of the pro-aggregatory thromboxane A_2, but also in endoperoxide metabolism being directed towards prostacyclin production when the alternative thromboxane pathway is blocked. Nijkamp, Moncada, White and Vane (1977) have shown that during selective inhibition of thromboxane A_2 synthesis with imidazole, formation of other products of endoperoxide metabolism is enhanced.

2.7.3.   ASPIRIN.

A further consequence of the discovery of prostacyclin has been the need to re-examine the use of aspirin as an antithrombotic compound.

Aspirin and other aspirin-like drugs inhibit generation of thromboxane A_2 in platelets, and that is why, prior to the discovery of prostacyclin, aspirin was widely promoted as an antithrombotic drug (Moncada and Vane, 1980b). However, since these agents exert their inhibitory effect by acetylation (Roth and Majerus, 1975) of cyclo-oxygenase, it is now clear that they also inhibit prostacyclin formation by the vessel wall. Many workers have investigated the possibility of adjusting the aspirin dosage schedule so as to produce a greater inhibitory effect on thromboxane A_2 than on prostacyclin production. This work is reviewed by Moncada and Vane (1980a). While it appears that the effect of aspirin on thromboxane A_2 synthesis by platelets is of longer duration than its inhibitory

effect on prostacyclin synthesis by the vessel wall, further studies are required to determine the dose regimen of aspirin which will inhibit thromboxane A_2 production, while permitting continuing prostacyclin synthesis (Preston, Whipps, Jackson, French, Wyld and Stoddard, 1981).

2.7.4.   <u>POSSIBLE DIETARY MANIPULATION.</u>

Dyerberg and Bang (1978) have suggested that the low incidence of acute myocardial infarction in Greenland Eskimos could be due not only to their plasma lipid profile but also to the relative lack of substrate required for the formation of proaggregatory prostaglandins by platelets. O'Grady (1980) has reviewed evidence that the plasma lipids of these Eskimos contain only small amounts of arachidonic acid and relatively high amounts of eicosapentaenoic acid, the latter being transformed in platelets to thromboxane A_3, which in contrast to thromboxane A_2 derived from arachidonic acid, is not pro-aggregatory. However, the vessel wall can utilise eicosapentaenoic acid to synthesise an anti-aggregatory substance, probably prostaglandin I_3 (Dyerberg, Bang, Stoffersen, Moncada and Vane, 1978). The possibility that dietary supplementation, or medication with eicosapentaenoic acid would provide prophylaxis against arterial thromboembolic disease remains to be explored.

2.8.   CHANGES IN PROSTACYCLIN AND/OR THROMBOXANE A$_2$ IN PATHOLOGICAL

SITUATIONS.

The discovery of prostacyclin opened a new field of investigation into whether or not prostacyclin and/or thromboxane levels may be altered in various pathological conditions.

One disorder which has received much attention is atherosclerosis .

2.8.1.   ATHEROSCLEROSIS.

Lipid peroxidation, induced by free radical formation, is known to occur in certain pathological conditions (Slater, 1972). The role of lipid peroxides in the development of atherosclerosis has been debated since Glavind, Hartmann, Clemmesen, Jessen and Dam (1952) described the presence of lipid peroxides in human atherosclerotic aortas and reported the peroxide content to be directly proportional to the severity of the atherosclerosis. Though this work was subsequently disputed, Moncada and Vane (1979a) in their review mention a number of authors whose work has supported the findings of Glavind et al. Recently it has been shown that a lipid peroxide 15-hydroperoxy arachidonic acid (Bunting, Gryglewski, Moncada and Vane, 1976, Gryglewski, Bunting, Moncada, Flower and Vane, 1976 and Moncada, Gryglewski, Bunting and Vane, 1976b) inhibits prostacyclin formation. Other fatty

acid peroxides and their methyl esters behave similarly (Salmon, Smith, Flower, Moncada and Vane, 1978) as do other substances related to atherosclerosis such as low density lipoproteins (Nordøy, Svensson, Wiebe and Hoak, 1978).

Accumulation of lipid peroxides in atheromatous plaques could predispose to thrombus formation by inhibiting prostacyclin formation by the vascular wall without impairing thromboxane A_2 generation by platelets (see Figure 4 proposed by Gryglewski, 1980). Indeed, Dembinska-Kiec, Gryglewska, Zmuda and Gryglewski (1977) have found that there is a reduction in prostacyclin formation by vessels of rabbits made atherosclerotic by diet. (Though after 5 months there is a tendency for PGI_2 synthesising capacity to recover (Gryglewski, Dembinska-Kiec, Zmuda and Gryglewska, 1978).) Similarly, it has been reported that human atherosclerotic tissue does not produce prostacyclin though normal vessel does (D'Angelo, Villa, Mysliewiec, Donati and de Gaetano, 1978). In addition, arteries of species susceptible to the development of atherosclerotic lesions have a lower capacity for the synthesis of PGI_2 (Sinzinger, Silberbauer, Winter and Clopath, 1979).

Since cell proliferation in vitro is inhibited by substances which stimulate cAMP formation, it has been suggested that prostacyclin may play a role in regulation of cell growth in the vascular wall, and that smooth muscle proliferation in atherosclerotic plaques (Bendit, 1977) might be a consequence of inhibition of prostacyclin generation by lipid peroxides (O'Grady, 1980).

It is interesting to note, that in addition to decreased
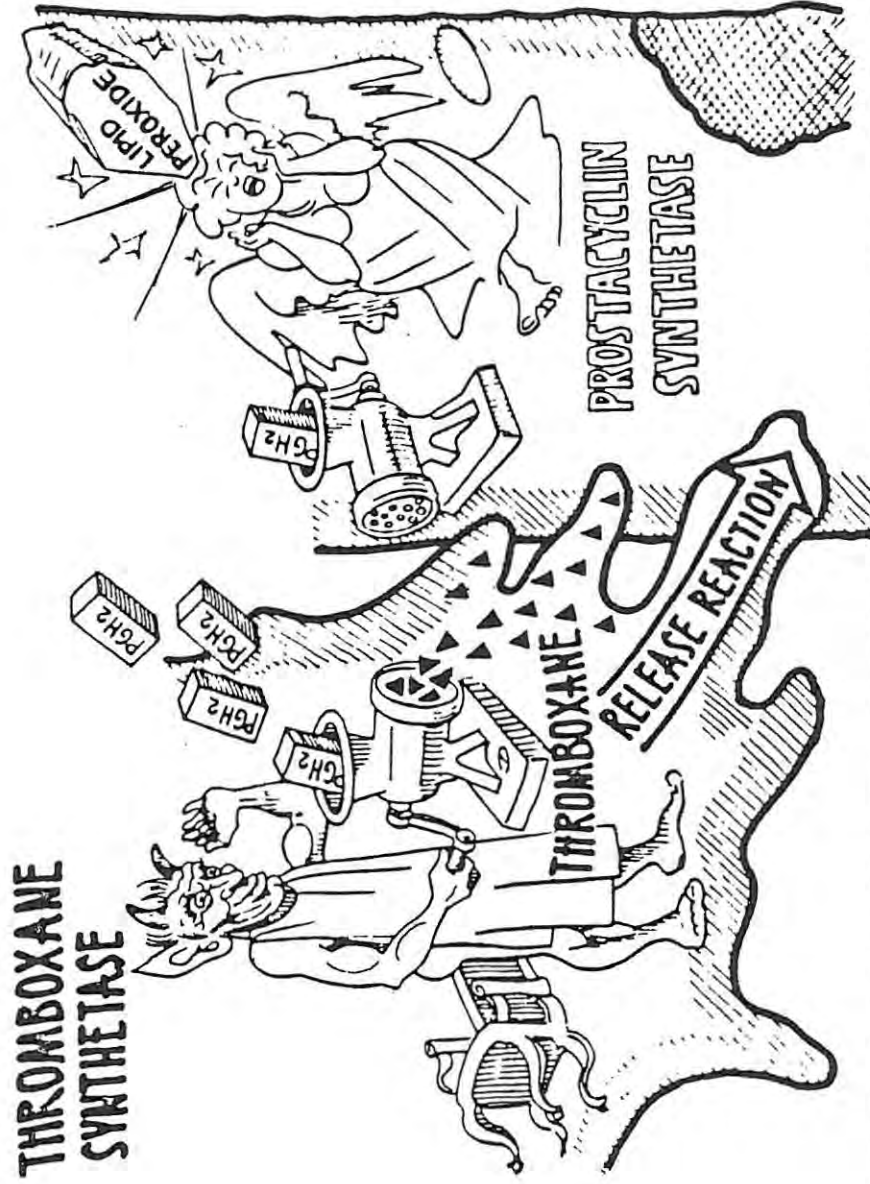
FIG.4: HOW ATHEROSCLEROSIS MAY BE INITIATED AS PROPOSED BY
GRYGLEWSKI (1980)

prostacyclin generation by the vasculature in rabbits made atherosclerotic by diet, at certain stages (Gryglewski, Dembinska-Kiec, Zmuda and Gryglewska, 1978) their platelets generate increased amounts of thromboxane A_2 and are more sensitive than controls to the actions of arachidonic acid, thromboxane A_2 and prostacyclin (Zmuda, Dembinska-Kiec, Chyckowski and Gryglewski, 1977).

2.8.2.   DIABETES.

The incidence of atherosclerosis and thrombosis is increased in patients with diabetes mellitus (Keen, 1976 and Timperley, Ward, Preston, Duckworth and O'Malley, 1976) and abnormalities of platelet function have been described in diabetic patients (Breddin, Grun, Krzywanek and Schremmer, 1976 and Ferguson, Mackay, Philip and Sumner, 1975). The possibility that disturbance in prostacyclin and/or thromboxane A_2 might be related to the development of diabetic angiopathy has been investigated. Reduced generation of prostacyclin by vascular tissue (Harrison, Reece and Johnson, 1978, Johnson, Harrison, Raferty and Elder, 1979, Silberbauer, Schernthaner, Sinzinger, Piza-Katzer and Winter, 1979 and Gerrard, Stuart, Rao, Steffes, Mauer, Brown and White, 1980), and elevated thromboxane generation by platelets (Butkus, Skrinska and Schumacher, 1980 and Gerrard, Stuart, Rao, Steffes, Mauer, Brown and White, 1980) have been reported for diabetic experimental animals and patients.

2.8.3. OTHER DISORDERS IN WHICH ABNORMAL LEVELS OF PROSTACYCLIN, THROMBOXANES OR ENDOPEROXIDES HAVE BEEN REPORTED.

Platelets of patients with arterial thrombosis and post-surgical deep vein thrombosis synthesise more prostaglandin endoperoxides, one minute after being stimulated with collagen, than do those of controls (Lagarde and Dechavanne, 1977). Platelets from patients who have survived myocardial infarction produce more thromboxane A_2 than those from controls and in addition, require significantly less thromboxane A_2 to initiate their aggregation (Szczeklik, Gryglewski, Musial, Grodzinska, Serwonska and Marcinkiewicz, 1978). Lewy, Smith, Silver, Wiener and Walinsky (1979) have demonstrated elevated thromboxane B_2 levels in the blood of patients with Prinzmetal's angina (Moncada and Vane, 1980a). Specimens of venous tissue from patients with renal failure have been shown to produce more prostacyclin-like activity than controls (Remuzzi, Cavenaghi, Mecca, Donati and de Gaetano, 1977). Remuzzi, Misiani, Marchesi, Livio, Mecca, de Gaetano and Donati (1978) suggest that patients with haemolytic-uraemic syndrome or related conditions lack a plasma factor which stimulates vascular prostacyclin activity. Defective prostacyclin activity could favour the formation of platelet thrombi in the microcirculation which is typical in such disorders. Patients with Bartter's syndrome excrete in the urine about four times as much 6-oxo-PGF$_{1\alpha}$ as do controls (Güllner, Cerletti, Bartter, Smith and Gill, 1979).

2.8.4.   HYPERTENSION.

Hypertension is characterised by an abnormal sustained elevation of the basal systemic arterial blood pressure and is associated with vascular complications accompanied by increased tendency of platelets to aggregate (Lentini and Bologna, 1974). This is, therefore, a disorder in which prostacyclin would obviously be of interest in view of its properties as a vasodilator and potent anti-aggregant, and its predominant production in the blood vessel wall.

In most forms of hypertension, cardiac output is normal, and the raised pressure is thus due to an increase in peripheral resistance (Pickering, 1974). The increase in peripheral resistance may result from morphological or functional changes of the arterioles, or from an alteration in any of the many complex and integrated mechanisms which function to maintain arterial pressure.

2.8.4.1. Morphological and Functional Changes in Arterioles.

Several changes in the arteriolar smooth muscle in hypertension have been described including a reduction in cAMP and increase of the muscle's permeability to various cations (Fasciolo, 1979). Folkow, Grimby and Thulesius (1958) list a number of authors who have suggested that a generalised hypertrophic thickening of the arteriolar wall takes place in chronic hypertension more or less independent of its original background. These authors point out that the existence of an increased wall mass causes a proportionally bigger lumen

decrease and hence resistance to flow for a given smooth muscle shortening, i.e. a structurally based "potentiation" of a vasoconstrictor response is obtained by virtue of an anatomical change in the vessel.

2.8.4.2. <u>Blood Pressure Control Systems - Alteration of which may be Involved in Hypertension</u>.

In certain types of secondary hypertension, that is hypertension which is secondary to some identifiable cause, e.g. phaeochromocytoma, involvement of one of these systems is clearly implicated. However, for the most part,particularly in primary or essential hypertension (which is the largest single form of hypertension), no cause has been clearly defined .

2.8.4.2.1. <u>Sympathetic Nervous System, Sympathomimetics and Adreno-receptors</u>.

Alpha and beta adrenoreceptors participate in blood pressure regulation (especially in the short term) and pharmacological manipulation of adrenergic mechanisms, both peripherally and centrally, lowers blood pressure in the long term. This manipulation is the basis of much successful antihypertensive therapy today.

Neural mechanisms in hypertension have recently been reviewed by Brody, Haywood and Touw (1980). Some data, such as direct recording of increased peripheral sympathetic nerve activity, supports the contribution of increased sympathetic activity to hypertension in Okamoto spontaneously hypertensive rats (SHR).

However, other data suggests that in conscious SHR, removal by ganglion blockade of sympathetic activity does not produce a greater fall in vascular resistance than that observed in normotensive, age matched controls. Certain studies suggest a central impairment of brain catecholamine function in SHR, characterised by a decrease in hypothalamic catecholamines and an increase in brain stem adrenaline and noradrenaline. However, the critical site(s) of central catecholamines in hypertension remain unclear.

In man, increased adrenal secretion of adrenaline and noradrenaline is responsible for the raised arterial pressure of phaeochromocytoma. However the role of the sympathetic nervous system and of adrenal medullary hormones in essential hypertension is debatable. Excessive vessel constriction is not due to overaction of sympathetic nerves, for it persists in hand, brain and kidney when sympathetic action is eliminated (Pickering, 1974). Estimations of plasma or urinary catecholamines give, at best, a shadowy indication of activity of the sympathetic system, and while some claims have been made that patients with essential hypertension have a greater output of catecholamines than age matched, normotensive patients (reviewed by Sleight, 1980), other authors (e.g. Reid, Jones and Hamilton, 1978) are not able to confirm this. Reid et al. also report that any increased pressor responsiveness to infused noradrenaline appears to be shared with other pressor agents and is probably secondary to hypertrophy of vascular smooth muscle.

It appears that there is, to date, no unequivocal evidence that increased activity of the sympathetic nervous system, increased

catecholamine levels or increased specific sensitivity of adrenoreceptors to catecholamines underlie the raised blood pressure of human essential hypertension.

2.8.4.2.2. Baroreceptor Reflexes

The existence of a long term influence by baroreceptors on blood pressure has been questioned, but some regard it as important. Their views are supported by the observations that in experimental animals interference with the baroreceptor reflex pathway centrally, or in the peripheral afferent arc, not only increases lability of arterial pressure but produces elevated arterial pressure in several species (Brody, Haywood and Touw, 1980). In addition, when the baroreceptors are exposed to a high arterial pressure, they are gradually "reset", so that eventually their output is similar to normal although the pressure stimulating them is abnormally high (Zanchetti, 1979). Thus sensitivity of the reflex varies inversely with arterial pressure (Pickering, 1974).

It is also unknown whether loss of reflex sensitivity is cause or effect, i.e. is hypertension caused by loss of reflex control or does hypertension damage the receptors by, for example, increasing the rigidity of the artery and thus the pressure increment required to produce a given stretch of the receptor? Both mechanisms may possibly be operative, producing a vicious cycle. Whatever the cause of hypertension in a given case, pressure could not be corrected efficiently if baroreceptor function were compromised.

2.8.4.2.3. Renin, Angiotensin and Aldosterone

Few would disagree that the kidney is of importance in the determination of arterial pressure. For example, it has been shown that the high blood pressure of selectively bred, spontaneously hypertensive rats can be lowered by transplanting the kidneys of normotensive rats and vice versa (Bianchi, Fox, di Francesco, Giovanetti and Pagetti, 1974). Since Goldblatt, Lynch, Hanzel and Summerville (1934) produced hypertension in dogs by constricting the renal arteries with clamps, it has become clear that many different renal lesions could lead to hypertension. The role of the renin-angiotensin system has received much attention. From its strategic origin in the kidney it serves a vital function in the homeostatic mechanisms of the body. It is a servomechanism regulating sodium exchange and blood volume through its action on receptor sites in the adrenal cortex, releasing aldosterone, an important regulator of water and electrolyte metabolism and the volume of the fluid compartments of the body. Angiotensin also acts on receptor sites of vascular smooth muscle, on the kidney and on the central nervous system; thus it may participate in the regulation of blood flow through the kidney, the tone of vascular smooth muscle, the production of renal prostaglandins and possibly, to a small degree, controls the modulation of arterial blood pressure by the central nervous system (Grollman, 1980).

As regards the importance of the renin-angiotensin system in hypertension, some authors, e.g. Page (1980) feel "it is highly

probable that the core of the mechanism of essential, renovascular and malignant hypertension is angiotensin." On the other hand, Grollman, in his recent survey of hypertension (1980), cites the mass of incontrovertible data available, proving that angiotensin II, or its active des-aspartic acid derivative angiotensin III, is not concerned in the pathogenesis of clinical or experimental hypertension and he points out that there is no correlation between the severity of hypertension and peripheral renin activity.

2.8.4.2.4. <u>Kinins and Prostaglandins</u>

While development of hypertension may reflect changes in one or more of several pressor hormones, e.g. overactivity of the renin-angiotensin system or a mineralocorticoid excess, a failure of opposing forces that normally act to lower blood pressure, may be of no less importance.

It is now believed that defects of vasodilatory systems, such as the prostaglandins and the kallikrein-kinin systems operating to offset vasoconstrictor influences, may be important in the aetiology of hypertension.

Renal prostaglandins are theoretically integrated with the kallikrein-kinin and renin-angiotensin systems in a vasoactive hormone complex summarised in Figure 5.

The role of prostaglandins and kinins in the regulation of blood pressure has recently been reviewed by McGiff and Quilley (1980). The kinins (such as bradykinin), released by the action of the enzyme kallikrein, are polypeptides having
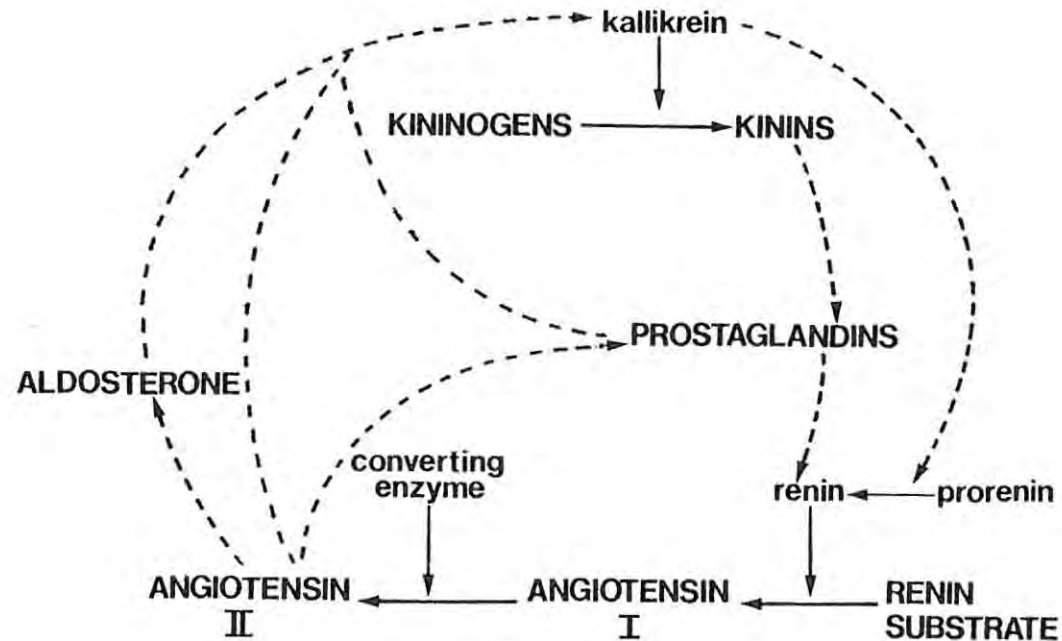
FIG.5: THE RENIN-KALLIKREIN-PROSTAGLANDIN SYSTEM.

(FitzGerald, Hossman Hummerich & Konrads 1980)

Kinins directly stimulate prostaglandin release which
in turn stimulates renin release. Angiotensin II stimulates
aldosterone, prostaglandin and kallikrein release.
Aldosterone is a potent activator of kallikrein which
in turn activates production of renin from prorenin.

vasodilator and diuretic properties; it has been suggested that they participate in the regulation of blood pressure. A defect of kallikrein release and subsequent deficient generation of kinins could contribute to the pathogenesis of some forms of hypertension. Excretion of kallikrein has been reported to be decreased in human and experimental forms of hypertension. A component of this possible role in blood pressure regulation is the ability of kinins to release prostaglandins. The released prostaglandins usually augment the action of kinins.

Local prostaglandins may be an important determinant of vascular reactivity. For example, prostaglandin E_2 released in response to a pressor stimulus (angiotensin II, noradrenaline or adrenergic nerve excitation), has been shown to attenuate the vasoconstrictor action of that stimulus (McGiff and Quilley, 1980). On the other hand, $PGF_{2\alpha}$ may enhance the pressor response of the vasculature (Hedqvist, 1976). These observations have suggested that an alteration in production or activity of various prostaglandins could be involved in hypertension.

Though Hornych (1978) has generally found PGE_2 to be significantly elevated in the peripheral and renal venous blood of hypertensive patients, basal urinary excretion of PGE_2 (which also usually has a direct vasodilator action on resistance vessels (Nowak, 1979)), has been shown to be low in an appreciable number of patients with essential hypertension (Weber, Siess and Scherer, 1980). Weber et al. have also reported a reduced capacity of the essential hypertensive kidney to produce vasodilating compounds (PGE_2 and kallikrein) upon challenge with furosemide.

The possible significance of renal prostaglandins in essential hypertension has been discussed recently by Weber, Siess and Scherer (1980):

In vivo and in vitro studies show that renal formation of prostaglandins, possibly in the vasculature of the cortex, represents an essential step in the mechanisms regulating the secretion of renin. Many of the experimental conditions known to be associated with alterations of renin secretion, renal blood flow or glomerular filtration rate have also been shown to lead to changes in renal prostaglandin formation. However, because of the complex interrelation it is difficult to delineate the relative importance of PGs and renin and the sequence of events operating in different functional states of the kidney.

It is suggested that an impairment in renal production of vasodilating and renin-stimulating PGs e.g. PGE_2 (which could also lead to unbuffered response to pressor substance) could be the common denominator for both the reduced renin secretion and increased vascular resistance, which have been reported to be associated in essential hypertension.

McGiff and Quilley, (1981) have highlighted the importance of species difference when using the rat as a model for human hypertension, particularly when studying renal prostaglandins. In the rat, unlike the human, prostaglandins of the E series constrict renal blood vessels and enhance the vasoconstrictor response to sympathetic nerve stimulation. Thus, they could actually contribute to elevation of blood pressure. This anomalous constrictor response of the renal vasculature of the rat to PGE_2, is exaggerated in the New Zealand strain of

genetically hypertensive rat (GH), where an abnormality of PG metabolism has been found which could account for the elevation in pressure. There is evidence for a vasodilator mechanism in the rat kidney, probably subserved by PGI_2 or its active metabolite 6-keto-PGE_1 which, unlike PGE_2, can dilate the renal vasculature in this species.

2.9.   <u>AIMS OF THE STUDY.</u>

There is clearly a mosaic of interrelated, vasoregulatory mechanisms, derangement of any of which may be related to the increased peripheral vascular resistance, which is characteristic of elevated arterial pressure.

The fact that prostacyclin is locally generated in the arterial wall and can relax arterial smooth muscle, suggests that it may play a part in the control of vascular tone. It is therefore a substance of obvious interest in hypertension. In fact, Moncada, Gryglewski, Bunting and Vane (1976a) suggested in their paper reporting the discovery of prostacyclin, that a deficiency could perhaps underlie some forms of hypertension.

The anti-aggregatory properties of prostacyclin are also of interest : platelet adhesiveness and ADP-induced aggregation in vitro have been reported to be increased in patients with hypertension (Coccheri and Fiorentini, 1971, Bologna, 1974 and Lentini and Bologna, 1974). It has been suggested (Lentini and Bologna, 1974) that this may be a major determinant of the thrombotic and atherosclerotic complications of hypertensive vascular disease.

The aims of this study were accordingly to investigate, using a rat model:

a) Whether there was any alteration in vascular (aortic) prostacyclin production in animals with elevated blood pressure.

b) Whether subsequent manoeuvres designed to lower elevated pressure affected aortic prostacyclin production.

c) The in vitro sensitivity of platelets of hypertensive animals to the effect of ADP and prostacyclin.

In the text, after consideration of general materials and methods, each study is presented individually together with the immediately obvious conclusions. All the findings and their implications are discussed in the final chapter of this thesis.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1.   <u>EXPERIMENTAL ANIMALS.</u>

The entire study involved the use of albino rats.   These were all housed under the same conditions, received a standard diet of United Oil Epol rat cubes and were allowed unrestricted access to tap water.

3.2.   <u>MEASUREMENT OF RAT BLOOD PRESSURE</u>

Arterial systolic blood pressures were measured in conscious animals by a modification of the tail cuff method developed in this laboratory by Lockett, Leary and Asmal (1979).   Briefly, the procedure is as follows : After spending approximately 20 minutes in a warming box at 39°C the rat is placed in a small, cylindrical, wire, restraining cage.   Its tail, after being passed through a small pressure cuff, has a Whitney mercury silastic strain gauge wound around it.   Output from the strain gauge and from a pressure transducer (connected to the tail cuff sphygmanometer) are recorded simultaneously on a potentiometric recorder (Figure 6).   Cuff pressure is raised to a point above the expected systolic pressure and slowly released.   The strain gauge registers pulsations once caudal arterial pressure exceeds cuff pressure.   Joining this point to the pressure curve gives the value of the systolic arterial pressure which is measured against 100 mm Hg standard.
An average of three readings was recorded as the blood pressure of the animal.
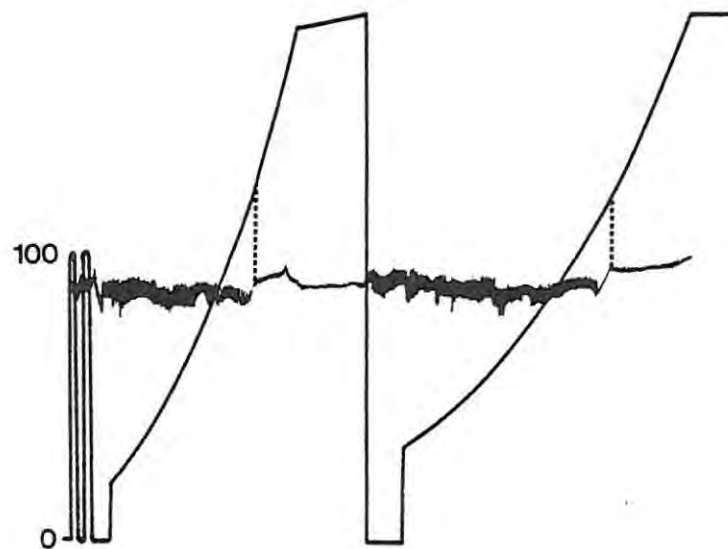
FIG.6: DIAGRAM OF RECORDER TRACING OF BLOOD PRESSURE
MEASUREMENT.

Strain gauge output (tail pulsations) is shown in the middle
of the trace. The sloping line running top to bottom indicates
pressure in mmHg. 100mm Hg standard is shown on the left.
The two measurements shown indicate a rat with systolic
arterial pressure of 120mmHg

3.3.   ASSAY OF PROSTACYCLIN

The ability of prostacyclin to inhibit the in vitro aggregation of blood platelets, provides a useful method for direct assay of the substance. Human blood platelets were used to detect prostacyclin in these studies.

3.3.1.   PREPARATION AND STORAGE OF PLATELET RICH AND PLATELET POOR PLASMA.

Human venous blood was obtained from healthy, male, adult Indian volunteers who had taken no drugs during the preceeding 2 weeks. On each occasion blood was collected from the antecubital vein and immediately added to 3,8°/o trisodium citrate (9 parts blood : 1 part citrate) as anticoagulant (Biggs, 1976).

After careful mixing, the sample was centrifuged at room temperature at 160g for 10 minutes (Rubin, Weston, Bullock, Roberts, Langley, White and Williams, 1977). Only the top two thirds of the generated platelet rich plasma (prp) was utilised in the study as leucocytes, present in the lower third, are a rich source of prostacyclin synthesising enzymes (Blackwell, Flower, Russel-Smith, Salmon, Thorogood and Vane, 1978).

A small portion of the prp was centrifuged for 15 minutes at 2000g (Sagel, Colwell, Crook and Laimins, 1975) at 4°C to yield a supernatant of platelet poor plasma (ppp). This low temperature causes better sedimentation of fibrinogen and cells (Marcus, 1978).

The remainder of the prp was dispensed in 450 µl quantities into siliconised glass cuvettes. These were kept at room temperature until used. Cooling of platelets below this temperature was considered inadvisable as considerable variation occurs in platelet shape during cooling (Zucker and Borrelli, 1954).

During this period, prp was kept under 5°/∘ carbon dioxide : 95°/∘ oxygen (Carbogen, Afrox) (Weksler, Ley and Jaffe, 1978). This procedure ensured that loss of carbon dioxide, and an associated rise in pH of the prp did not occur. Han and Ardlie (1974) have reported that the pH of prp increases during storage at room temperature and that this change is associated with increased response to ADP.

Care was taken that the assay was completed and platelets were used within 120 minutes of drawing blood because Cronberg (1971) has shown that when plasma is stored for this period, though the number of aggregating platelets remains the same, 'stickiness' decreases.

It has been known since the observations of Ranvier and Vulpian in 1873 that platelets adhere to glass surfaces (Ulutin, 1976), therefore blood and plasma were handled exclusively with plastic and siliconised glass apparatus throughout these studies.

3.3.2.   PLATELET COUNTS.

Platelet counts were carried out on both prp and ppp. Plasma was diluted 1 in 20 with trisodium citrate 3,2°/∘ in a plastic

tube and, after mixing, a sample was transfered to a Newbauer counting chamber using a capillary tube. The chamber was placed in a petri dish containing damp filter paper (to provide a moist atmosphere to prevent drying) and left for 40 minutes. Ashman (1976) has shown that at least 40 minutes is required for all platelets to settle. After this time platelets were counted, in duplicate, under x40 magnification.

Only prp with counts between 300 000 and 500 000 /mm^3 (i.e. within normal range) were used in these studies. Ppp never contained more than 2000 platelets/mm^3.

3.3.3. AGGREGATION OF PLATELETS.

3.3.3.1. Aggregant.

A number of substances, including epinephrine, serotonin (Mitchell and Sharp, 1964), collagen (Wilner, Nossel and Le Roy, 1968) and arachidonic acid (Moncada, Gryglewski, Bunting and Vane, 1976a) can be used to aggregate platelets in vitro. Adenosine 5'-diphosphate (ADP) (Gaarder, Jonsen, Laland, Hellem and Owren, 1961) was used as aggregant in these studies. Adenosine 5'-diphosphate grade I sodium salt was obtained from Sigma Ltd., St. Louis, U.S.A. Sigma reports that decomposition can be reduced by adjusting the pH to 6,8 and storing the solution at -20°C. A stock solution of ADP (1 mg/ml) was therefore prepared in tris acid maleate buffered saline (Sano, Boxer, Boxer and Yokoyama 1971) at pH 6,8 and 0,5 ml aliquots were stored at -20°C. Immediately before use, aliquots were diluted with the same buffered saline to produce the required concentrations of ADP.

3.3.3.2   Photometric Measurement of Aggregation.

The photometric method first described by Born (1962), is valuable for investigating platelet aggregation in vitro and for the quantitative study of substances influencing this process.The method depends on the continuous measurement of changes in transmission of light of constant wave length, through a suspension of platelets. When platelets aggregate, the optical density decreases because the light scattering power of the comparatively few coarse particles present at the end of the experiment, is less than that of the many small particles (free platelets) whose diameter approaches the wavelength of light (O'Brien, 1962).

Born first measured changes in transmission in an absorptiometer, but subsequently platelet aggro-meters have been designed to regulate the many factors that must be kept constant during aggregation tests. A dual channel model 340 Chronolog Aggro-meter was used in these studies. This instrument was thermostatically controlled so that all tests were carried out at a constant temperature (37°C). In addition, each cuvette of prp was allowed to equilibrate in a water bath at 37°C for 4 minutes before insertion into the instrument. Temperature control is important as speed of aggregation is influenced by temperature (Born, 1962 and O'Brien, 1962).

Stirring (collision) of platelets is required for aggregation to occur on the addition of ADP (O'Brien, 1962 and Born and Cross, 1963) and different stirring rates result in different

rates of aggregation (Born, 1962 and O'Brien, 1962). In these studies with the Chronolog model 340 Aggro-meter, a constant stir rate of 1000 rpm was provided in each channel by the addition of a small teflon covered magnetic stirrer bar to the prp sample.

The extent of aggregation induced by ADP is dependant upon the concentration of platelets (Born and Cross, 1963 and Rossi and Louis, 1977). Concentration was kept constant from sample to sample by aliquoting exactly 450 µl of prp into each cuvette from a well mixed prp pool (as already described).

Changes in light transmission were monitored on a two pen recorder coupled with the aggro-meter. A device on the instrument allowed for zeroing of each channel on the recorder prior to each study. The recorder scale of both channels was calibrated at the beginning of every study by adjusting the sensitivity to give a standard, nearly full scale deflection, between prp and ppp. (i.e. to allow 5°/○ light transmission with prp and 95°/○ transmission with ppp).

Platelets were aggregated by addition of 20 µl quantities of ADP. This produced characteristic curves on the chart recorder:- first a rapid small decrease in light transmission, caused by a change in shape of the platelets (Born, 1970 and Michal and Born, 1971), overtaken within a few seconds by the much larger increase in light transmission associated with the formation of aggregates (Figure 7).

Calculations were made by assuming that the ppp blank represents 100°/○ aggregation and that the percentage change in absorbance was equivalent to the percentage aggregation.
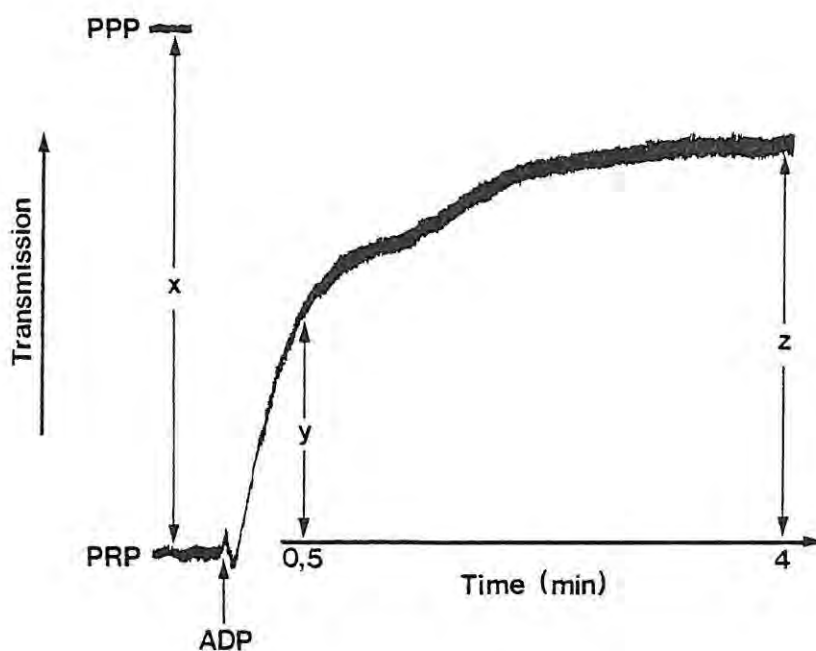
% Aggregation calculated as:

$$\frac{y}{x} \times 100 \quad \text{at} \quad 0,5\,\text{min}$$

$$\frac{z}{x} \times 100 \quad \text{at} \quad 4\,\text{min}$$

(PRP and PPP represent 0 and 100% aggregation respectively)

FIG.7: DIAGRAM OF CHARACTERISTIC RECORDER TRACING SHOWING THE CHANGE IN LIGHT TRANSMISSION WITH TIME ON THE ADDITION OF ADP TO HUMAN PRP.

This was based on the fact that the absorbance of many prp suspensions will approach zero if treated with adequate aggregating agent (Sagel, Colwell, Crook and Laimins, 1975).

Two parameters of the curve were used for quantitation and comparison:

a) the initial rate of aggregation as indicated by the extent of aggregation 0,5 minutes after the addition of ADP (Born and Cross, 1963) and

b) the extent of permanent aggregation 4 minutes after the addition of ADP (Sagel, Colwell, Crook and Laimins, 1975) (Figure 7).

3.3.3.3.   Comparison of the Two Channels of the Aggro-meter.

A dual channel instrument was chosen for these studies since it allowed comparison of prostacyclin production by aortas from a control and a test animal in parallel.

Three preliminary experiments were conducted to confirm that results from the two channels did not differ appreciably. Each of these involved one volunteer's platelets which were aggregated by varying concentrations of ADP, in duplicate, in the two channels.

The mean values of percentage aggregation for the two channels were 40,95 and 39,99 respectively, the mean difference being 0,96. The mean percentage variation between channels was thus 2,4°/∘. This was felt to be within the limits of experimental error; in addition, when estimating PGI_2 production, test and control animals were deliberately randomised by

alternating between the two channels from experiment to experiment.

3.3.3.4. Choice of ADP Concentration.

The result of the study comparing the two channels of the aggro—meter also confirmed the finding of Born and Cross (1963) that, up to a point, as the concentration of added ADP in the plasma increases the rate at which, and the extent to which, platelets aggregate also increases. Figure 8 shows the concentration of ADP plotted against percentage aggregation as measured at 0,5 and 4 minutes respectively (results for one volunteer).

The 'plateau' nature of both these concentration effect curves indicates the importance of chosing a submaximal dose of ADP if the effects of an inhibitor of aggregation are to be investigated. Maximal concentrations with responses on the plateau portion of the curve, may give the same response (i.e. slightly further to the left on the plateau) in the presence of an inhibitor and therefore inhibitory activity would not be detected. Thus, in all studies, platelets were aggregated with a submaximal dose of ADP.

3.3.4. IHIBITION OF AGGREGATION BY AORTIC INCUBATE.

Moncada, Higgs and Vane reported in 1977 that an inhibitor of platelet aggregation (prostacyclin) was generated by incubation
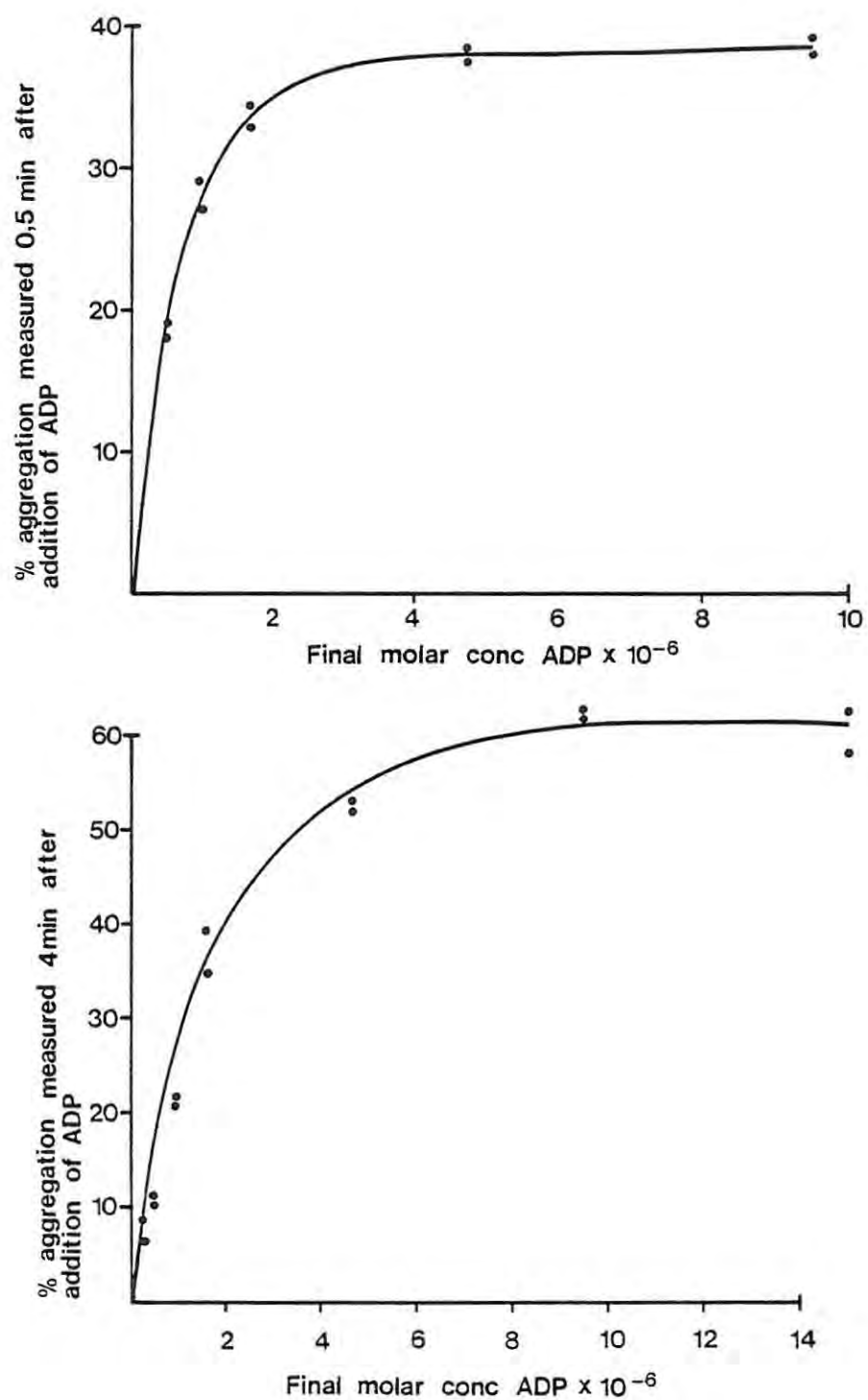
FIG.8: INCREASING AGGREGATION ACHIEVED WITH INCREASING
CONCENTRATIONS OF ADP. MEASUREMENTS MADE 0,5
AND 4 MIN AFTER ADDITION OF ADP.

of fresh rings of human artery in tris buffer. The method used in these studies was based on this work.

3.3.4.1. Collection and Storage of Vessels.

After an overnight fast, paired test and control animals were sacrificed by a blow to the neck. The abdominal aortas, from the right renal artery to the bifurcation into the common iliac arteries, were removed, cleared, and rinsed with ice cold tris buffer (0,5 mmol/l, pH 7,5). Each aorta was adjusted to a wet weight of 10-20 mg. (Weight adjustments were made by trimming the aorta at the proximal end to ensure that a comparative portion as well as weight of aorta was always used. It has been suggested that there may be a segmental distribution of enzyme activity in blood vessels (Skidgel and Printz, 1978).) Aortas were then stored in 1,5 ml tris buffer on ice until tested (within 30 minutes).

3.3.4.2. Production of Inhibitory Incubate.

Each aorta was cut once longitudinally to produce two approximately equal strips (in some studies 3 additional transverse cuts were made to produce 8 strips). Strips were used in preference to the rings of Moncada, Higgs, and Vane (1977) since they could be produced more reproducibly. If, as has been suggested (Moncada and Vane, 1977), prostacyclin is produced by vessels in response to damage, then the number of cuts and surface area exposed per vessel would be important in

determining the amount of prostacyclin released. As these studies required quantitative comparison the use of strips aimed to keep such variation to a minimum.

Strips of aorta were incubated in 700 µl tris buffer at room temperature. Use of ambient temperature meant that no comparison of results from day to day was possible and emphasises the necessity of using a dual channel aggro-meter for assay of a control and test animal in parallel.

Removal and assay of 20 µl aliquots of incubate during preliminary experiments, indicated that prostacyclin was not distributed homogenously throughout the incubation mixture. This problem was overcome by the addition of a small, plastic covered magnetic stirrer bar, which also stimulated prostacyclin production mechanically. An identical stir rate in both test and control incubate was ensured by the use of a common magnetic stirrer plate.

3.3.4.3.   Measurement of the Inhibitory Activity of Aortic Incubate.

At various times after incubation began (usually 2 , 8 and 14 minutes) 20 µl samples of the incubate were removed and added to prp 1 minute before the addition of ADP. The percentage inhibition of platelet aggregation produced by each sample 0,5 and 4 minutes after the addition of ADP, was assessed by comparing the extent of aggregation at both times, with the corresponding control values. Control values were the percentage aggregation at 0,5 and 4 minutes for control curves, obtained by adding 20 µl of tris at room temperature to prp

1 minute before the addition of ADP (Figure 9). A diagram of a typical recorder tracing is shown in Figure 10. As can be seen, a control curve was recorded before and after each assay. Control values (for each channel) were the means of the values for these 2 curves.

The second rat in each experiment was always sacrificed 7,5 minutes after the first, to allow time for the preparation of the first aorta before storage. There was thus a corresponding 7,5 minute delay in the incubation of the second aorta, to ensure that both vessels were stored for the same length of time. Animals from each group, viz normotensive and hypertensive were sacrified first in alternate experiments.

3.3.5.   CONVERSION OF PERCENTAGE INHIBITION TO ABSOLUTE UNITS.

The values of percentage inhibition, as measured at 0,5 and 4 minutes for each sampling time 2, 8 and 14 minutes, were converted into absolute units (ng prostacyclin/ml). This was achieved using the concentration effect curves obtained when synthetic prostacyclin sodium salt was used to inhibit aggregation in the same platelets. Use of the same platelets was obligatory, since platelets provide an assay material which varies considerably from batch to batch (Maguire and Michal, 1968). Standard curves were therefore prepared with every experiment.

3.3.5.1.   Storage and Stability of Prostacyclin Sodium Salt.

Prostacyclin sodium salt was supplied by Upjohn (Kalamazoo).

$$\% \, AGG_C = \frac{y}{x} \times 100$$

$$\% \, AGG_I = \frac{b}{a} \times 100$$

$$\% \, Inhibition = \frac{\% \, AGG_C - \% \, AGG_I}{\% \, AGG_C} \times 100$$
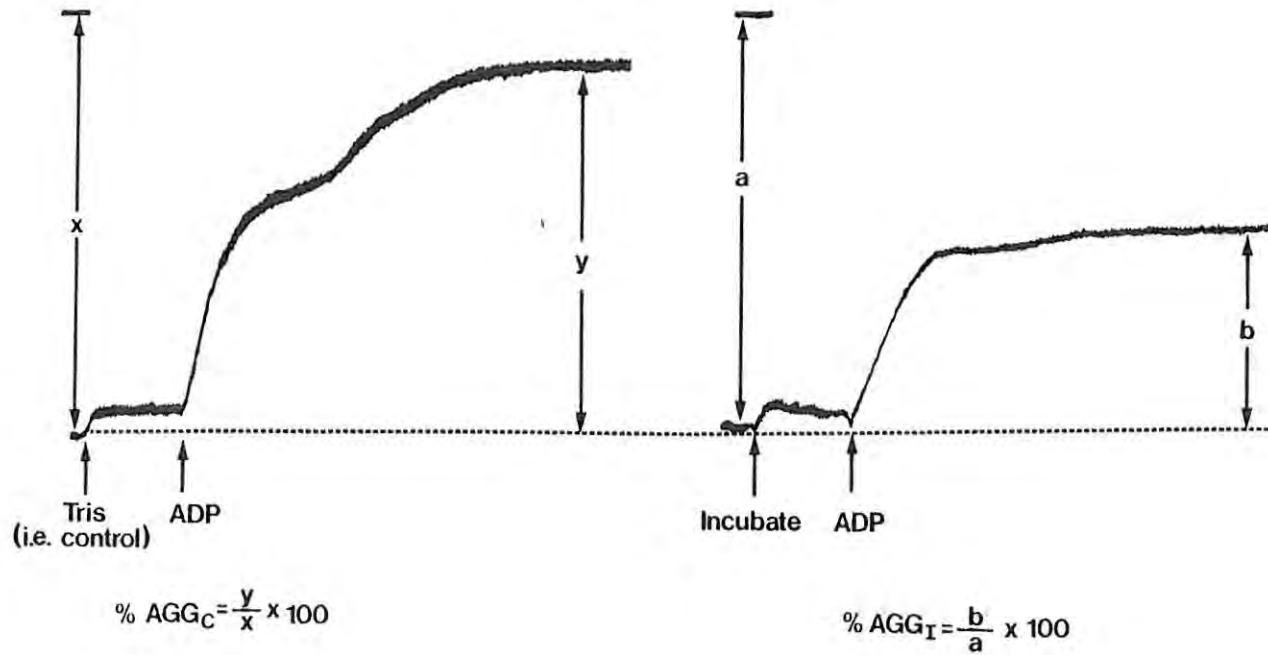
FIG.9: PRINCIPLE OF CALCULATION OF % INHIBITION OF AGGREGATION PRODUCED BY SAMPLE OF AORTIC INCUBATE. THE CALCULATION 4 MIN AFTER THE ADDITION OF ADP IS SHOWN. A VALUE WAS ALSO CALCULATED AT 0,5 MIN FOR EACH CURVE.

Approximately 1 mg aliqouts of the solid were dispensed into plastic tubes and stored under nitrogen at -20°C. Checking purity by techniques within the scope of this laboratory (viz TLC) proved impossible because of the rapid breakdown of prostacyclin. It was thus necessary to assume that purity prevailed under the storage conditions described. This assumption was confirmed with Dr.Pike of Upjohn (personal communication).

Because of the instability of prostacyclin in solution, a 1 mg/ml solution was only prepared immediately before use, by addition of the required volume of tris buffer (0,5 mmol/l, pH 9,9) to a previously weighed aliquot. Tris buffer (0,5 mmol/l, pH 8,6) was used to serially dilute this to give approximately 10 solutions, ranging in concentration from 1 to 100 ng/ml. Solutions of prostacyclin were stored on ice throughout. Prostacyclin has a half life of about 10 minutes in tris buffer at pH 7,5 at 24°C. It is considerably more stable at lower temperature and higher pH (Chapter 2.4.). Hence the use of low temperature and high pH in preparation of solutions. pH 8,6 was used in preference to pH 9,9 in preparation of the final dilutions for addition to platelets, in order not to overtax the buffering capacity fo the prp.

3.3.5.2. <u>Measurement of the Inhibitory Effect of Prostacyclin Sodium Salt.</u>

20 µl samples of each of the 10 solutions over the range 1 - 100 ng/ml were added to prp 1 minute before the addition

of ADP. The inhibitory activity of each was assessed by comparing, with control values, the percentage aggregation 0,5 and 4 minutes after the addition of ADP.

Control values were obtained from calculation of the percentage aggregation at 0,5 and 4 minutes for control curves, obtained by adding 20 µl tris buffer (pH 8,6 on ice) to prp 1 minute before the addition of ADP. The mean values from two control curves (one at the beginning and one at the end) were used to provide control values for each channel.

3.3.5.3. Concentration Effect Curves.

A concentration effect curve (ng prostacyclin/ml versus percentage inhibition of aggregation) was drawn daily for each of the parameters 0,5 and 4 minutes. From these curves values of percentage inhibition, at 0,5 and 4 minutes respectively, were converted into ng prostacyclin/ml. The mean of these two values gave the value of ng prostacyclin/ml for each sampling time, viz 2 , 8 and 14 minutes. After correction for volume and weight, the final results were quoted as ng prostacyclin/mg wet weight of aorta at each sampling time.

3.3.6. SPECIFICITY OF THE ASSAY.

Conversion of the inhibitory activity of the aortic incubate to absolute units of prostacyclin, presupposed that the incubate contained prostacyclin as the sole inhibitory substance. Obviously other substances may be present, and any which

inhibits ADP-induced aggregation of human prp in vitro, could interfere in the assay. This would be especially true if the substance were a potent inhibitor and/or were present in appreciable amounts.

ADP-ase could be expected to affect the aggregation process (a) by decreasing the amount of ADP and (b) by increasing adenosine and AMP which are inhibitors of ADP induced aggregation. (Born and Cross, 1973).

Apyrase was obtained from Sigma Ltd., St. Louis, USA. A 1 mg/ml solution (containing 0,75 units of ADP-ase activity/ml) was prepared in tris buffer (0,5 mmol/l, pH 7,5). 20 µl of this solution added to prp 1 minute before the addition of ADP had very little inhibitory effect ($7^\circ/\circ$) on the percentage aggregation as measured at 0,5 minutes. However, it inhibited by $63^\circ/\circ$, aggregation as measured 4 minutes after the addition of ADP. It is therefore possible that if ADP-ase were present to any extent in the aortic incubate, it could be responsible for part of the inhibitory effect observed.

PGE_1 has been shown to inhibit ADP-induced aggregation in human prp (Kloeze, 1967) and Smith, Silver, Ingerman and Kocsis (1974) and Nishizawa, Miller, Gorman, Bundy, Svensson and Hamberg (1975) have shown that PGD_2 is even more potent than PGE_1, in this regard.

Miller, Aiken, Shebuski and Gorman (1980) have shown prostacyclin to be about 70 times and 48 times more potent than PGE_1 and D_2 respectively in inhibiting human platelet aggregation induced by ADP. This means that either of these two substances would contribute to the inhibitory effect

observed, only if they were present in the incubate in higher concentrations than PGI_2.

ADP-ase, PGE_1 and PGD_2 therefore all have the capacity to interfere with the assay if present in the aortic incubate in appreciable amounts.

While an ADP-ase is present in the blood vessel wall, it is unlikely that it contributes significantly to the wall's anti-aggregatory activity (Chapter 2.6.1). PGE_1 and PGD_2 do not arise in appreciable amounts from intrinsic endoperoxides (Needleman, Whitaker, Wyche, Watters, Sprechler and Raz, 1980). In addition, PGE_1 is probably not likely to be present to any extent, as rats have large amounts of the Δ^5-desaturase enzyme system. (Stone, Willis, Hart, Kirtland, Kernoff and McNicol, 1979). This means that dihomo gamma linolenic acid synthesis is probably directed largely to the formation of arachidonic acid rather than PGE_1.

However, to confirm that the anti-aggregatory activity being detected in these studies was due to PGI_2, the incubate was tested to see if it possessed some of the other properties of prostacyclin besides that of inhibition of platelet aggregation. As a further confirmation, when radio-immuno-assay reagents became available for measurement of the stable metabolite of prostacyclin, 6-keto-$PGF_{1\alpha}$, the incubate was assayed by this method in parallel with the usual assay in six experiments.

3.3.6.1.   Effect of Boiling on Inhibitory Activity of Incubate.

Moncada, Gryglewski, Bunting and Vane (1976 a and b) have reported that the anti-aggregatory activity of prostacyclin
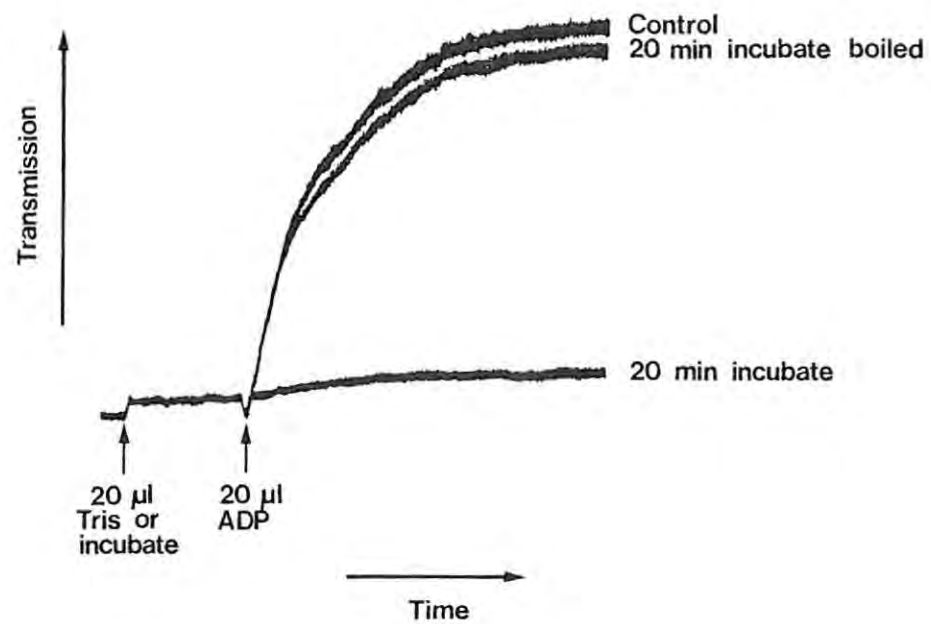
FIG. 11: EFFECT OF 15 SEC OF BOILING ON THE ANTI-AGGREGATORY
ACTIVITY OF THE AORTIC INCUBATE

disappears on boiling for 15 seconds. Two experiments were conducted to determine whether the aortic incubate behaved similarly. Aortic strips were incubated in tris buffer as previously described. After 20 minutes, 600 µl of incubate were transferred to a siliconised glass tube containing a few anti-bumping granules. 20 µl samples of this were tested for anti-aggregatory activity and were found to cause total inhibition. The remainder was boiled for 15 seconds. After cooling to room temperature, addition of 20 µl samples to prp 1 minute before the addition of ADP showed the inhibitory activity to have been almost totally abolished (Figure 11).

3.3.6.2. Stability of Inhibitory Incubate at Room Temperature(22°C) and on Ice (1°C).

Moncada, Gryglewski, Bunting and Vane (1976a) showed that the anti-aggregatory activity of PGI_2 was preserved much longer in buffered solution when kept at 1°C, than when allowed to stand at 22°C.

In one experiment, a 5 minute aortic incubate was divided into two. One half was stored on ice and the other allowed to stand on the bench at room temperature. Each was tested for its inhibitory activity at 0 , 7 and 16 minutes after storage began. As shown in Figure 12, inhibitory activity (as measured both 0,5 and 4 minutes after addition of ADP) decayed at 22°C but not at 0°C.
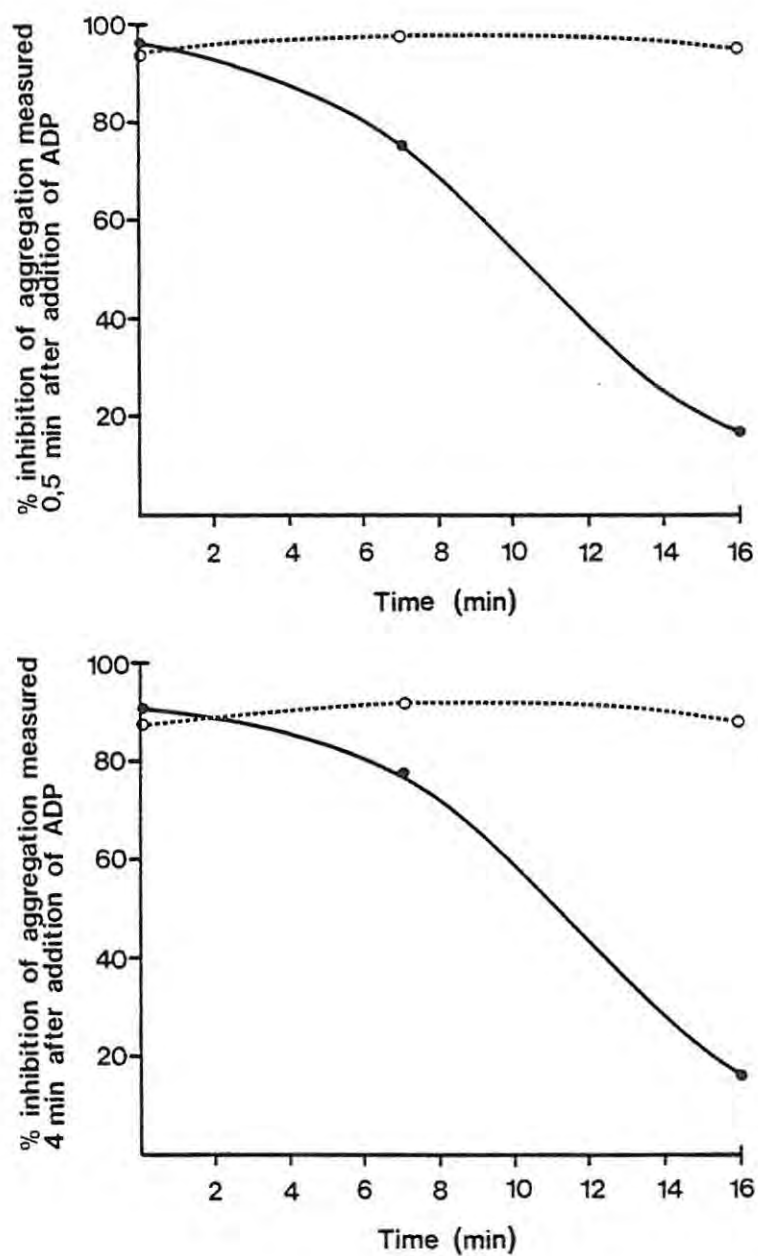
FIG.12: DECAY OF ANTI-AGGREGATORY ACTIVITY OF
AORTIC INCUBATE WITH TIME AT ROOM
TEMPERATURE (●—●) AND ON ICE (○···○)
MEASUREMENTS MADE 0,5 AND 4 MIN AFTER
ADDITION OF ADP.

3.3.6.3.   Comparison of the Decay Rate at Room Temperature, of the Aortic
            Incubate and Standard Prostacyclin.

If the aortic incubate and a standard prostacyclin solution
owed their inhibitory activity to the same substance, then
their anti-aggregatory potency should decline in parallel,
provided they were both held under identical conditions.

In each of two experiments 500 µl of a 14 minute aortic
incubate and 500 µl of a solution of prostacyclin sodium salt
(100 ng/ml) prepared using the same tris buffer (pH 7,5), were
allowed to stand side by side on the bench. Each was assayed
for its ability to inhibit aggregation at intervals over the
next hour.

While decay rates varied from day to day, depending upon the
ambient temperature, within each experiment decay rates for the
incubate and standard substance were similar.

Percentage inhibition was measured both 0,5 and 4 minutes after
the addition of ADP. Figure 13 shows the decay curves for
incubate and standard, obtained using each of these parameters
respectively. (Results from one of the two experiments.)

3.3.6.4.   Comparison of Concentration Effect Curves of Aortic Incubate
            and Standard Prostacyclin.

If the anti-aggregatory activity of the incubate were due to
prostacyclin, then the incubate and a solution of the standard
substance should have comparable concentration effect and log
concentration effect curves.
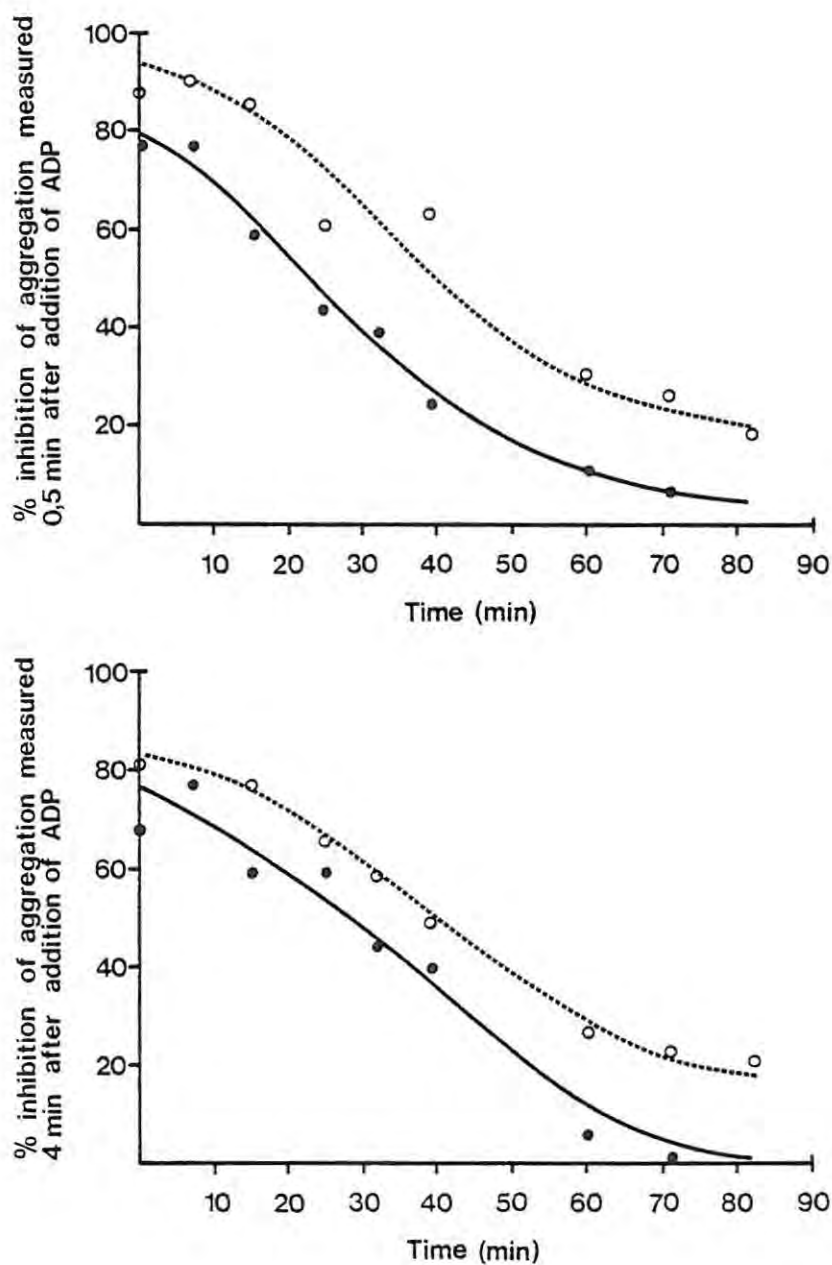
FIG. 13: DECAY OF ANTI-AGGREGATORY ACTIVITY OF AORTIC
INCUBATE (●—●) AND OF PROSTACYCLIN SODIUM SALT
(○···○) AT ROOM TEMPERATURE. MEASUREMENTS MADE
0,5 AND 4 MIN AFTER ADDITION OF ADP.

In one experiment a 14 minute aortic incubate and a standard prostacyclin sodium salt solution (100ng/ml) were prepared using tris buffer pH 7,5. These solutions were both serially diluted with the same tris buffer. All solutions were kept on ice and were assayed in parallel for their inhibitory activity, as measured by their effect on percentage aggregation 0,5 and 4 minutes after the addition of ADP. Hyperbolic dilution versus effect curves were obtained. The sigmoidal log dilution versus effect curves as obtained by 0,5 and 4 minute measurements, are shown in Figure 14.

Comparison of curves for standard and incubate shows that both are capable of the same maximum effect and that the central nearly linear portions of the curves, are almost parallel.

The similarity of these curves, supports the idea that inhibitory activity of the incubate may be due to prostacyclin.

3.3.6.5. <u>Effect of Indomethacin on the Inhibitory Activity of the Aortic Incubate.</u>

Aspirin-like drugs inhibit prostaglandin production by blocking the conversion of arachidonic acid to cyclic endoperoxides, PGG_2 and H_2 (Chapter 2). While it is uncertain how important this pathway is for aortic microsomes (Gryglewski, Bunting, Moncada, Flower and Vane, 1976), Bunting, Gryglewski, Moncada and Vane (1976) were able to show that rabbit arterial rings washed with a solution containing 1 µg/ml indomethacin, did not generate PGI_2 either spontaneously or when incubated with arachidonic acid. Indomethacin would thus be expected to affect the inhibitory activity of the incubate used in the present studies.
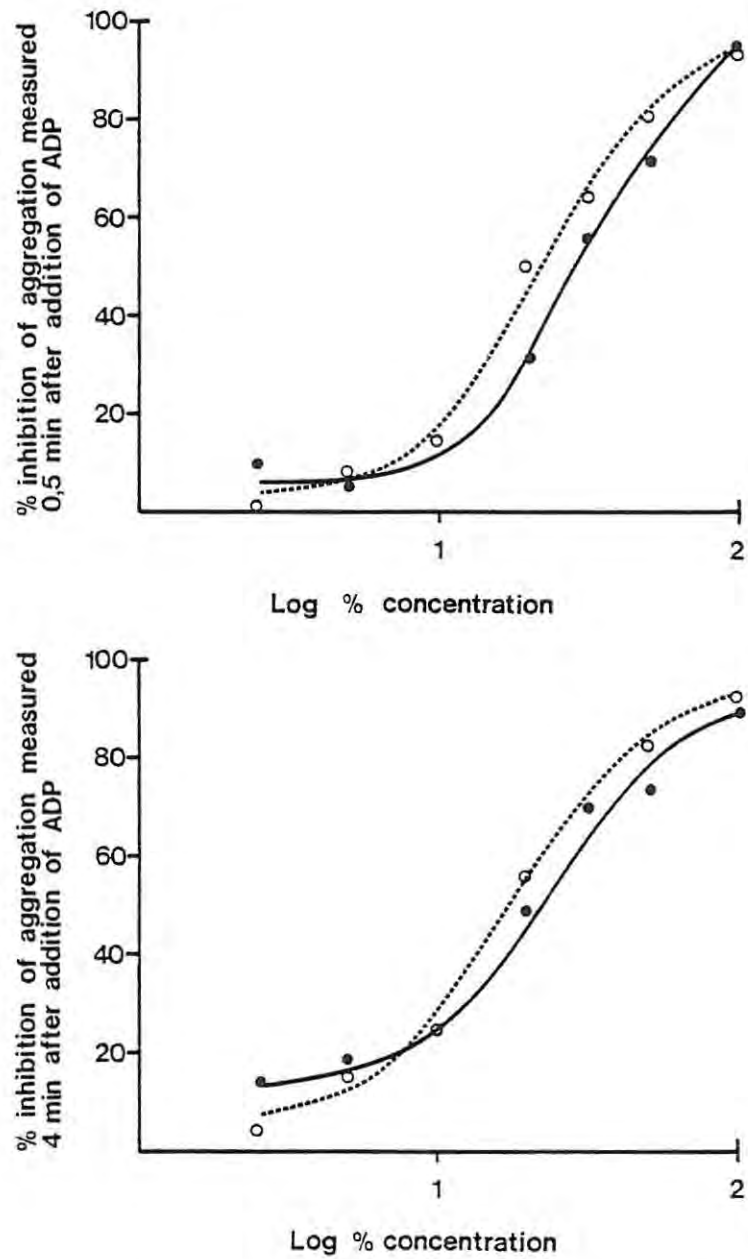
FIG.14: LOG CONCENTRATION EFFECT CURVES FOR AORTIC
INCUBATE (o--o) AND STANDARD PROSTACYCLIN
SODIUM SALT (•—•). ANTI-AGGREGATORY EFFECT
MEASURED 0,5 AND 4 MIN AFTER ADDITION OF ADP

An experiment was conducted in which a rat abdominal aorta, prepared as usual, was cut longitudinally into two approximately equal strips. Each was incubated for 20 minutes in 700 µl tris buffer, one buffer containing indomethacin in a concentration of 5 µg/ml. 20 µl samples of each were tested at intervals for their anti-aggregatory activity.

Figure 15 shows the inhibitory ability of both incubates after correction for tissue weight. With advancing time, while the inhibitory activity of the control incubate increased, that of the indomethacin incubate decreased. As both these curves are a function of production and decay of inhibitory activity, the findings indicate that indomethacin had the ability to block production of the inhibitory activity, adding further evidence to the view that this activity was due to a prostaglandin.

3.3.6.6. Effect of Tranylcypromine on the Inhibitory Activity of the Aortic Incubate.

Gryglewski, Bunting, Moncada, Flower and Vane (1976) preincubated aortic microsomes with various drugs for 3 minutes prior to the addition of PGG_2 or H_2, to investigate the ability of these agents to inhibit the conversion of endoperoxides to PGI_2. They found that tranylcypromine, but no other mono-amine-oxidase inhibitor, reduced conversion by 50°/. at a concentration of 160 µg/ml and prevented it totally at 500 µg/ml.

To investigate the effect of tranylcypromine in the present studies the following experiments were conducted:-
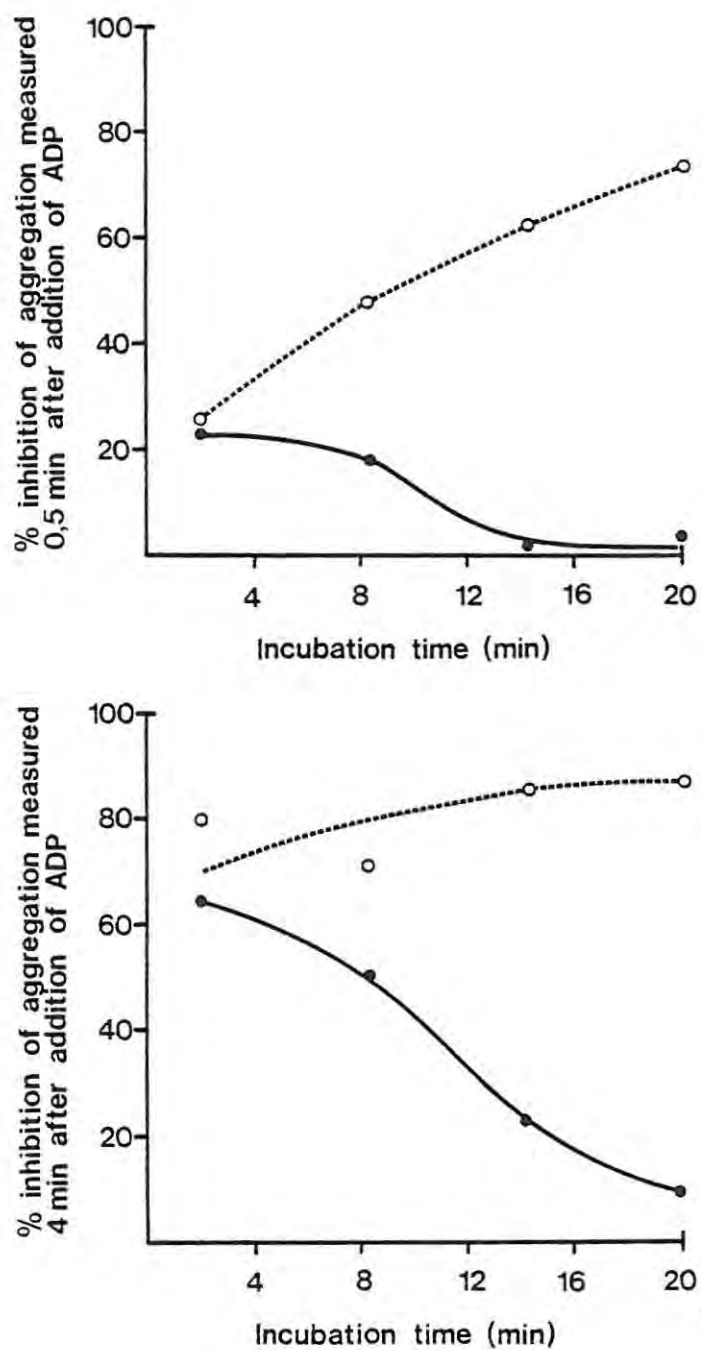
FIG.15: REDUCED ANTI-AGGREGATORY ACTIVITY OF
AORTIC INCUBATE CONTAINING INDOMETHACIN
5μg/ml (●—●) COMPARED TO CONTROL (○···○)
MEASUREMENTS MADE 0,5 AND 4 MIN AFTER
ADDITION OF ADP

In one experiment, a rat abdominal aorta was cut longitudinally into two approximately equal strips. Each strip was incubated in 700 μl of tris buffer (pH 7,5), one buffer containing tranylcypromine at a concentration of 160 μg/ml. During a 16 minute incubation period, 20 μl samples were removed at intervals from each incubate and assessed as usual for anti-aggregatory activity. Figure 16a illustrates the reduced ability of the incubate containing tranylcypromine, to inhibit platelet aggregation as compared to the control (activities corrected to the same weight of vessel). The experiment was repeated, this time using a tranylcypromine concentration of 500 μg/ml. The higher concentration had an even greater inhibitory effect on the production of anti-aggregatory activity (Figure 16b - once again after being corrected for weight).

Due to the difference in experimental design, a quantitative comparison of these results with those of Gryglewski, Bunting, Moncada, Flower and Vane (1976) was obviously not possible. However, the same qualitative effect of tranylcypromine, a specific inhibitor of prostacyclin synthetase, is a further indicator to the identity of the inhibitory activity of the aortic incubate.

3.3.6.7.   The Hypotensive Action of the Incubate.

Pace-Asciak, Carrara and Nicolaou (1978) have shown that prostacyclin has a potent ability to lower arterial blood pressure and is of equal potency via the carotid artery or the
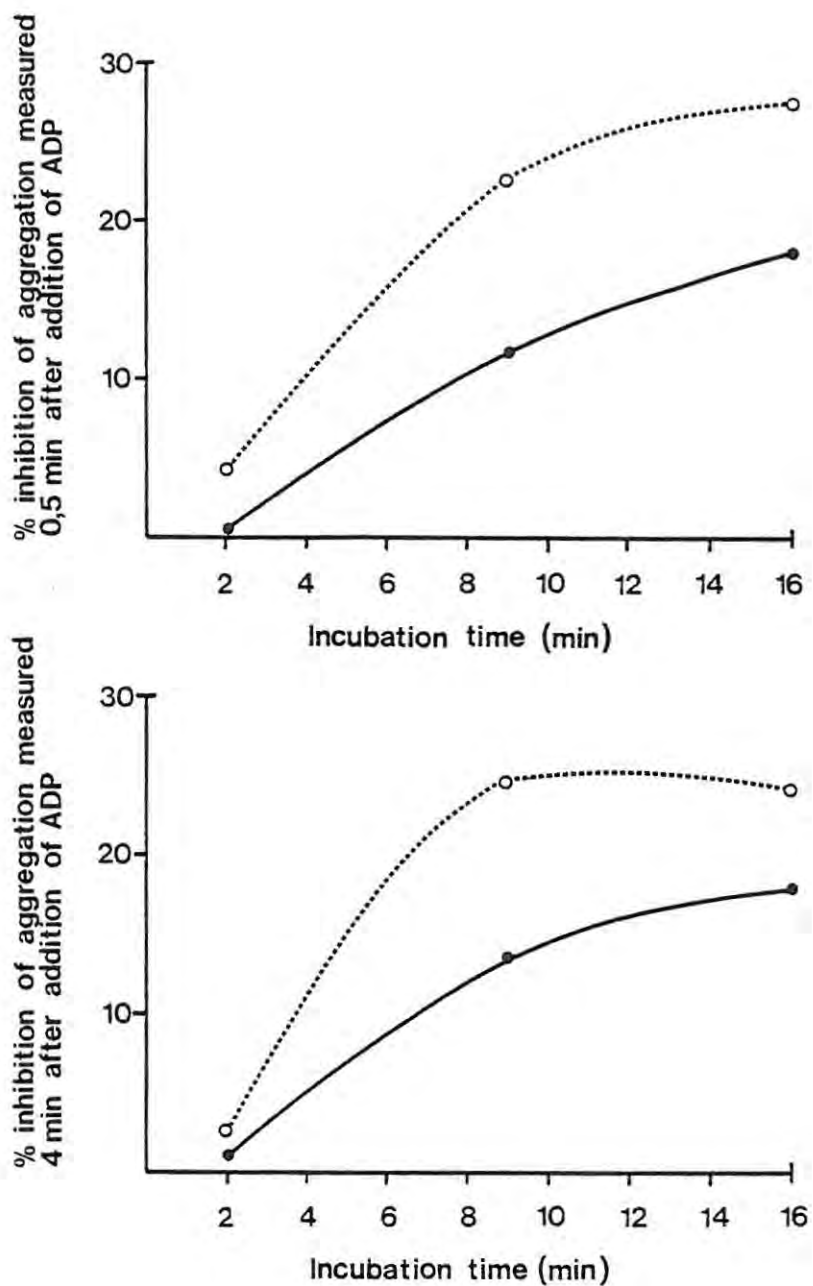
FIG.16a: REDUCED ANTI-AGGREGATORY ACTIVITY OF
AORTIC INCUBATE CONTAINING TRANYLCYPROMINE
160μg/ml (●—●) COMPARED TO CONTROL (○⋯○)
MEASUREMENTS MADE 0,5 AND 4 MIN AFTER
ADDITION OF ADP.
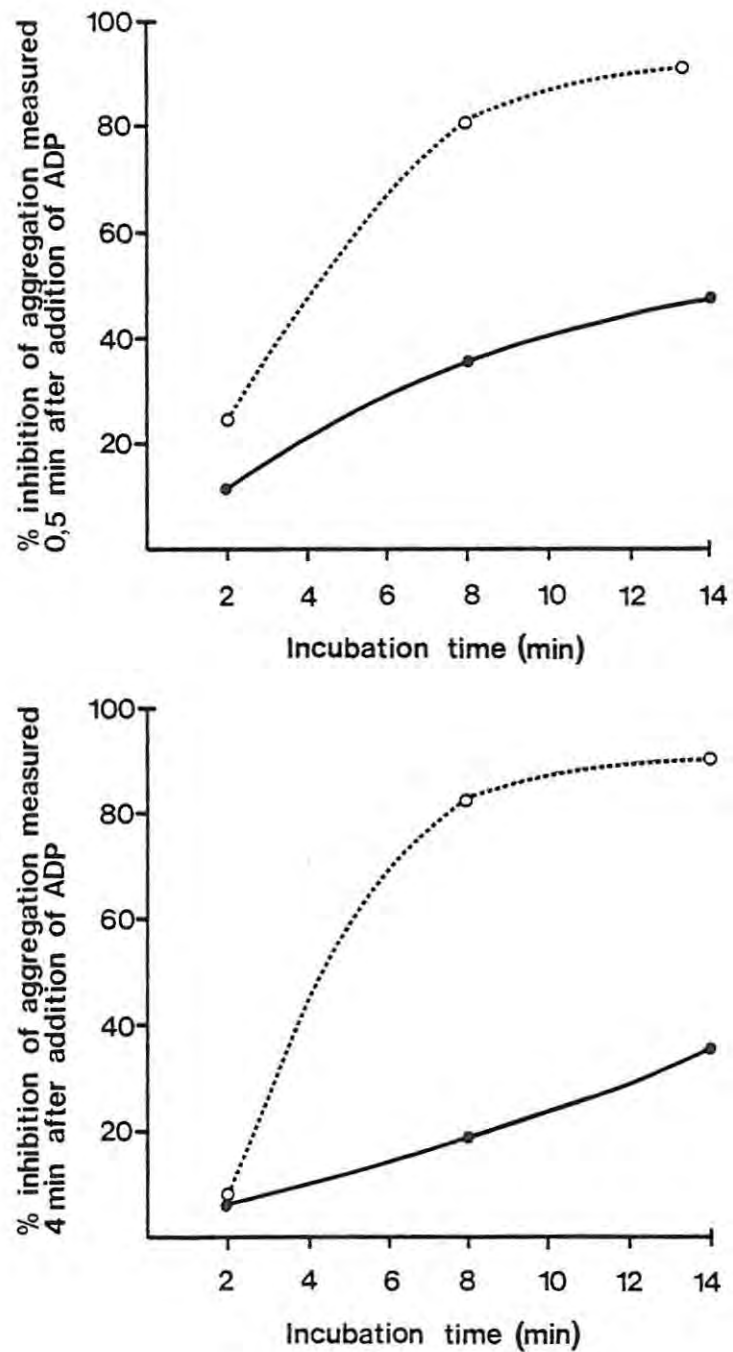
FIG.16b: REDUCED ANTI-AGGREGATORY ACTIVITY OF AORTIC
INCUBATE CONTAINING TRANYLCYPROMINE 500μg/ml
(●—●) COMPARED TO CONTROL (○⋯○). MEASUREMENTS
MADE 0,5 AND 4 MIN AFTER ADDITION OF ADP.

jugular vein, indicating a lack of pulmonary inactivation.

The aortic incubate used in the present studies was tested for its hypotensive activity as follows:-

A hypertensive rat was anaesthetised with intraperitoneal pentobarbital 60 mg/kg. (Sagatal, May and Baker). 30-50 units of heparin (Pularin, Glaxo Allenbury) were administered I.V. The throat was opened and a tracheostomy performed. A fine plastic cannula was introduced into the left carotid artery and the blood pressure was measured continuously by a Stratham strain-gauge tranducer and traced on a Unicorder U400 pen recorder. Another cannula was introduced into the right jugular vein for the purpose of administration of substances to be tested.

When steady pressure was recorded, 0,1 ml of normal saline mixed with 0,1 ml of tris buffer (pH 7,5) was injected slowly into the jugular cannula. This procedure acted as a control for the second injection of 0,1 ml saline mixed with 0,1 ml of a 15 minute incubate prepared as usual in the same tris buffer. When pressure had returned to normal, the procedure was repeated with injections being administered in random order. Figure 17 shows the effect on the blood pressure of these injections. It is evident that even after passage through the lungs, the incubate has hypotensive activity.

3.3.6.8. <u>Comparative Assays of Prostacyclin and 6-keto-PGF$_{1\alpha}$ in the Aortic Incubate.</u>

When reagents became commercially available for the radio-immuno-assay (RIA) of 6-keto-PGF$_{1\alpha}$, the hydrolysis
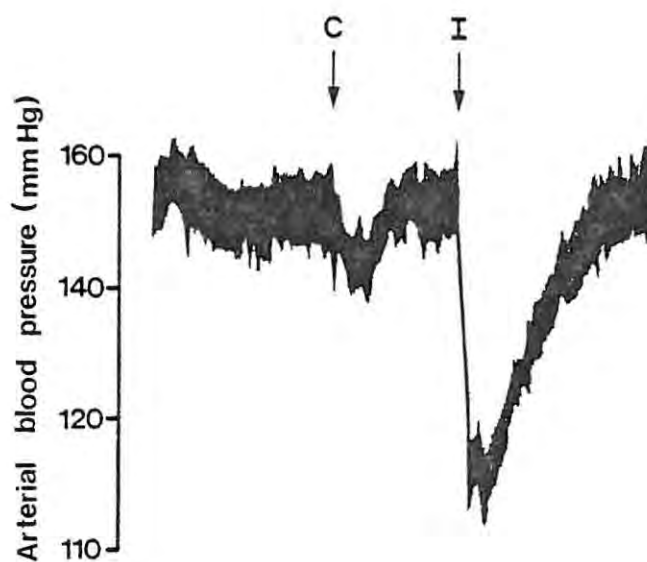
FIG.17: RAT ARTERIAL BLOOD PRESSURE SHOWING THE
EFFECT OF INTRAJUGULAR ADMINISTRATION OF
0,2ML SALINE & TRIS (1:1) C, AND 0,2ML SALINE
AND AORTIC INCUBATE (1:1)I

product of prostacyclin (Chapter 2.4.), the aortic incubate was assayed in parallel by this method and by the usual method in six experiments.

Each abdominal aorta, prepared as usual, was incubated in 700µl tris buffer at room temperature. At each of the sampling times 2, 8, 14, 20 and 38 minutes, two samples were withdrawn. One was assayed immediately for prostacyclin (by the usual method) and the other was reserved for RIA. Samples for RIA were allowed to stand on the bench for approximately 2 hours prior to assay. The study reported in Chapter 3.3.6.3 showed most of the anti-aggregatory activity of prostacyclin and of the aortic incubate to have disappeared within 2 hours at room temperature. This was considered as an indication of almost total conversion of any prostacyclin present, to 6-keto-PGF$_{1\alpha}$. (While their studies involved the use of arachidonate as aggregant, Pace Asciak and Nashat (1977) have shown that 6-ketoPGF$_{1\alpha}$ has only weak anti-aggregatory activity).

Reagents for RIA were supplied by New England Nuclear, Boston, USA (kit number NEK - 008) and the assay was carried out according to the instruction manual. The antibody was stated to show cross reactivity of less than 3°/₀ with PGs E$_2$, F$_{2\alpha}$, A$_2$, A$_1$ and TxB$_2$.

The cross reactivity of the antibody with other substances of particular interest in this study was also checked. This was 10°/₀ for PGE$_1$ and was insignificant for PGD$_2$ and ADP-ase.

Figure 18 shows the trend of prostacyclin and 6-keto-PGF$_{1\alpha}$ (measured as ng/mg wet weight of aorta) present at the various sampling times .
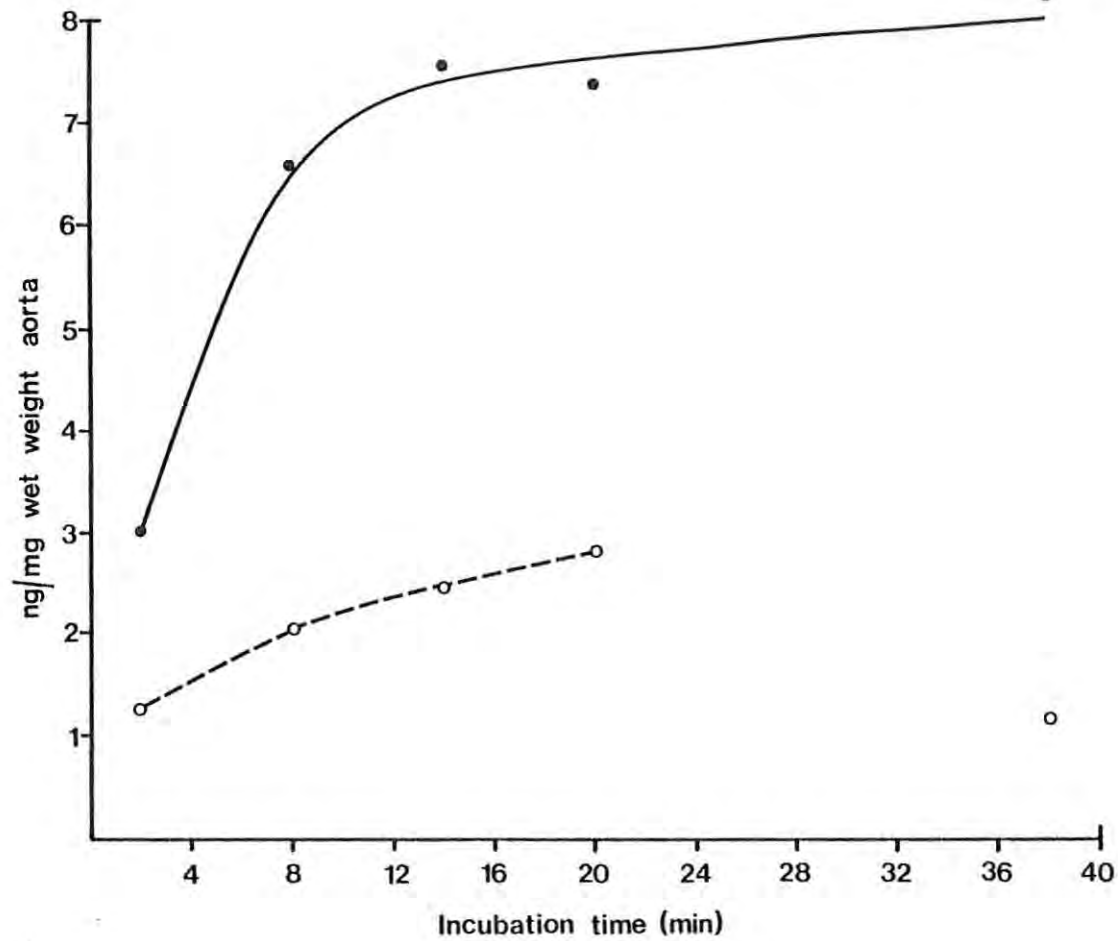
FIG.18: PROSTACYCLIN (∘--∘) AND 6·KETO-PGF$_{1\alpha}$ (•—•) (ng per mg wet weight aorta) FOUND IN THE INCUBATE AT VARIOUS TIMES.

The increase in the 6-keto-PGF$_{1\alpha}$ as prostacyclin increased over the first 20 minutes would be expected, as prostacyclin undergoes hydrolysis in aqueous solutions to 6-keto-PGF$_{1\alpha}$ (Chapter 2.4.).

At the 2 minute sampling time, amounts of 6-keto-PGF$_{1\alpha}$ were already greater than prostacyclin, probably due to 6-keto-PGF$_{1\alpha}$ present initially in the vessel, plus prostacyclin converted to 6-keto-PGF$_{1\alpha}$ in the first two minutes.

As expected the two curves are not parallel. The lower curve (prostacyclin) is a function of production and rapid breakdown, whereas the upper curve (6-keto-PGF$_{1\alpha}$) is steeper as it is a function of production only (6-keto-PGF$_{1\alpha}$ being more stable). The curves therefore diverge further with time . By 38 minutes, values of prostacyclin in the incubate have dropped, as the rate of breakdown has exceeded the rate of production. This is reflected in the plateau value of 6-keto-PGF$_{1\alpha}$ at 38 minutes. At this point, as there is little further prostacyclin to convert to 6-keto-PGF$_{1\alpha}$, levels of the latter have become fairly constant.

Features of the curves are thus consistent with the idea that prostacyclin was present in the aortic incubate.

3.3.6.9. Conclusion.

None of the tests conducted in Sections 3.6.1 to 3.6.8 provide absolute proof that the sole anti-aggregatory substance in the aortic incubate is prostacyclin. However, together they all provide circumstantial evidence that expression of anti-aggregatory activity in terms of absolute values of prostacyclin is acceptable.

CHAPTER 4

AORTIC RELEASE OF PROSTACYCLIN

IN GENETICALLY HYPERTENSIVE RATS

4.1.   INTRODUCTION.

There may be some hereditary predisposition to the development of hypertension in man, as reflected by its varying incidence in different races and high family incidence (Grollman, 1980). In the rat, genetically determined hypertension is well documented and by selective breeding, strains of hypertensive rats can be developed. The spontaneously hypertensive rat provides a convenient model for human essential hypertension (Grollman, 1972) with similar clinical course and pathological findings. This model was therefore chosen for the investigation of aortic prostacyclin production in animals with elevated blood pressure.

4.2.   MATERIALS AND METHODS.

The systemic arterial blood pressures of eight, nine month old, Wistar rats of the New Zealand genetically hypertensive strain (GH) and eight, age and sex matched normotensive Wistar control rats (N), were measured on three successive occasions at two day intervals, by the method described in Chapter 3.2. The last measurement (which followed an overnight fast) was recorded immediately prior to assay of prostacyclin production by aortas of pairs of rats (one GH and one N) — see Chapter 3.3.

4.3.   RESULTS.

The results of the eight experiments in this study can be found in Appendix I. They are summarised in Tables 1 and 2.

The weight variation between the two groups (Table 1) was considered acceptable in view of the fact that animals were strictly age matched (Pace-Asciak, 1979 and Panganamala, Hanumaiah and Merola, 1981 have shown that there is a clear, direct relationship between aortic prostacyclin production and rat age). Of the three blood pressure measurements, only the last, immediately prior to the assay of aortic prostacyclin, is recorded (Table 1). The first two, while similar to the third, were performed mainly to familiarise the animals with the procedure.

While for both N and GH rats more prostacyclin was detected with increasing incubation time (Table 2), at all three sampling times aortas of GH rats released more prostacyclin than those of their matched normotensive controls. This response was fairly consistent and when analysed by the Wilcoxon matched-pairs signed-ranks test, reached a level of significance of $p < 0,05$ at the 2 minute sampling time and $p < 0,02$ at the 8 and 14 minute sampling time.

4.4.   CONCLUSION.

Contrary to expectation, the aortas of GH rats were shown to consistently release greater amounts of prostacyclin than those of matched normotensive controls.

	MEAN		DIFFERENCE WITHIN PAIRS	
	N	GH	MINIMUM	MAXIMUM
WEIGHT (g)	350	304	22	60
BLOOD PRESSURE (mmHg)	121	199	55	100

TABLE 1 :   WEIGHTS AND BLOOD PRESSURES OF NORMOTENSIVE (N)

AND GENETICALLY HYPERTENSIVE (GH) RATS RECORDED

IMMEDIATELY BEFORE ASSAY OF AORTIC PROSTACYCLIN.

INCUBATION TIME (min)	ng PGI_2 PRODUCTION/mg WET WT. AORTA			
	MEAN		MEAN DIFFERENCE WITHIN PAIRS (NUMBER OF PAIRS IN BRACKETS)	
	N	GH	N > GH	GH > N
2*	1,66	2,31	0,30(2)	0,97(6)
8**	3,17	3,94	0,20(1)	0,91(7)
14**	3,80	4,37	0,03(1)	0,66(7)

TABLE 2 :   PROSTACYCLIN PRODUCTION BY AORTAS OF NORMOTENSIVE(N)

AND GENETICALLY HYPERTENSIVE (GH) RATS.

COMPARING N TO GH *$p < 0,05$ **$p < 0,02$

These results are qualitatively consistent with the findings of other authors who have studied spontaneously hypertensive rats: Pace-Asciak, Carrara, Rangaraj and Nicolaou (1978) have reported that intact rings and homogenates of aorta from spontaneously hypertensive rats of the Wistar, Aoki-Okamoto strain contained enhanced capacity over normal rats to convert arachidonic acid into prostacyclin and Okuma, Yamori, Ohta and Uchino (1979) have shown that prior to stroke, elevated amounts of 'prostacyclin-like' substance were produced by spontaneously hypertensive rats (stroke-prone and stroke-resistant) of the Wistar-Kyoto colony.

CHAPTER 5

AORTIC RELEASE OF PROSTACYCLIN

IN EXPERIMENTAL RENAL HYPERTENSION

5.1.   INTRODUCTION.

The relationship between elevated pressure and aortic release
of prostacyclin was further investigated using a second rat
model.

In 1939, Page reported that application of cellophane or
surgical silk to one kidney, produces hypertension which can be
intensified and usually stabilised by extension of the
procedure to, or removal of, the contralateral kidney. The
perinephritis caused by the cellophane, results in the
formation of a fibrocollagenous shell which constricts the
renal parenchyma. Whether it is compression of the kidney or
of the pedicle which is important in the production of this
hypertension is disputed. However as far as is known, it
behaves in all ways like that due to renal artery constriction
(Pickering, 1968) which, in turn, is like that found in man
with respect to a number of features (Pickering, 1974).

5.2.   MATERIALS AND METHODS.

The systemic arterial pressures of a group of male, weanling
Wistar rats (University of Natal inbred strain), approximately
150g in weight, were recorded. The animals were then
anaesthetised with intraperitoneal pentobarbital 6mg/100g.
Working aseptically, a left uninephrectomy was performed in
every animal through a midline abdominal incision. The right
kidney was mobilised in all animals. While half the animals
acted as sham controls (N), in the other half (ERH) (randomly

selected) the right kidneys were wrapped carefully with sterile braided silk suture (Mersilk, Ethnor) and were then returned to the abdominal cavities. Wounds were closed with interrupted sutures.

Ten weeks elapsed before blood pressures of these animals were re-recorded and aortic prostacyclin production was measured.

5.3.   RESULTS.

The full results for the five experiments in this study can be found in Appendix II. Once again, in view of careful age matching, weight variations were considered acceptable (Table 3).

Baseline pressures of the whole group, at the time of entry into the study, were mean 89 (minimum 84, maximum 96)mm Hg., i.e. all normal. As shown in Table 3, binding the kidney and removal of the contralateral kidney, produced experimental renal hypertension (ERH) in those animals on which the procedure was performed. Pressures of the sham group, in which uninephrectomies only had been performed although increasing with age, remained within normotensive range.

Aortic prostacyclin production by ERH and N rats is summarised in Table 4. At all sampling times, aortas of rats with experimentally elevated pressure released more prostacyclin than those of their matched controls. Five pairs of data cannot be analysed statistically by the Wilcoxon matched-pairs signed-ranks test, groups of this size being too small to reach a 5°/∘ level of significance. However, the fact that ERH aortas consistently produced more prostacylin than N aortas

	MEAN		DIFFERENCE WITHIN PAIRS	
	N	ERH	MINIMUM	MAXIMUM
WEIGHT (g)	287	229	43	77
B.P.10 WEEKS POST-OP	110	144	10	50

TABLE 3 : WEIGHTS AND BLOOD PRESSURES (B.P.) mmHg OF RATS WITH
EXPERIMENTAL RENAL HYPERTENSION (ERH) AND THEIR
MATCHED NORMOTENSIVE CONTROLS (N) IMMEDIATELY
PRIOR TO ASSAY OF AORTIC PROSTACYCLIN.

INCUBATION TIME (min)	ng PGI_2 PRODUCTION/mg WET WT. AORTA			
	MEAN		MEAN DIFFERENCE WITHIN PAIRS (NUMBER OF PAIRS IN BRACKETS)	
	N	ERH	N > ERH	ERH > N
2	3,04	4,90	0,03(1)	2,34(4)
8	5,90	7,56	(0)	1,66(5)
18	6,19	8,05	(0)	1,86(5)

TABLE 4 : PROSTACYCLIN PRODUCTION BY AORTAS OF RATS WITH
EXPERIMENTAL RENAL HYPERTENSION (ERH) AND
THEIR MATCHED CONTROLS (N).

in all five experiments, would indicate that the results for this small sample are likely to be representative of the true situation.

5.4.   CONCLUSION.

Aortas of rats with experimental renal hypertension, were shown to produce more prostacyclin than those of their matched controls. This finding, together with that for genetic hypertension (Chapter 4) and with the fact that similar results have been reported for rats with DOCA-saline hypertension (Friedman, Webster, Hensby and Lewis, 1980), indicates that a variety of forms of hypertension in rats are associated with elevated aortic prostacyclin production. This is in complete contrast to the original hypothesis of Moncada, Gryglewski, Bunting and Vane (1976a) viz. that the vasodilator and anti-aggregant prostacyclin may be lacking in certain forms of hypertension.

CHAPTER 6

EFFECT OF PRESSURE REDUCTION WITH HYPOTENSIVE AGENTS

ON AORTIC PROSTACYCLIN RELEASE

IN GENETICALLY HYPERTENSIVE RATS

6.1.   INTRODUCTION.

To further elucidate the relationship between elevated arterial pressure and elevated aortic production of prostacyclin, the effect of pressure reduction (with hypotensive agents) on the ability of the aortas of spontaneously hypertensive rats to produce prostacyclin, was investigated.

Freis (1972) has reported that a combination of reserpine, hydralazine and chlorothiazide added to drinking water, effectively lowers the blood pressure of spontaneously hypertensive rats without apparent toxicity to the animals. In this study, the regimen was altered by substitution of suitable amounts of furosemide for the chlorothiazide.

6.2.   MATERIALS AND METHODS.

Eighteen, two month old, male, Wistar rats of the New Zealand genetically hypertensive strain were divided randomly into two groups. The systemic arterial pressures of conscious animals were recorded. Both groups received the same diet and unlimited access to drinking water. However, while the control group (C) drank tap water only, the treatment group (T) received drinking water containing a combination of reserpine (Serpasil, Ciba Giegy), dihydralazine methane sulphonate (Nepresol, Ciba Giegy) and furosemide (Lasix, Hoechst) in concentrations of 1,4mg, 100mg and 40mg/l respectively. The injectable form of each of these agents was added to tap water without any precipitation. However, the mixture was prepared

freshly each day in an attempt to ensure maximum stability. Pressures of both groups of animals were recorded at weekly intervals. When the pressures of the treated group had been controlled below 120mmHg for one week (this required approximately one month of treatment), aortas of paired C and T animals were assayed for aortic production of prostacyclin.

6.3.   RESULTS.

Weights and blood pressures of C and T animals are summarised in Table 5. While pressures were similar in the two groups on entry into the study, the drug regimen sucessfully lowered pressures of the test group to within normotensive range. Prostacyclin release by the aortas of the treated and control groups can be seen in Table 6. While at all sampling times the means indicate a tendency for treated aortas to produce less prostacyclin than their matched controls, there was no consistent trend in this direction in the nine experiments. Analysis of the data by the Wilcoxon matched-pairs signed-ranks test, showed the difference between T and C groups (at all sampling times) to be non-significant at the 5°/° level. (Full results in Appendix III).

6.4.   CONCLUSION.

The hypotensive agents employed effectively lowered the pressures of the treated group compared to controls. However, despite the pressure difference between the two groups there

	MEAN		DIFFERENCE WITHIN PAIRS	
	T	C	MINIMUM	MAXIMUM
WEIGHT (g)	218	229	0	75
B.P..BEFORE TREATMENT	168	159	3	30
B.P. POST TREATMENT	105	172	48	100

TABLE 5 :   WEIGHTS AND BLOOD PRESSURES (B.P.) mmHg OF

GENETICALLY HYPERTENSIVE CONTROL (C) AND

TREATED (T) RATS.

INCUBATION TIME (min)	ng PGI$_2$ PRODUCTION/mg WET WT. AORTA			
	MEAN		MEAN DIFFERENCE WITHIN PAIRS (NUMBER OF PAIRS IN BRACKETS)	
	T	C	T > C	C > T
2	0,46	0,63	0,10(3)	0,29(6)
8	1,62	1,96	0,46(2)	0,57(7)
14	2,12	2,39	0,21(2)	0,42(7)

TABLE 6 :   AORTIC PROSTACYCLIN PRODUCTION BY CONTROL (C) AND

TREATED (T) GENETICALLY HYPERTENSIVE RATS

was no consistent, statistically significant difference in aortic prostacyclin generation.

Since completion of this study, it has become clear that at least two of the drugs administered to the treated group may influence prostaglandin levels, irrespective of their effect on arterial pressure.

There is evidence, (reviewed by Ciabattoni, Pugliese, Cinotti, Stirati, Ronci, Castrucci, Pierucci and Patrono, 1979), that furosemide causes generalised activation of the renal prostaglandin system. The excretion rate of 6-keto-PGF$_{1\alpha}$, like other members of the renal prostaglandin system, increases during the first fifteen minutes following furosemide injection. Whether there is any extra-renal effect of furosemide, especially after oral administration as would be important in this study, is unknown. However, the findings reported above do not augur well for this model providing information about the direct relationship between arterial pressure and prostacyclin production.

Förster, Taube and Fahr (1980), while reporting that dihydralazine (4mg/kg) given intravenously to spontaneously hypertensive rats decreased the blood pressure by 12 - 22°/° and increased aortic prostacyclin synthesis by 80°/°, have not indicated what the cause/effect relationship may be between these three variables. Haeusler and Gerold (1979) and McGiff and Quilley (1980) suggest that the hypotensive effects of the vasodilators hydralazine and dihydralazine, may actually be mediated through the vasodilator prostaglandins, indomethacin having completely reversed within fifteen minutes, the

hypotension produced by either of these agents in anaesthetised dogs.

It is therefore clear, that as these drugs may affect prostaglandins directly, no conclusions about the direct relationship between arterial pressure and prostacyclin release can be drawn from this study. Accordingly, despite the results obtained, the possibility cannot be excluded, that reduction of raised arterial pressure may have some effect on the capacity of the aorta to release prostacyclin.

CHAPTER 7

EFFECT OF MECHANICAL PRESSURE REDUCTION

ON AORTIC PROSTACYCLIN RELEASE

IN GENETICALLY HYPERTENSIVE RATS

7.1.   INTRODUCTION.

The unsatisfactory nature of the previous study, stimulated consideration of further models of pressure reduction.

One possibility which was pursued, was that of saline hypertension which could be reversed on reduction of the salt intake. However, the animals used for this study (University of Natal inbred strain) were not salt-sensitive and did not develop satisfactorily elevated pressure. In retrospect, it was felt that even this model would have involved sodium chloride as a further variable besides the two being investigated, namely, pressure and prostacyclin. Accordingly, a mechanical method of pressure reduction was eventually selected.

Goldblatt and Kahn (1938) have reported that constriction of the aorta of dogs immediately above the renal arteries, produced hypertension above and immediate hypotension below the clamp. Pressure reduction in abdominal aortas of genetically hypertensive rats, was achieved by the use of this method.

7.2.   MATERIALS AND METHODS.

The systemic arterial pressures of sixteen, approximately fourteen week old, male, Wistar rats of the New Zealand genetically hypertensive strain were recorded. Pairs of rats were then anaesthetised with intraperitoneal pentobarbital (6mg/100g). Working aseptically, the aorta, just below the diaphragm, was mobilised in both animals through a mid-line

abdominal incision. It was then constricted above the hepatic artery and as near to the diaphragm as possible, by a silver clip (Scoville Lewis JN 1300 IW). In one animal, which acted as a sham control (S), the clip was immediately removed. In the other (C) it was left in position thus producing hypotension distally. Wounds were closed with interrupted sutures. Eighteen hours after the operation, distal aortic pressures were measured by the tail cuff method in both conscious animals which had been fasted overnight. After their weights had been recorded the two animals were sacrificed and their aortic prostacyclin production measured.

7.3.   RESULT.

In both groups post-operative tail pressures were reduced compared to pre-operative values, possibly due to a residual effect of the barbiturate anaesthetic or constriction of the aortas due to handling. However, comparing C to S, while pressures were very similar pre-operatively, post-operatively those of the C group were much lower than S controls due to the application of the clip (Table 7).

The aortas from the group in which pressures had been reduced mechanically, generated less prostacyclin than those of their matched controls. This tendency was consistent at the 8 and 14 minute sampling times and when analysed by the Wilcoxon matched-pairs signed-ranks test, reached a level of significance of $p < 0,02$ (Table 8). Full results for the eight experiments appear in Appendix IV.

	MEAN		DIFFERENCE WITHIN PAIRS	
	C	S	MINIMUM	MAXIMUM
WEIGHT (g)	291	295	0	50
PRE OP B.P. (mmHg)	178	180	0	12
POST OP B.P. (mmHg)	95	160	34	93

TABLE 7 :   WEIGHTS AND BLOOD PRESSURES (B.P.) mmHg OF

CLIPPED (C) AND SHAM (S) RATS.

	ng PGI_2 PRODUCTION/mg WET WT. AORTA			
INCUBATION TIME (min)	MEAN		MEAN DIFFERENCE WITHIN PAIRS (NUMBER OF PAIRS IN BRACKETS)	
	C	S	C > S	S ⩾ C
2	1,50	1,57	0,26(4)	0,41(4)
8*	3,14	3,75	0,14(1)	0,72(7)
14**	3,54	4,04	(0)	0,50(8)

TABLE 8 :   AORTIC PROSTACYCLIN PRODUCTION BY CLIPPED (C) AND

SHAM (S) RATS.   COMPARING C TO S *p < 0,02   **p < 0,01.

7.4.   <u>CONCLUSION.</u>

Blood flow, pressure and pulse pressure were consistently reduced in C abdominal aortas, as was the subsequent ex vivo capacity of aortas to produce prostacyclin. These results indicate that local pressure/pulse pressure and the subsequent ability of the vessel to generate prostacyclin may be related.

CHAPTER 8

RESPONSE OF GENETICALLY HYPERTENSIVE RAT PLATELETS

TO PROSTACYCLIN AND ADP

8.1.   <u>INTRODUCTION.</u>

In an attempt to discover more about the possible significance of raised aortic generation of prostacyclin in hypertension, the sensitivity of platelets of spontaneously hypertensive rats to prostacyclin was investigated.

It would appear obvious that in any pathological condition, haemostatic balance must be influenced not only by vascular prostacyclin production, but also by platelet sensitivity to prostacyclin (Burghuber, Sinzinger, Silberbauer, Wolf and Haber, 1981). For example, in diabetes both decreased vascular prostacyclin generation (see Chapter 2.8.2.) and decreased sensitivity of platelets to prostacyclin (Klein, Sinzinger, Kaliman and Schernthaner, 1980) have been reported.

When atherosclerosis is induced in rabbits, decreased prostacyclin generation occurs together with increased sensitivity of platelets to prostacyclin (Chapter 2.8.1).

Since the sensitivity of human platelets to prostacyclin, has been shown to be decreased during long term intra-arterial infusion of the substance in peripheral vascular disease (Sinzinger, Silberbauer, Horsch and Gall, 1981), the possibility existed that increased aortic generation of prostacyclin in hypertension, might be associated with reduced platelet sensitivity to prostacyclin. A comparison of the effect of prostacyclin on the in vitro, ADP-induced aggregation of platelets, of male Wistar rats of the New Zealand genetically hypertensive strain (GH) and those of matched normotensive Wistar control rats (N), was used to test this hypothesis.

8.2.   MATERIALS AND METHODS.

Systemic arterial blood pressures were measured in twenty one, three month old, male animals from each group (GH and N).

In each of seven experiments three animals from each group were fasted overnight, weighed and anaesthetised with intraperitoneal pentobarbital (6mg/100g). Through a midline incision, a plastic cannula was placed in the abdominal aorta and blood was collected into disposal plastic syringes containing heparin (10U/ml of blood). (Heparin was chosen as anti-aggregant because heparinised rat platelet rich plasma is much more sensitive to aggregating agents than citrated platelet rich plasma (Hwang, 1980).)

Platelet rich plasma (prp) was prepared by centrifugation of blood at 160g for 18 minutes. Further centrifugation of remaining blood at 2000g (at 4°C) for 15 minutes, yielded platelet poor plasma (ppp). For the aggregation test, prp obtained from the three rats in each group was pooled. Ppp for each group was obtained by pooling ppp from the appropriate animals. Details of handling and storage of prp and ppp were as described in Chapter 3.3.1. Platelet counts were performed as described in Chapter 3.3.2. except that prp was diluted 1 in 40 rather than 1 in 20 with trisodium citrate, to facilitate counting of the large number of platelets.

Aggregation of pooled prp from the three rats in each group, was carried out in a dual channel aggro-meter as previously described (Chapter 3.3.3.2.). ADP (final concentration 1µM) was added to prp, one minute after the addition of tris buffer
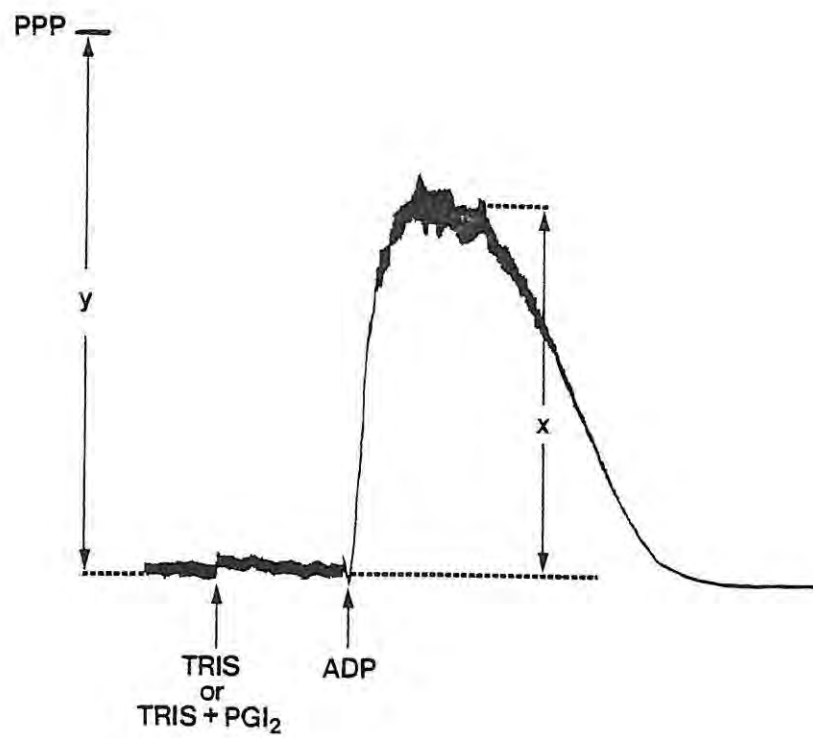
$$\% \text{ AGGREGATION} = \frac{x}{y} \times 100$$

FIG.19: DIAGRAM OF RECORDER TRACING SHOWING AGGREGATION OF RAT PRP.

or an equal volume of the same buffer containing various concentrations of prostacyclin sodium salt (Chapter 3.3.5.1). Maximum aggregation was measured as a percentage of the distance between prp and ppp as shown in Figure 19.

8.3.   RESULTS.

Weights, blood pressures and platelet counts, which can be seen in full in Appendix V, are summarised in Table 9. Platelet counts in prp differed by no more than 4°/o in the two groups. As illustrated in Figure 20 (full results in Appendix V), the response of the platelets to ADP did not differ significantly between the two groups, whether ADP followed tris buffer alone or buffer containing various concentrations of prostacyclin. (The slightly greater aggregation for GH platelets may have been due to the slightly higher platelet count.)

	N			GH		
	MEAN	MIN	MAX	MEAN	MIN	MAX
WEIGHT (g)	263	220	300	261	230	320
B.P. (mmHg)	115	80	125	173	158	190
PLATELET COUNT x 10^3	1469	996	2134	1532	1156	2226

TABLE 9 :   WEIGHTS, BLOOD PRESSURES AND PLATELET COUNTS OF GENETICALLY HYPERTENSIVE (GH) AND NORMAL (N) RATS.
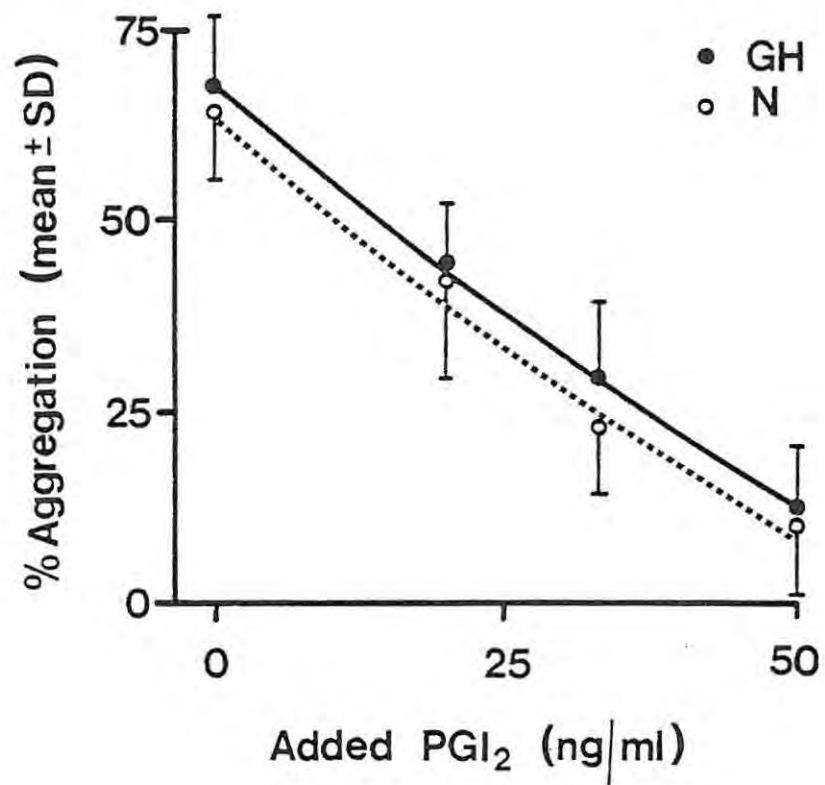
FIG.20: EFFECT OF PGI$_2$ ON ADP INDUCED
AGGREGATION IN RAT PLATELETS.

8.4.   <u>CONCLUSION</u>.

Contrary to expectation the results indicate that platelets from both GH and N rats are equally sensitive, in vitro, to the aggregatory action of ADP and the anti-aggregatory effect of prostacyclin.

CHAPTER 9

DISCUSSION

9.1.   ELEVATED AORTIC GENERATION OF PROSTACYCLIN IN HYPERTENSION

This study was originally initiated by an interest in the properties of prostacyclin, in relation to the features of hypertension. The possibility existed that a lack of this vasodilatory, anti-aggregatory prostaglandin might be an aetiological factor in certain forms of hypertension.

The findings resulting from testing this hypothesis were completely contrary to expectation. In both genetically hypertensive rats (Chapter 4) and in rats with experimental renal hypertension (Chapter 5), the ability of the abdominal aorta to generate prostacyclin, was found to be consistently greater than that of matched controls.

While these studies were in progress various other authors published their investigations on this subject. Though it is difficult to make quantitative comparisons between results obtained, due to the use of different strains of animals, forms of hypertension and methodology, many findings are qualitatively consistent with those reported in the present studies.

Pace-Asciak, Carrara, Rangaraj and Nicoloau (1978) reported that intact rings and homogenates of spontaneously hypertensive rats (SHR) of the Wistar, Aoki-Okamoto strain, possessed enhanced capacity to convert arachidonic acid to PGI_2. In fact, prostacyclin was the major prostaglandin formed by SHR, arachidonic acid metabolism being uniquely directed along this pathway (Pace-Asciak, Carrara and Nicoloau, 1978 and Pace-Asciak, Carrara, Rangaraj and Nicoloau, 1978).

Pace-Asciak and Carrara (1980) were further able to show, that a positive correlation exists between rise in blood pressure and aortic synthesis of PGI_2 and that the unique specificity to PGI_2 production appears to be acquired with age. In another study involving SHR, this time from the Wistar Kyoto colony, Okuma, Yamori, Ohta and Uchino (1979) showed that aortas of both stroke-prone and stroke-resistant animals, generate elevated amounts of 'prostacyclin-like' substance. Detre, Leithner, Winter, Sinzinger, Silberbauer, Fródy and Jellinek (1980), who induced malignant renal hypertension by aortic ligature between the origins of the renal arteries (Rojo-Ortega method), noted elevated PGI_2 formation by the aortic wall both above and below the ligature in the first phase of hypertension. Elevated PGI_2 has also been reported in other forms of hypertension in experimental animals: Treatment of rats for 10 oestral cycles with an oestrogen-progestogen combination was found by Roncaglioni, di Minno, Pangrazzi, Reyers, Mussoni, de Gaetano and Donati (1980) to trigger a vascular response, characterised by increased prostacyclin activity in arterial walls and increased systolic blood pressure. Increased aortic PGI_2 activity has been shown by a number of authors to occur in DOCA-saline or saline induced hypertension (Friedman, Webster, Hensby and Lewis, 1980, Yoshimura, Kaimasu, Kitani, Kawamura, Takeda, Sasaki, Kajita, Nakagawa and Ijichi, 1980 and Limas, Goldman, Limas and Iwai, 1981).

Despite these reports in support of the findings presented in this thesis there are a few interesting conflicting results which should be mentioned:

Firstly, there are two groups of workers who have actually reported results which contrast completely with those reported above. Markov (1978) found that biosynthesis of prostacyclin from arachidonic acid was decreased in aortic homogenates of SHR, and Inoue, Hayashi, Hara, Watanabe, Enomoto and Nomura (1979) reported that indomethacin pretreated aortic rings from Wistar Kyoto rats produced more PGI_2-like substance than SHR.

Secondly, there are two groups of workers, who although reporting elevated aortic prostacyclin production early in hypertension, found, unlike Pace-Asciak and Carrara (1980) who studied SHR, that these elevations were not ·sustained indefinitely, despite continued elevation of blood pressure. This phenomenon was noted by Detre, Leithner, Winter, Sinzinger, Silberbauer, Fódy and Jellinek (1980) in that, by the thirty-fifth day of Rojo-Ortega hypertension, vessel wall PGI_2 content had returned to normal or even subnormal values. Similar to this was the finding of Yoshimura, Kaimasu, Kitani, Kawamura, Takeda, Sasaki, Kajita, Nakagawa and Ijichi (1980) that, after eight weeks of salt loading, though pressure was significantly raised, elevated prostacyclin activity was not sustained and levels actually fell to appproximately 80 percent of normal. Also interesting is the finding of these authors that two weeks of salt depletion, which had no effect on blood pressure, resulted in significantly elevated PGI_2 activity. These results would indicate that salt has an effect on prostacyclin generation irrespective of blood pressure, possibly mediated by the renin-angiotensin-aldosterone system.

Friedman, Webster, Hensby and Lewis (1980) support this view in reporting that in man, salt loading, which lowers renin levels, decreases prostacyclin. In the long term Rojo-Ortega model of hypertension some factor other than pressure, such as renin levels, may also influence prostacyclin.

Skidgel and Printz (1980) have shown that the elevated prostacyclin producing capacity in SHR arteries, is related to enhanced cyclo-oxygenase activity and not prostacyclin synthetase activity, which is essentially the same as that for normal controls. Though these findings do not seem quite consistent with the report of Pace-Asciak, Carrara, Rangaraj and Nicoloau (1978), that in adult SHR arachidonate metabolism is uniquely directed towards one prostaglandin, namely 6-keto-$PGF_{1\alpha}$, they are in accord with the findings of the same authors, that SHR aortas have increased capacity to convert arachidonic acid to prostacyclin. They also correlate with the findings of authors who have studied production of 'PG-like' material (rather than prostacyclin specifically) by aortic strips of hypertensive rats. Rioux, Quirion and Regoli (1977) have reported elevated release of 'PG-like' material (analysis of which suggested the presence of PGE_2 and E_1) from aortic strips of SHR, renal hypertensive and DOCA-salt hypertensive rats. If cyclo-oxygenase is the important variable, then perhaps the methodology of Inoue, Hayashi, Hara, Watanabe, Enomoto and Nomura (1979), namely pretreatment with indomethacin, may, by inhibition of cyclo-oxygenase, have obscured the difference in PGI_2 generation reported by other workers for SHR.

9.2.   STIMULUS TO ELEVATED AORTIC PROSTACYCLIN GENERATION

It is interesting to note that Rioux, Quirion and Regoli (1977) found that production of 'PG-like' material by aortic strips of renal and DOCA-salt hypertensive rats, was largely reduced when hypertension was interrupted, indicating an association between the elevated production of 'PG-like' material and the elevated pressure.   Limas, Goldman, Limas and Iwai (1981) have suggested that the enhanced PGI_2 synthesis, which they found in salt loaded, salt sensitive Dahl rats, may be secondary to the hypertension and Friedman, Webster, Hensby and Lewis (1980) in their similar study, were also of this opinion in view of the fact that salt loading, per se, may be expected to decrease prostacyclin.

Various suggestions have been put forward as to the possible stimulus to elevated prostacyclin production in hypertension.

Since they were able to show that norepinephrine stimulated $6\text{-keto-PGF}_{1\alpha}$ formation from arachidonic acid by aortic homogenates of both SHR and control rats (probably at the cyclo-oxygenase level), Pace-Asciak, Carrara, Rangaraj and Nicoloau (1978) have suggested that the enhancement of biosynthetic capacity in hypertensive aortas, might represent an adaptive response to elevated plasma catecholamine levels during hypertension. Proliferation of aortic endothelial cells is another proposed explanation (Friedman, Webster, Hensby and Lewis, 1980). There is also the possibility, that increased mechanical stimulation of the aorta resulting from the pressure elevation, may be related to the increased PGI_2 release.

The release of many prostaglandins is altered by mechanical stress of the secreting cells - to quote Vane: "Now, we know that whenever you stretch a tissue or touch it, or cause even the slightest disturbance, there is an exaggerated PG release" (Aiken, Gorman and Shebuski, 1979). There is evidence that this may also be the case for prostacyclin release by vascular tissue: ten Hoor, de Deckere, Haddeman, Hornsta and Vendelmans-Starrenburg (1978) have reported that production of PGI_2 by pieces of rat aorta, increases after damaging the endothelium with sand paper, and Hamilton, Rosza, Hutton, Chow, Dandona and Hobbs (1981) have shown that direct trauma of the hepatic vein caused a small increase in the PGI_2 activity of the vessel measured one week later. It has also been noted (Gryglewski, 1979), that increased ventilation of the lung increases prostacyclin release and that in isolated, perfused rabbit aorta pulsations stimulate PGI_2 release into the perfusate (Voss, Haddeman, Don, and ten Hoor, 1980).

In hypertension, due to elevated pressure, there is an increased mechanical force acting upon the vessel wall. In addition, the pulse pressure is often elevated such that there is increased excursion of the arterial diameter with associated extra transmural stress. It is possible that PGI_2 production is stimulated as a direct consequence of these increased mechanical stresses on the vessel wall.

To investigate this possibility, pressures and pulse pressures were reduced mechanically in abdominal aortas of genetically hypertensive rats (Chapter 7). In this model, blood flow through the abdominal aorta and thus pressure and pulse pressure, are diminished immediately the clip is applied. After

eighteen hours exposure to this reduced pressure and pulse pressure, these aortas released consistently less PGI_2 than did matched controls which had been subjected to identical surgical manipulation. These results indicate, that the reduction in mechanical stress, may have removed a stimulus to prostacyclin production and that there may be a direct relationship between the mechanical effect of elevated pressure and the increased ability of the vessel to generate PGI_2.

Of interest, in relation to this possible mechanical explanation, is the finding of Skidgel and Printz (1980), that unlike arteries, veins of SHR do not differ from those of normal rats in their cyclo-oxygenase activity. Clearly, veins of the two groups are not subject to the difference in transmural stress that is apparent between the arteries of the two groups. Similarly, Detre, Leithner, Winter, Sinzinger, Silberbauer, Fódy and Jellinek (1980) showed, that except for significantly elevated levels at day one, production of PGI_2 by inferior vena cava, was normal in renovascular hypertension induced by the Rojo-Ortega method for a period of thirty-five days, during which aortic PGI_2 was elevated.

There is also an inherent difference in prostacyclin generating capacity between arteries and veins in general. Although Moncada, Higgs and Vane (1977) reported that more prostacyclin was generated by human venous than arterial tissue, two independent groups have reported the opposite for rat vessels. Villa, Mysliwiec and de Gaetano (1977) found that rat aorta generated more 'prostacyclin-like' activity than vena cava and Skidgel and Printz (1978 and 1980) have shown that arteries of

rats have a greater capacity than veins to utilise arachidonic acid and PGH_2 in the formation of prostacyclin. The findings of these two groups, if it were not for the contradictory results of Moncada, Higgs and Vane (1977), would make it tempting to speculate that the different transmural stress between arteries and veins may account, at least in part, for their difference in PGI_2 generation.

It appears that PGI_2 production by veins may be subject to a pressure effect, for Hamilton, Rosza, Hutton, Chow, Dandona and Hobbs (1981) have shown that rat portal vein, in which portal hypertension has been induced by ligature one week previously, shows significantly elevated prostacyclin compared with control vessel exposed to the same surgical procedure. Skidgel and Printz (1978) have also found that the thoracic aorta of the rat has a greater capacity than the abdominal portion to convert PGH_2 to 6-keto-$PGF_{1\alpha}$. The thoracic portion is clearly under the greater stress, as it is exposed to variations in thoracic pressure due to respiration and experiences back pressure from the closing of the right atrial valves.

Thus increased direct transmural stress, appears attractive as an explanation for the elevated PGI_2 release in hypertension. However, the studies of Detre, Leithner, Winter, Sinzinger, Silberbauer, Fódy and Jellinek (1980) do not support this idea. These workers induced renovascular hypertension by application of a clip between the renal arteries. Above the clip they recorded elevated pressure and elevated PGI_2 formation, results which would be consistent with the findings reported in this thesis. However, they report an initial fall

in pressure below the clip (as recorded via the femoral artery), during which time PGI_2 production was elevated above normal values. It is possible that in this model of hypertension some other factor also effects prostacyclin release, for it is in this same study that by thirty-five days, despite a still elevated pressure, PGI_2 production by aorta both above and below the clip had returned to normal or below normal. This study, together with that of Yoshimura, Kaimasu, Kitani, Kawamura, Takeda, Sasaki, Kajita, Nakagawa and Ijichi (1980), in which after eight weeks of salt loading, pressure was elevated but PGI_2 had returned to normal, indicates that other factors beyond local mechanical effect are involved in the in vivo regulation of vascular PGI_2 generation.

Synthesis of PGI_2 by pig endothelial cell cultures and rat aortic rings, is stimulated in vitro by human plasma and there is evidence that plasma stimulation of PGI_2 synthesis may occur in vivo (Remuzzi, Misiani, Marchesi, Livio, Mecca, de Gaetano and Donati, 1978). These authors have suggested the existence of a plasma factor, which stimulates PGI_2 activity and which may be lacking in such disorders as haemolytic uraemic syndrome (HUS). If perhaps the increased PGI_2 release in hypertension were related to an elevation of this plasma factor, reduced plasma flow resultant on application of the clip (in the study in Chapter 7) could remove the stimulus to increased PGI_2 generation. This idea however, would not correlate with the fact that veins of SHR and normal rats (which would, like arteries, be exposed to different amounts of plasma factor) do not differ in prostacyclin producing capacity. It would also not be consistent with the proposal

of Remuzzi, Misiani, Marchesi, Livio, Mecca, de Gaetano and Donati (1978) that the hypertension in HUS may be due to defective PGI_2 release, due to lack of the plasma factor. Noradrenaline (Horton, Pipili and Poyser, 1980 and Pace-Asciak, Carrara, Rangeraj and Nicoloau, 1978), nerve stimulation (Horton, Pipili and Poyser, 1980) and angiotensin II (Gryglewski, Korbut and Splawinski, 1979) have also been shown to stimulate PGI_2 production in various situations. While therefore, there are clearly a number of factors which may interact to regulate PGI_2 release, it would appear that fluctuations in renin levels and mechanical transmural stress may be particularly important.

9.3.   BIOLOGICAL SIGNIFICANCE OF THE ELEVATED VASCULAR RELEASE OF PROSTACYCLIN IN HYPERTENSION.

In experiments in which increased aortic PGI_2 generation in experimental hypertension have been reported, results have been statistically significant. However, what is really important is whether or not differences obtained between test and control groups are of biological significance, i.e. are they quantitatively large enough to exert a noteable effect? Unfortunately this is very difficult to assess, for the absolute amounts of endogenous PGI_2 required to cause any particular response are not known. There are many experiments in which the effects of exogenous prostacyclin have been quantitated, but obviously extrapolation from these should be made with caution. In addition, it is not certain whether the amounts of PGI_2 being measured in vitro accurately reflect the

in vivo situation. Pace-Asciak, Carrara, Rangaraj and Nicoloau (1978) have shown that as little as a 5 mm section of SHR aorta, can produce enough prostacyclin to cause a 30 mmHg fall in blood pressure and they feel that this indicates the great capacity for PGI_2 production in vivo. However, there are two points which should be considered. Firstly the aortic rings in these experiments were incubated with arachidonic acid - a false situation as the quantity of substrate may be rate-limiting in vivo. Quirion, Rioux and Regoli (1978) have shown that SHR aortic strips produce twice as much 'prostaglandin-like' material when preincubated with arachidonic acid. Secondly, the use of aortic rings may not be truly representative. Certainly, preparation of the tissue is important, for Pace-Asciak, Carrara, Rangaraj and Nicoloau (1978) have reported that the products formed from arachidonate by aortic homogenates, are slightly different from those formed by rings. Further, ten Hoor, de Deckere, Haddeman, Hornstra and Quadt (1980) have reported that when expressed per cm^2 surface area, PGI_2 production by pieces of aorta is about five times that of pulsating perfused aorta. This finding would indicate, that investigators may be detecting amounts greatly in excess of those produced in vivo. On the other hand, in terms of detecting quantitative differences between two groups, these authors note that while changes in dietary linoleic acid did not significantly influence PGI_2 production of pieces of aorta, a significant increase was detected in the pulsating perfused aorta model. They suggest that the moderate activation of the perfused aorta, by simulation of physiological

pulsations, makes it possible to detect more subtle changes in PGI_2 production than in a more vigorously stimulated system. This would indicate that differences in PGI_2 production between hypertensive and control vessels, may be even greater than most workers have detected. It appears that in future studies, a truer reflection of the in vivo situation might be obtained, by use of the clearly more physiological model of perfused aorta, rather than by use of rings or strips.

Any conclusion about the meaning of the elevated levels of aortic PGI_2 production in hypertension which have been reported to date, should obviously be drawn bearing in mind the shortcomings of the methodology and the uncertainty about the biological significance of the differences.

9.4.   MEANING OF THE ELEVATED LEVELS OF AORTIC PROSTACYCLIN IN HYPERTENSION

If the consistent difference in prostacyclin generation which has been reported between hypertensive and control vessels, is in fact large enough to exert biological effect, then it is interesting to speculate on the possible function of the elevated levels in hypertension.

There has been much interest in a possible systemic role for circulating prostacyclin. Now however, evidence is mounting against the concept of PGI_2 functioning as a circulating hormone, either with a vasodepressor function, or as a regulator of platelet aggregability (Chapter 2.5). It therefore appears likely, that synthesis and action of PGI_2

is most often part of a localised response to a local stimulus, e.g regulation of vascular tone or thrombus formation at a regional level. For this reason, amounts synthesised by blood vessels may be of particular importance.

Since all the studies that have been done to date on prostacyclin in experimental hypertension, have involved vessels (e.g. aorta) which are not a major site of vascular resistance regulation, the functional implications of the results depend on the extent to which they reflect changes of PGI_2 production in resistance vessels. Skidgel and Printz (1980) have shown that cyclo-oxygenase activity is enhanced in homogenates of carotid artery of SHR as well as in aortic homogenates, indicating that the phenomenon may extend to all arteries. However, in the future, this will have to be confirmed for resistance vessels.

9.4.1. POSSIBLE EFFECT ON THE VASCULATURE.

Though there is ample evidence that prostacyclin generally dilates vascular beds and lowers blood pressure (Chapter 2.6.2), Levy (1980) has cautioned against ascribing to it a generalised vasodilator role, in view of the heterogeneity of individual blood vessel responses in a variety of species. Of particular relevance to the present study, may be his finding that PGI_2 (500-5000 ng/ml) produced a concentration dependent increase in contractile tension of isolated thoracic aortic strips, from normotensive rats and SHR. (No significant difference in response was noted between aortas from the two

groups of animals.) If large enough amounts of PGI_2 are generated by rat vessels and Levy's results are extrapolated to the in vivo situation, it would appear that for the thoracic aorta at least, vaso-constriction could be affected. On the basis of these findings, the elevated aortic generation of PGI_2 found in hypertension might appear to participate in the genesis or maintenance of hypertension. However, Kiprov and Somova, who noted in 1975 that the vasodepressor prostaglandins E and A paradoxically contracted isolated aortic smooth muscle strips of both normotensive rats and SHR, felt that this tissue could not be used as an indicator of vascular reactivity especially of resistance vessels, because of the paradoxical response.

If endogenous PGI_2 actually exerts a vasodilator role in the resistance vessels of the rat, then it may be that the enhanced synthesis in hypertension serves as an adaptive response to attenuate elevations in blood pressure, or keep them in 'control'. This hypothesis has been proposed by Pace-Asciak (1979). Release of PGI_2 has been shown to be increased in response to such stimuli as noradrenaline (Pace-Asciak, Carrara, Rangeraj and Nicoloau, 1978 and Horton, Pipili and Poyser, 1980), nerve stimulation (Horton, Pipili and Poyser, 1980) and angiotensin II (Gryglewski, Korbut and Splawinski, 1979 and Mullane and Moncada, 1980) and most evidence points to the fact that prostacyclin attenuates the vasoconstrictor action of these stimuli (Rascher, Dietz, Schömig, Burkart, Lüth, Mann and Weber, 1979, Carmignani, Marchetti and Caprino, 1980, Fischetti, Carmignani, Marchetti, Ranelletti and Caprino,

1980, Mullane and Moncada, 1980, Okuna, Kondo, Suzuki and Saruta, 1980 and Yabek and Avner, 1980). This property, i.e. modulation of pressor responses would be consistent with the protective role proposed by Pace-Asciak (1979). If the elevated PGI_2 were protective in nature, then the effect could be amplified by the altered sensitivity of the vasculature to prostacyclin which has been reported in hypertension. Pace-Asciak, Carrara and Nicoloau (1978) have shown, using anaesthetised animals, that SHR are much more responsive than normals to the hypotensive action of injected PGI_2. Similar findings have been reported by Casals-Stenzel and Morton (1979) for infusions of PGI_2 into conscious renal and DOC-salt hypertensive rats. The studies of Schölkens (1978) are also consistent with these findings:- rats with spontaneous hypertension and chronic renal hypertension appeared more sensitive than normal rats to the antihypertensive activity of PGI_2. It is unclear whether this increased response to prostacyclin in hypertension is actually related to the elevated pressure. Though the fall in blood pressure achieved 24 hours after the removal of the clip in 1 clip-2 kidney hypertension in the rat, is accompanied by a reduction of the heightened hypotensive response to PGI_2 (ten Berg, de Jong and Nijkamp, 1980), the increased response of SHR is abolished by feeding a diet containing α-linoleic acid, even though the pressure remains elevated (Feinberg, Trachte, Curtis and Lefer, 1980).

In line with the possible protective role for elevated prostacyclin, Pace-Asciak (1979) has suggested that stimulation

of PGI_2 production before deviation of vascular resistance in hypertension, may be useful. Certainly, Weeks and Sutter (1979) were able to demonstrate that continuous I.V. infusion of prostacyclin into concious rats, inhibited the development of one-kidney Goldblatt renal hypertension. Although they found no lowering of blood pressure with a comparative dose in sham operated rats, the doses used did have potential depressor activity which was masked by compensatory mechanisms. To exclude the possibility that the vasodilator action of PGI_2 may have merely provided compensation for the pressor influence following clipping of the renal artery, they studied sodium nitroprusside similarly (Sutter and Weeks, 1980) and found that it did not inhibit the development of .hypertension as prostacyclin had done. They therefore concluded that PGI_2 may play a specific role in preventing the onset of renal hypertension in the rat.

Another interesting study, which would also support the 'protective role' theory, is that of Terragno (1980). On inducing renovascular hypertension, while he found vascular PGI_2 production to be elevated, he found it to be even higher in animals which never developed raised pressure, despite manipulation aimed at inducing hypertension. He interpreted this result as indicating, that elevated prostacyclin levels may be protective and in some cases, may actually inhibit the development of hypertension.

Against the possible 'protective role' theory are such results as those of Detre, Leithner, Winter, Sinzinger, Silberbauer, Fódy and Jellinek (1980) and Yoshimura, Kaimasu, Kitani,

Kawamura, Takeda, Sasaki, Kajita, Nakagawa and Ijichi (1980) in which, after a number of weeks of certain forms of experimental hypertension, though pressure was still elevated, aortic PGI_2 elevation had returned to normal or below normal. Also, of course, one cannot be impressed by a protective mechanism that generally does not work, i.e. animals have an increased ability to generate PGI_2 but they are still hypertensive.

It appears that trying to assign a clear vascular function to the elevated aortic generation of PGI_2 found in hypertension, may be premature. Further studies will obviously provide additional insight. For example, metabolites of PGI_2 may be functionally important (Chapter 2.5) e.g. Gimeno, Sterin-Borda, Borda, Lazzari and Gimeno (1980) have reported that human plasma transforms PGI_2 into a platelet anti-aggregatory substance, which actually contracts isolated bovine coronary arteries (this may be 6-keto-PGE_1). Also, there are many vasoactive substances, levels of which may be important in relation to those of prostacyclin in the vasoconstriction of hypertension. Of particular interest are levels of TXA_2, since it is the balance between TXA_2 and PGI_2 which is more likely to be important in regulation of vascular tone, than are absolute amounts of either substance alone (Chapter 2). Unfortunately, measurement of thromboxane levels in hypertension have not kept pace with those of prostacyclin. Matsumoto, Nukada, Uyama, Yoneda, Imaizumi, Miyamoto and Kayama (1980) found that human platelets collected from control or hypertensive subjects, did not differ significantly in their ability to generate TXB_2 from arachidonic acid or PGH_2.

However, Inoue, Hayashi, Hara, Watanabe, Enomoto and Nomura (1979) have indicated that TXA_2 generation in platelets of SHR is increased. Should this be so, and if it also applies to other forms of experimental hypertension, then the elevated PGI_2 recorded may be nothing more than a mechanism to counterbalance the vasoconstrictor effects of excess TXA_2. Evidence reviewed by Ally and Horrobin (1980), indicates that in addition to its formation in platelets, TXA_2 is produced by vessel walls, in amounts which though small, are adequate to exert actions on both vascular reactivity and on platelets. Levels of vessel and platelet TXA_2 and hence the TXA_2/PGI_2 ratio need further attention and clarification if the relevance of the elevated levels of PGI_2 in hypertension, is to be established.

9.4.2. POSSIBLE EFFECT ON PLATELETS.

Another potential role for the elevated prostacyclin reported in hypertension, is at the level of platelet function, since prostacyclin has the capacity to increase platelet cAMP levels and inhibit platelet aggregation (Chapter 2.6.1). Clearly, one factor which might influence platelet response to the elevated levels of PGI_2, would be the sensitivity of the platelets to prostacyclin (Burghuber, Sinzinger, Silberbauer, Wolf and Haber, 1981). The possibility that the increased responsiveness of hypertensive vessels to the hypotensive effect of PGI_2, might amplify the proposed protective effect of the elevated levels of prostacyclin on the vasculature, has already been discussed (Chapter 9.4.1).

The sensitivity of genetically hypertensive rat platelets, to the anti-aggregatory effect of prostacyclin was investigated (Chapter 8). Sinzinger, Silberbauer, Horch and Gall (1981) have reported decreased sensitivity of human platelets to PGI_2, during long-term intra-arterial prostacyclin infusion in peripheral vascular disease. The possibility therefore existed, that increased aortic generation of PGI_2 in hypertension, might be associated with reduced platelet sensitivity. The results of the study however, invalidated this hypothesis. In fact, platelets of hypertensive rats showed sensitivity almost identical to that of normal controls.

Measurements of vascular PGI_2 in relation to platelet prostacyclin sensitivity have been made in other disorders. In diabetics, both decreased vascular prostacyclin generation and decreased platelet sensitivity, i.e. a 'double defect' have been reported and when atherosclerosis is induced in rabbits, though decreased vascular PGI_2 generation occurs, platelets are more sensitive to prostacyclin (Chapter 8.1). Bearing in mind the disadvantage of making comparisons when studies have involved different species, it is still interesting that in three disorders which are all associated with a high risk of arterial thrombosis, namely hypertension, diabetes and atherosclerosis, changes in vessel prostacyclin production and platelet sensitivity to prostacyclin do not appear to be consistent. In fact, the normal sensitivity of platelets in hypertension, would tend to indicate that the elevated vascular generation of PGI_2 may exert a protective role at the platelet level.

However, once again, consideration of prostacyclin in isolation obviously gives an incomplete picture. Generation of, and platelet sensitivity to TXA_2, will be of the utmost importance in determination of the TXA_2/PGI_2 balance, which is more likely to be relevant than levels of either substance alone, in the regulation of thrombus formation. In atherosclerosis in rabbits there is increased platelet production of, and sensitivity to, TXA_2 (Chapter 2.8.1) and in diabetic patients increased platelet synthesis of TXA_2 (Chapter 2.8.2). Thus in diabetics and in experimental atherosclerosis (most particularly at three months after feeding the atherogenic diet (Gryglewski, Dembinska-Kiec, Zmuda and Gryglewska, 1978)), it appears that the TXA_2/PGI_2 balance may be tipped in favour of thrombus formation.

In hypertension, if it is possible to extrapolate to experimental animals, the findings of Matsumoto, Nukada, Uyama, Yoneda, Imaizumi, Mijamoto and Kayama (1980) that for human subjects platelet thromboxane generation is normal, then the increased vascular generation of PGI_2 may continue to appear protective at the platelet level. However, it appears on a species basis, that the findings of Inoue, Hayashi, Hara, Watanabe, Enomoto and Nomura (1979) (that SHR platelets generate increased TXA_2) are more likely to extend to other models of experimental hypertension. If this is so, then any effect of the elevated prostacyclin on platelets may be balanced or even overshadowed by TXA_2. An elevated TXA_2/PGI_2 ratio would be in line with the observation of an increased tendency to arterial thrombosis in hypertension and

would correlate better with the type of balance recorded for diabetes and atherosclerosis. Clearly, thromboxane production (by both platelet and vessel) and platelet sensitivity to TXA_2, need further investigation in the models in which elevated prostacyclin has been reported.

Even after establishment of the TXA_2/PGI_2 ratio, there will be many other factors to be integrated with this knowledge. It has been shown, for example, that epinephrine blocks PGI_2 disaggregation of ADP aggregated platelets (Rao, Reddy and White, 1980), i.e. there are other vasoactive compounds (the exact involvement of which in hypertension is still to be established) which may interact with the arachidonate derivatives and platelet function. In addition, not all platelet behaviour is dependent on prostaglandin pathways and of course, in occlusive arteriolar disease events in the lumen (thrombosis) invariably co-exist with disease of the wall (atheroma).

9.5.   CONCLUSION

These studies have revealed, that aortas of rats with various forms of experimental hypertension, have an increased capacity for generation of the vasodilatory, anti-aggregatory substance prostacyclin. The findings are consistent with those of most other workers. Further, it has been shown that the elevated ability to release PGI_2, may be directly related to the mechanical effect of the increased transmural stress associated with elevated pressure.

Assuming that the established increased capacity of the aorta for prostacyclin generation extends to other arteries, various suggestions have been put forward as to the possible local function and consequent relevance of these elevated levels. Pace-Asciak (1979) has proposed that the raised levels may be a protective response to elevated pressure, attenuating further pressure rises. The results presented in this thesis, that hypertensive rat platelets are not less responsive than those of normals, to the anti-aggregatory effects of prostacyclin, would indicate that the 'protective role' may extend to platelet function.

Despite this proposed explanation, it appears that it will be possible to draw more valid conclusions about the findings once thromboxane levels and thus the TXA_2/PGI_2 ratio in hypertension have been established. It is possible, that despite elevated generation of PGI_2 by the vessel wall, the TXA_2/PGI_2 balance may be tipped in favour of the vasoconstrictive, proaggregatory substance TXA_2.

The discovery of the thromboxanes and prostacyclin has uncovered a new dimension of cardiovascular research but many questions remain unanswered. While studies with rats do contribute to our understanding, of greater interest will be the situation in humans, where direct measurement of vascular prostacyclin producing capacity is obviously more difficult than in experimental animals. Though plasma 6-keto-$PGF_{1\alpha}$ levels have been reported recently to be low in patients with essential hypertension (Uehara, Ishii, Takeda, Ikeda, Atarshi and Murao, 1981), one cannot be certain whether difference in

species or methodology, is responsible for results which conflict with studies in the rat.

In the future, further studies in this field are likely to provide information leading to a better understanding of the pathophysiology of such disorders as hypertension.

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- APPENDIX I -

EXPT. NO.	N		GH	
	WEIGHT (g)	BLOOD PRESSURE (mmHg)	WEIGHT (g)	BLOOD PRESSURE (mmHg)
1	322	135	300	200
2	330	120	290	195
3	360	112	300	200
4	360	130	320	210
5	370	110	310	210
6	340	118	300	210
7	360	135	310	190
8	360	110	300	175

WEIGHTS AND BLOOD PRESSURES OF GENETICALLY HYPERTENSIVE
RATS (GH) AND MATCHED NORMOTENSIVE CONTROLS (N).

	ng PROSTACYCLIN RELEASED / mg WET WEIGHT AORTA					
	N			GH		
EXPT. NO.	INCUBATION TIME (MIN)			INCUBATION TIME (MIN)		
	2	8	14	2	8	14
1	1,77	3,41	3,77	2,43	3,99	4,22
2	2,24	2,35	3,36	2,02	4,68	4,81
3	0,69	2,80	3,68	2,36	3,57	4,03
4	0,57	3,14	3,52	1,30	3,69	4,35
5	1,66	3,00	4,05	2,77	4,43	4,81
6	2,41	4,27	4,36	3,40	4,39	4,62
7	0,99	2,37	3,29	1,62	2,93	3,79
8	2,93	4,02	4,39	2,55	3,82	4,36

ng PROSTACYCLIN RELEASED / mg WET WEIGHT AORTA
OF GENETICALLY HYPERTENSIVE RATS (GH)
AND MATCHED NORMOTENSIVE CONTROLS (N)
AT VARIOUS SAMPLING TIMES.

– APPENDIX II –

EXPT. NO.	N		ERH	
	WEIGHT (g)	B.P. 10 WEEKS POST-OP (mmHg)	WEIGHT (g)	B.P. 10 WEEKS POST-OP (mmHg)
1	295	106	236	130
2	325	115	282	125
3	275	110	227	160
4	275	110	215	160
5	265	108	188	145

WEIGHTS AND BLOOD PRESSURES (B.P.) OF RATS
WITH EXPERIMENTAL RENAL HYPERTENSION (ERH)
AND MATCHED NORMOTENSIVE CONTROLS (N).

EXPT. NO.	ng PROSTACYCLIN RELEASED / mg WET WEIGHT AORTA					
	N			ERH		
	INCUBATION TIME (min.)			INCUBATION TIME (min.)		
	2	8	18	2	8	18
1	2,28	4,70	4,86	2,41	6,13	7,48
2	3,85	6,17	6,32	7,18	8,14	8,61
3	2,90	6,80	6,35	5,47	7,84	8,06
4	2,20	5,96	6,45	5,51	8,63	8,77
5	3,98	5,85	6,97	3,95	7,05	7,32

ng PROSTACYCLIN RELEASED / mg WET WEIGHT AORTA
OF RATS WITH EXPERIMENTAL RENAL HYPERTENSION (ERH)
AND MATCHED NORMOTENSIVE CONTROLS (N)
AT VARIOUS SAMPLING TIMES.

— APPENDIX III —

EXPT. NO.	T			C		
	WEIGHT (g)	B.P. BEFORE TREATING (mmHg)	B.P. AFTER TREATING (mmHg)	WEIGHT (g)	B.P. BEFORE TREATMENT PERIOD (mmHg)	B.P. AFTER TREATMENT PERIOD (mmHg)
1	200	155	105	205	145	160
2	210	170	105	225	150	190
3	202	150	110	235	138	170
4	210	175	100	285	165	200
5	220	190	110	230	160	160
6	220	162	120	220	180	168
7	220	165	95	220	157	168
8	260	172	90	220	175	165
9	220	175	106	220	164	165

WEIGHTS AND BLOOD PRESSURES (B.P.) OF TREATED (T)
AND UNTREATED CONTROL (C) RATS.

EXPT. NO.	ng PROSTACYCLIN RELEASED / mg WET WEIGHT AORTA					
	T			C		
	INCUBATION TIME (MIN)			INCUBATION TIME (MIN)		
	2	8	14	2	8	14
1	0,28	1,80	2,38	0,23	1,87	2,39
2	0,45	0,83	1,47	0,33	1,06	1,57
3	0,00	1,42	2,07	0,17	1,95	2,30
4	0,90	2,08	2,20	0,78	1,80	1,98
5	0,80	1,94	1,94	0,92	2,06	2,24
6	0,59	2,57	2,66	1,14	2,93	3,17
7	0,53	0,66	0,91	0,60	1,00	1,75
8	0,29	2,59	2,93	0,56	1,96	2,73
9	0,34	0,68	2,49	0,90	3,02	3,41

ng PROSTACYCLIN RELEASED / mg WET WEIGHT AORTA
OF TREATED (T) AND UNTREATED CONTROL (C) RATS
AT VARIOUS SAMPLING TIMES.

- APPENDIX IV -

EXPT. NO.	C			S		
	WEIGHT (g)	PRE-OP. B.P. (mmHg)	POST-OP. B.P. (mmHg)	WEIGHT (g)	PRE-OP. B.P. (mmHg)	POST-OP. B.P. (mmHg)
1	320	218	116	320	212	150
2	300	182	107	290	194	165
3	305	172	95	300	172	159
4	308	170	95	310	170	162
5	240	168	90	290	172	152
6	270	158	90	260	161	147
7	295	189	75	290	182	168
8	292	166	90	300	174	178

WEIGHTS AND BLOOD PRESSURES (B.P.) OF CLIPPED (C)
AND SHAM (S) RATS.

EXPT. NO.	ng PROSTACYCLIN RELEASED / mg WET WEIGHT AORTA					
	C			S		
	INCUBATION TIME (MIN)			INCUBATION TIME (MIN)		
	2	8	14	2	8	14
1	1,61	2,68	3,08	1,20	2,90	3,28
2	0,97	2,86	2,90	1,27	2,72	2,90
3	1,51	3,47	3,76	1,26	4,12	4,29
4	2,00	4,06	4,47	1,63	4,40	4,91
5	1,67	3,09	3,96	2,40	3,76	4,24
6	0,76	2,11	2,81	0,74	3,86	3,96
7	2,02	3,31	3,50	2,09	4,15	4,38
8	1,47	3,54	3,83	1,99	4,11	4,36

ng PROSTACYCLIN RELEASED / mg WET WEIGHT AORTA
OF CLIPPED (C) AND SHAM (S) RATS
AT VARIOUS SAMPLING TIMES.

– APPENDIX V –

	N			GH		
EXPT. NO	WEIGHT (g)	BLOOD PRESSURE (mmHg)	POOLED PLATELET NO.X10³	WEIGHT (g)	BLOOD PRESSURE (mmHg)	POOLED PLATELET NO.X10³
1	240	80		320	170	
	230	95	996	250	158	1176
	270	111		260	160	
2	290	106		290	180	
	250	120	1334	240	180	1322
	260	125		240	180	
3	280	118		240	175	
	270	115	1296	240	170	1156
	300	125		240	188	
4	260	115		230	170	
	280	119	1540	260	166	1420
	230	122		265	179	
5	270	116		265	169	
	300	124	1302	270	183	1482
	265	125		240	176	
6	280	96		280	160	
	260	115	1682	250	160	1942
	240	118		310	190	
7	260	115		240	172	
	270	120	2134	280	168	2226
	220	125		270	180	

WEIGHTS, BLOOD PRESSURES AND PLATELET COUNTS OF
GENETICALLY HYPERTENSIVE (GH) AND NORMAL (N) RATS.

EXPT. NO.	MAXIMUM AGGREGATION MEASURED AS $^\circ/^\circ$ OF DISTANCE PRP TO PPP							
	N				GH			
	CONC. ADDED PGI_2 (ng/ml)				CONC. ADDED PGI_2 (ng/ml)			
	0	20	33,33	50	0	20	33,33	50
1	68,12	48,33	26,72	19,05	65,33	44,10	19,06	13,79
2	47,02	18,07	9,73	0,00	63,59	29,94	33,06	13,85
3	64,49	49,04	31,96	5,68	49,75	41,24	21,74	3,32
4	63,46	40,97	33,71	24,04	68,48	45,88	45,64	27,67
5	59,45	40,00	19,51	8,17	73,04	53,73	27,51	15,39
6	69,61	–	25,24	0,00	70,82	–	36,72	4,41
7	74,88	56,73	15,07	9,71	80,39	50,52	15,71	5,05

$^\circ/^\circ$ AGGREGATION ACHIEVED WITH 1 μM ADP
IN NORMAL (N) AND GENETICALLY HYPERTENSIVE (GH) RAT PLATELETS
IN THE PRESENCE OF VARYING CONCENTRATIONS OF PGI_2.