PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Prostacyclin: Its Biosynthesis, Actions and Clinical Potential [and Discussion]

S. Moncada, J. R. Vane, Elspeth B. Smith, D. B. Longmore and H. O. J. Collier

Phil. Trans. R. Soc. Lond. B 1981 **294**, 305-329
doi: 10.1098/rstb.1981.0108

Phil. Trans. R. Soc. Lond. B **294**, 305–329 (1981)
Printed in Great Britain

Prostacyclin: its biosynthesis, actions and clinical potential

By S. Moncada and J. R. Vane, F.R.S.
Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.

Prostacyclin (PGI_2) is the product of arachidonic acid metabolism generated by the vessel wall of all mammalian species studied, including man. Prostacyclin is a potent vasodilator and the most potent inhibitor of platelet aggregation so far described. Prostacyclin inhibits aggregation through stimulation of platelet adenyl cyclase leading to an increase in platelet cyclic AMP. In the vessel wall, the enzyme that synthesizes prostacyclin is concentrated in the endothelial layer. Prostacyclin can also be a circulating hormone released from the pulmonary circulation. Based on these observations we proposed that platelet aggregability *in vivo* is controlled via a prostacyclin mechanism.

The discovery of prostacyclin has given a new insight into arachidonic acid metabolism and has led to a new hypothesis about mechanisms of haemostasis. Reductions in prostacyclin production in several diseases, including atherosclerosis and diabetes, have been described and implicated in the pathophysiology of these diseases. Additionally, since prostacyclin powerfully inhibits platelet aggregation and promotes their disaggregation, this agent could have an important use in the therapy of conditions in which increased platelet aggregation takes place and in which, perhaps, a prostacyclin deficiency exists.

Prostacyclin has been used beneficially in humans during extracorporeal circulation procedures such as cardiopulmonary bypass, charcoal haemoperfusion and haemodialysis. Its possible use in other conditions such as peripheral vascular disease or transplant surgery is at present being investigated.

During 1975, Moncada and coworkers began to look for biosynthesis of thromboxane A_2 (TXA_2) by various tissues other than platelets. Vascular tissues did not generate TXA_2, but the cascade bioassay technique (Vane 1964) showed that microsomal fractions of blood vessels converted the endoperoxide precursor enzymically into an unknown product that was labile and relaxed the coeliac and mesenteric arteries of the rabbit (Moncada *et al.* 1976*a*). They called this substance PGX, and showed also that it inhibited platelet aggregation; in fact it was the most potent inhibitor of platelet aggregation known, being 30–40 times more potent than PGE_1 (Moncada & Vane 1978). In later work PGX was characterized further; it potently relaxed coronary (Dusting *et al.* 1977*a*) as well as splanchnic vascular strips *in vitro* (Bunting *et al.* 1976), dilated vascular beds *in vivo* (Armstrong *et al.* 1977, 1978; Dusting *et al.* 1978*c*) and had strong antithrombotic activity *in vivo* (Higgs *et al.* 1977; Ubatuba *et al.* 1979). Furthermore, it was the major metabolite of arachidonic acid in vascular tissues (Johnson *et al.* 1976; Salmon *et al.* 1978). PGX was the unstable intermediate in the formation of 6-oxo-$PGF_{1\alpha}$, a compound described by Pace-Asciak (1976) as a product of prostaglandin (PG) endoperoxides in the rat stomach. The work that led to the elucidation of the structure of PGX was carried out as a collaborative effort between scientists from the Wellcome Research Laboratories and from the Upjohn Company (Johnson *et al.* 1976). PGX was then renamed prostacyclin with the abbreviation of PGI_2. It has now been given the approved name of epoprostenol, but the trivial name of prostacyclin will be used throughout this review.

The discovery of prostacyclin, together with the isolation and characterization of the prostaglandin endoperoxides and TXA_2 which preceded it (Hamberg & Samuelsson 1973; Hamberg *et al.* 1974, 1975; Nugteren & Hazelhof 1973), have added substantially to our understanding of platelet – vessel wall interactions, and opened new lines of research in haemostasis and thrombosis. Another consequence that is also gathering momentum is a better understanding of the basis of some diseases. In this chapter we shall deal mainly with the way in which the balance between aggregatory and anti-aggregatory metabolites of arachidonic acid affects the processes of haemostasis and thrombosis. We shall discuss the regulation and pharmacological manipulation of prostacyclin biosynthesis as well as disturbances in its biosynthesis in some pathological conditions. The therapeutic potential of prostacyclin as an antithrombotic agent will be addressed in the last section.

The ability of the vessel wall to synthesize prostacyclin is greatest at the intimal surface and progressively decreases towards the adventitia (Moncada *et al.* 1977). Cultures of cells from vessel walls also show that endothelial cells are the most active producers of prostacyclin (MacIntyre *et al.* 1978; Weksler *et al.* 1977*b*); moreover, this production persists after numerous subcultures *in vitro* (Christofinis *et al.* 1979).

Initially it was demonstrated that vessel microsomes in the absence of cofactors could utilize prostaglandin endoperoxides, but not arachidonic acid, to synthesize prostacyclin (Moncada *et al.* 1976*a*). Later it was shown that fresh vascular tissue could utilize both precursors, although the endoperoxides were much better substrates (Bunting *et al.* 1976). Moreover, vessel microsomes, fresh vascular rings or endothelial cells treated with indomethacin could, when incubated with platelets, generate a prostacyclin-like anti-aggregating activity (Bunting *et al.* 1976, 1977; Gryglewski *et al.* 1976). The release of this substance was inhibited by 15-hydroperoxyarachidonic acid (15-HPAA) and other fatty acid hydroperoxides known to be selective inhibitors of prostacyclin formation (Gryglewski *et al.* 1976; Moncada *et al.* 1976*b*; Salmon *et al.* 1978). From all these data we concluded that the vessel wall can synthesize prostacyclin from its own endogenous precursors, but also that it can utilize prostaglandin endoperoxides released by the platelets, thus suggesting a biochemical cooperation between platelet and vessel wall (Moncada & Vane 1978, 1979*b*).

This hypothesis was challenged by Needleman *et al.* (1978), who demonstrated that while arachidonic acid was rapidly converted to prostacyclin by perfused rabbit hearts and kidneys, PGH_2 was not readily transformed. They concluded that some degree of vascular damage is necessary for the endoperoxide to be utilized by prostacyclin synthetase. On the other hand, incubation of platelet-rich plasma (p.r.p.) with fresh, indomethacin-treated arterial tissue leads to an increase in platelet cyclic AMP (cAMP) (Best *et al.* 1977) that parallels the inhibition of the aggregation and can be abolished by previous treatment of the vascular tissue with tranylcypromine, a less active inhibitor of prostacyclin formation (Gryglewski *et al.* 1976). Furthermore, Tansik *et al.* (1978) showed that lysed aortic smooth muscle cells could be supplied with prostaglandin endoperoxides by lysed human platelets to form prostacyclin. Finally, undisturbed endothelial cell monolayers readily transform PGH_2 to prostacyclin (Marcus *et al.* 1978).

Needleman *et al.* (1979) and Hornstra *et al.* (1979), using vessel microsomes or fresh vascular tissue, concluded that endoperoxides from platelets cannot be utilized by other cells under their experimental conditions. However, more recently, Marcus *et al.* (1979; see also Marcus *et al.*, this symposium) showed that feeding of endoperoxides to endothelial cells suspended in p.r.p.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

takes place *in vitro*, but only when the platelet number is around normal blood levels. Too high a platelet concentration induces a platelet–platelet interaction that limits the platelet – endothelial cell reaction. It should be stressed, however, that the possibility of platelet-released endoperoxides being utilized by endothelial cells has not yet been tested *in vivo*. Adherence of the platelet to the vessel wall could well provide the proximity that would be needed for such 'cooperation'.

It is also possible that formed elements of blood such as the white cells, which produce endoperoxides and TXA_2 (Davison *et al.* 1978; Goldstein *et al.* 1977; Higgs *et al.* 1976) could interact with the vessel wall to promote formation of prostacyclin. Moreover, leucocytes themselves generate prostacyclin in whole blood, especially in the presence of thromboxane synthetase inhibitors (Flower & Cardinal 1979). Thus, prostacyclin might regulate white cell behaviour (Higgs *et al.* 1978*b*; Weksler *et al.* 1977*a*), and help to control white cell activity during the inflammatory response. Interestingly, an artificial surface, when exposed to blood *in vivo*, initially becomes coated with platelets, but this coat is slowly replaced by a pavement of white cells. The white cell pavement is then unattractive to platelets, and this could be due to prostacyclin generation by the leucocytes.

Bradykinin and angiotensin release prostaglandins from the kidney (Aiken & Vane 1973; McGiff *et al.* 1970, 1972), lungs (Vane & Ferreira 1976) and other organs *in vivo* (Ferreira *et al.* 1973). Before the discovery of prostacyclin, Gimbrone & Alexander (1975) had demonstrated that angiotensin II stimulated the generation of an immunoreactive, PGE-like substance by human umbilical endothelial cells in culture. Needleman and coworkers (Blumberg *et al.* 1977; Needleman 1976; Needleman *et al.* 1975) had also described the release by angiotensin and bradykinin of a PGE-like substance from rabbits' isolated perfused hearts and mesenteric vessels. The PGE-like substance was characterized by bioassay on gastrointestinal tissues and chromatographic mobility on thin-layer plates. It is now clear that these techniques do not readily distinguish between prostacyclin (or 6-oxo-$PGF_{1\alpha}$) and PGE_2 (Moncada & Vane 1978; Omini *et al.* 1977), and Needleman *et al.* (1978) have now shown that bradykinin or angiotensin II release a prostacyclin-like substance from Langendorff-perfused hearts of rabbits. Moreover, Dusting and coworkers (Dusting & Mullins 1980; Dusting *et al.* 1981) demonstrated that angiotensins I and II release much more prostacyclin than PGE_2 from perfused isolated mesenteric vasculature of rats, and prostacyclin was identified as the major prostacyclin released from the pulmonary circulation of the dog *in vivo* by antiotensin I or II (Mullane & Moncada 1980*b*). In contrast, the isolated perfused kidney of the rabbit converts exogenous arachidonate predominately into prostacyclin, but PGE_2 is the main prostanoid released by bradykinin and angiotensin II into the venous effluent (Needleman *et al.* 1978). However, perfusion of isolated kidneys with albumin-free Krebs's solution produces a large increase in glomerular filtration rate so that medullary perfusion is enhanced. Since PGE_2 is prevalent in the medulla and the effluent assayed is a mixture of venous and urinary outflow, this technique could account for the large quantities of PGE_2. In the canine kidney *in vivo*, prostacyclin and not PGE_2 was identified as the main prostaglandin released into renal venous blood by angiotensin and bradykinin (Mullane & Moncada 1980*b*). Small quantities of PGE_2 (approximately 10% of those of prostacyclin) were observed in some experiments by these workers. These findings have renewed interest in the concept proposed by Vane & McGiff (1975) that prostaglandins released by angiotensin and bradykinin may modulate or partly mediate the renal and vascular actions of these peptides.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The pulmonary circulation has long been recognized for its ability to transform arachidonic acid rapidly into more polar products. Indeed most known metabolites of arachidonic acid have at one time or another been proposed as major products generated by the lungs. Isolated perfused lungs of guinea pigs, rats and rabbits release prostaglandins E_2 and $F_{2\alpha}$, TXA_2, lipoxygenase metabolites of arachidonic acid, prostacyclin and metabolites of all these substances when they are challenged with histamine, bradykinin, 5-hydroxytryptamine, arachidonic acid or anaphylactic shock. These products are also generated when pulmonary tissue is subjected to mechanical trauma. Gryglewski (1979) has recently reviewed evidence that isolated perfused lungs of cats, rats, rabbits and guinea-pigs release spontaneously a prostacyclin-like substance, and little other arachidonate-derived material, when perfused through the pulmonary artery with Krebs's solution. Prostacyclin has been identified in the pulmonary effluent by relaxation of bovine coronary artery strips, by disaggregation of platelet clumps, and by mass spectrometric quantification of the stable degradation product of prostacyclin, 6-oxo-$PGF_{1\alpha}$. The output of prostacyclin is blocked by cyclo-oxygenase inhibitors and is stimulated by low concentrations of arachidonic acid (100 ng/ml), angiotensin I, angiotension II or bradykinin.

The release of prostacyclin induced by angiotensin I is blocked by the converting enzyme inhibitor, captopril, in isolated guinea pig lungs, rat mesenteric vasculature (Dusting & Mullins 1980; Dusting *et al.* 1981; Grodzinska & Gryglewski 1980; Gryglewski 1979) and the pulmonary and renal circulation of anaesthetized dogs *in vivo* (Mullane & Moncada 1980*a*). Prostacyclin release induced by angiotensin I or II is also abolished by the receptor antagonists saralasin or [Sar^1–Ala^8]-angiotensin II in both the rat and the dog (Dusting 1981*a*; Dusting *et al.* 1981; Gryglewski 1979). Thus, prostacyclin is released by activation of an angiotensin II receptor, and is not released directly by angiotensin I. Activation of the angiotensin II receptor appears to be linked to a phospholipase, since angiotensin II-stimulated prostacyclin release can be abolished by dexamethasone or mepacrine (Dusting 1981*a*).

Other peptides or amines tested do not release prostacyclin from perfused lungs (Gryglewski 1979). Noradrenalin and vasopressin do not release prostacyclin from rat mesenteric vessels, despite their potent vasoconstrictor effects (Dusting & Mullins 1980; Dusting *et al.* 1981). Moreover prostacyclin release into the circulation of the dog was not observed after injections of adrenalin, noradrenalin or 5-hydroxytryptamine (Mullane & Moncada 1980*b*), despite changes in systemic blood pressure.

Therefore, the release of prostacyclin induced by angiotensin and bradykinin does not appear to be a simple consequence of the mechanical events associated with alterations in vessel diameter. These observations, together with the finding that low concentrations of prostacyclin are released from the lungs *in vivo*, prompted the proposal that the pulmonary endothelium may be regarded as an endocrine organ regulating platelet behaviour (Gryglewski *et al.* 1978*a*, *b*; Moncada *et al.* 1978).

In rats and dogs, prostacyclin is a much more powerful vasodepressor agent than PGE_2, but only when the two substances are given intravenously, and not if they are given into the aorta (Armstrong *et al.* 1977, 1978). Using dogs, we showed by direct bioassay in circulating blood that prostacyclin escapes the pulmonary inactivation process (Dusting *et al.* 1977*b*), which normally removes 95 % or more of PGE_2 or $PGF_{2\alpha}$ in a single circulation *in vivo*. Thus, prostacyclin can recirculate (Dusting *et al.* 1978*b*). Furthermore, infused arachidonic acid is converted into prostacyclin in passage across the lung circulation *in vivo* (Dusting *et al.* 1978*a*; Mullane *et al.* 1979). Therefore, prostacyclin generated in the lung or elsewhere would not be confined to a local site of action, and is potentially a circulating hormone.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Gryglewski *et al.* (1978*a*) developed a technique for continuously measuring platelet aggregation in circulating blood of anaesthetized cats, and showed that arterial blood contained higher concentrations of an anti-aggregatory substance than mixed venous blood. They concluded that the arterial–venous difference was due to prostacyclin released from the lungs since the difference was abolished by aspirin or by incubating the blood at 37 °C for 10 min, during which time prostacyclin activity disappears (Dusting *et al.* 1977*b*, 1978*b*). Moncada *et al.* (1978) applied this technique to anaesthetized rabbits, and came to the same conclusion, since the greater disaggregatory activity present in arterial blood was abolished by an antibody raised against 5,6-dihydro prostacyclin (6β-PGI_1), which cross-reacts with prostacyclin. Moreover, the prostacyclin-like, disaggregatory substance in arterial blood is increased during hyperventilation of the lungs, or after pulmonary embolism by intravenous injection of air (Gryglewski 1979). In a recent study, 6-oxo-$PGF_{1\alpha}$, measured by mass spectrometry, was at a higher concentration in the arterial than in the venous side of the circulation of five patients undergoing cardiac catheterization (Hensby *et al.* 1979*a*). Thus, the lungs may constantly release small amounts of prostacyclin into the passing blood. This, combined with 50 % overall inactivation in one circulation through peripheral tissues (Dusting *et al.* 1978*b*), would account for higher levels in arterial than in venous blood.

Three reservations about these results should be mentioned. First, in the studies with anaesthetized cats and rabbits, blood was drawn through an extracorporal circuit with a peristaltic pump. Under such conditions, the circulating blood volume would be slightly reduced and this may lead to a stimulation of the renin–angiotensin system, which in turn could stimulate prostacyclin release (see below). In addition, it is now well recognized that surgical procedures in anaesthetized small animals can exaggerate the contribution of prostaglandins to renal homoeostasis (Terragno *et al.* 1977), and by analogy, the same may be true for the lungs. Secondly, in these extracorporeal experiments platelet emboli dislodged from the collagen strips return to the animal in the venous blood. The trapping of platelet emboli in the lungs may be an additional stimulus for generation of prostacyclin under these conditions (Aiken 1979). Platelet emboli are also generated, and returned to the animal in other extracorporeal systems, particularly when venous blood is reoxygenated for bioassay on a cascade of smooth muscle strips. Thirdly, the biotransformation of prostacyclin in the human circulation is not yet fully understood, and the assumption that 6-oxo-$PGF_{1\alpha}$ determined in blood samples is a reliable index of concentrations of active prostacyclin in circulating blood may not be valid. Recent studies of human platelet aggregation performed within 3 min of withdrawal of arterial or venous blood (Steer *et al.* 1980) led to the conclusion that circulating levels of prostacyclin in resting man were too low to influence aggregability of platelets, but again it is important to note that these tests were performed *in vitro*. Studies in which levels of prostacyclin or its metabolites have been determined in man have failed to clarify the situation. Prostacyclin-like activity was detectable in human venous blood used to superfuse various tissues sensitive to prostacyclin (Neri Serneri *et al.* 1980). The level rose by several nanograms per millilitre with relief of ischaemia, and was reduced by pretreatment with indomethacin. However, in a study in which 6-oxo-$PGF_{1\alpha}$ in human blood samples was measured by mass spectrometry, levels of 80 pg/ml in venous blood and approximately double that in arterial blood were obtained (Hensby *et al.* 1979*a*). Although these levels are lower than those achieved by bioassay, they are still much higher than those obtained by measuring the daily turnover in urine of a metabolite of prostacyclin (Oates *et al.* 1980). Further work is necessary to establish clearly the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

routes of catabolism of both prostacyclin and 6-oxo-$PGF_{1\alpha}$ in the human circulation, to determine whether there is an effective level of circulating prostacyclin in normal man at rest or during exercise.

Prostacyclin is the most potent endogenous inhibitor of platelet aggregation yet discovered. It is 30–40 times more potent than PGE_1 (Moncada & Vane 1977). and more than 1000 times more active than adenosine (Born 1962). *In vivo*, prostacyclin applied locally in low concentrations inhibits thrombus formation due to ADP in the microcirculation of the hamster cheek pouch (Higgs *et al.* 1977) and given systemically to the rabbit it prevents electrically induced thrombus formation in the carotid artery and increases bleeding time (Ubatuba *et al.* 1979). The duration of these effects *in vivo* in short: they disappear within 30 min of administration. Prostacyclin disaggregates platelets *in vitro* (Moncada *et al.* 1976*b*; Ubatuba *et al.* 1979), in extracorporeal circuits where platelet clumps have formed on collagen strips (Gryglewski *et al.* 1978*a*, *c*), and in the circulation of man (Szczeklik *et al.* 1978*b*). Moreover, it inhibits thrombus formation in a coronary artery model in the dog when given locally or systemically (Aiken *et al.* 1979) and protects against sudden death (thought to be due to platelet aggregation) induced by intravenous arachidonic acid in rabbits (Bayer *et al.* 1979).

Prostacyclin is unstable and its activity disappears within 15 s on boiling or within 10 min at 22 °C at neutral pH. In blood at 37 °C, the activity of prostacyclin (as measured by bioassay on vascular smooth muscle) has a half-life of 2–3 min (Dusting *et al.* 1977*b*, 1978*b*). It has been reported that prostacyclin has an extended stability in plasma or blood (Gimeno *et al.* 1980; Wynalda & Fitzpatrick 1980) and that this may be associated with binding to albumin or with metabolism to 6-oxo-PGE_1 (Blasko *et al.* 1980). The relevance of these observations to the actual biological activity remains unclear. Alkaline pH increases the stability of prostacyclin (Cho & Allen 1978; Johnson *et al.* 1976) so that at pH 10.5 at 25 °C, it has a half-life of 100 h. It can be stabilized as a pharmaceutical preparation by freeze-drying and can be reconstituted in an alkaline glycine buffer for use in man.

The generation of prostacyclin is an active mechanism by which the vessel wall could be protected from deposition of platelet aggregates. Prostacyclin formation thus provides an explanation of the long recognized fact that contact with healthy vascular endothelium is not a stimulus for platelet clumping. An imbalance between formation of prostacyclin and TXA_2 could be of dramatic consequence.

Vascular damage leads to platelet adhesion but not necessarily to thrombus formation. When the injury is minor, platelet thrombi are formed which break away from the vessel wall and are washed away by the circulation. The degree of injury is an important determinant, and there is general agreement that for the development of thrombosis, severe damage or physical detachment of the endothelium must occur. All these observations are in accord with the differential distribution of prostacyclin synthetase across the vessel wall, decreasing in concentration from the intima to the adventitia (Moncada *et al.* 1977). Moreover, the pro-aggregating elements increase from the subendothelium to the adventitia. These two opposing tendencies render the endothelial lining anti-aggregatory and the outer layers of the vessel wall thrombogenic (Moncada *et al.* 1977).

The ability of the vascular wall actively to prevent aggregation has been postulated before (Saba & Mason 1974). For instance, the presence of an ADPase in the vessel wall has led to the suggestion that this enzyme, by breaking down ADP, limits platelet aggregation (Heyns *et al.* 1974; Lieberman *et al.* 1977). We have confirmed the presence of an ADPase in the vessel wall.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

However, the anti-aggregating activity is mainly related to the release of prostacyclin, for 15-HPAA or 13-hydroperoxylinoleic acid (13-HPLA), two inhibitors of prostacyclin formation that have no activity on ADPase, abolish most if not all of the anti-aggregatory activity of vascular endothelial cells (Bunting *et al.* 1977). Similar results have been obtained with an antiserum which cross-reacts with and neutralizes prostacyclin *in vitro* (Bunting *et al.* 1978). Endothelial cells pretreated with this antiserum can no longer inhibit ADP-induced aggregation (Bunting *et al.* 1978; Christofinis *et al.* 1979).

It is not yet clear whether prostacyclin is responsible for all the thromboresistant properties of the vascular endothelium and it would be unusual for an important biological principle to rely on a single mechanism. However, Czervionke *et al.* (1979), using endothelial cell cultures, have demonstrated that platelet adherence in the presence of thrombin increases from 4% to 44% after treatment with 1 mM aspirin. This increase was accompanied by a decrease in 6-oxo-$PGF_{1\alpha}$ formation from 107 nM to less than 3 nM and could be reversed by addition of 25 nM endogenous prostacyclin. This suggests that prostacyclin, although not responsible for all the thromboresistant properties of vascular endothelium, plays an important role in the control of platelet aggregability.

Prostacyclin inhibits platelet aggregation (platelet–platelet interaction) at much lower concentrations than those needed to inhibit adhesion (platelet–collagen interaction) (Higgs *et al.* 1978*a*). This suggests that prostacyclin can allow platelets to stick to vascular tissue and to interact with it, while at the same time preventing or limiting thrombus formation. Certainly, platelets adhering to a site where prostacyclin synthetase is present could well feed the enzyme with endoperoxide, thereby producing prostacyclin and preventing other platelets from clumping onto the adhering platelets, limiting the cells to a monolayer. Weiss & Turitto (1979) have observed some degree of inhibition of platelet–endothelium interactions with low concentrations of prostacyclin at high shear rates, but at none of the concentrations used could they observe total inhibition of platelet adhesion.

Prostacyclin inhibits platelet aggregation by stimulating adenylate cyclase, leading to an increase in cAMP levels in the platelets (Gorman *et al.* 1977; Tateson *et al.* 1977). In this respect prostacyclin is much more potent than either PGE_1 or PGD_2 (Tateson *et al.* 1977). 6-Oxo-$PGF_{1\alpha}$ has relatively weak antiaggregatory activity and is almost devoid of activity on platelet cAMP (Tateson *et al.* 1977).

Prostacyclin is not only more potent than PGE_1 in elevating cAMP but the elevation persists longer. The elevation induced by PGE_1 in platelets *in vitro* starts falling after 30 s, while prostacyclin stimulation is not maximal until after 30 s. It is then maintained for 2 min after which it gradually wanes over 30 min (Gorman *et al.* 1977). Prostacyclin also strongly stimulates adenylate cyclase in isolated membrane preparations (Gorman *et al.* 1977).

Prostacyclin, PGE_1 and PGD_2 stimulate adenylate cyclase by acting on two distinct receptors on the platelet membrane (Miller & Gorman 1979; Whittle *et al.* 1978). PGE_1 and prostacyclin act on one, whereas PGD_2 acts on another. This is shown by differences in activity in different species (Whittle *et al.* 1978), and by the use of a prostaglandin antagonist (Eakins *et al.* 1976) that selectively prevents the inhibition of platelet aggregation induced by PGD_2 but not that induced by prostacyclin or PGE_1 (Whittle *et al.* 1978). Moreover, studies of agonist-specific sensitization of cAMP accumulation in platelets show that PGE_1 or PGE_2 can desensitize for subsequent PGE_1 or prostacyclin activation, and that subthreshold concentrations of prostacyclin desensitize for PGE_1 stimulation. PGD_2, however, desensitizes to a further dose of PGD_2 but not

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

to PGE_1 or prostacyclin. These results suggest (Miller & Gorman 1979; Whittle *et al.* 1978) that the receptor in platelets previously described as a PGE_1 receptor (MacDonald & Stuart 1974) is, in fact, a prostacyclin receptor.

There have not been many detailed studies of the mechanism of action of prostacyclin. In contrast to TXA_2, it enhances Ca^{2+} sequestration (Kazer-Glanzman *et al.* 1977). Moreover, inhibitory effects on platelet phospholipase (Lapetina *et al.* 1977; Minkes *et al.* 1977) and platelet cyclo-oxygenase have been described (Malmsten *et al.* 1976). All these activities are related to its ability to increase cAMP levels in platelets. Moreover, prostacyclin inhibits endoperoxide-induced aggregation, which suggests additional sites of action still undefined but dependent on the cAMP effect (Minkes *et al.* 1977). These observations have extended and given important biological significance to the original observation of Vargaftig & Chignard (1975), who demonstrated that substances such as PGE_1 that increase cAMP in platelets inhibit the release of TXA_2 (measured as rabbit aorta contracting activity) in platelets. Prostacyclin, by inhibiting several steps in the activation of the arachidonic acid cascade, exerts an overall control of platelet aggregability *in vivo*.

The fact that prostacyclin increases cAMP levels in cells other than platelets (Gorman *et al.* 1979; Hopkins *et al.* 1978) and the possibility that in those cells an interaction with the thromboxane system could lead to a similar control of cell behaviour to that observed in platelets, suggests that the prostacyclin–thromboxane A_2 system has wider biological significance in cell regulation.

Prostacyclin relaxes *in vitro* most vascular strips, including rabbit coeliac and mesenteric arteries (Bunting *et al.* 1976), bovine coronary arteries (Dusting *et al.* 1977*a*; Needleman *et al.* 1978), human and baboon cerebral arteries (Boullin *et al.* 1979), and lamb ductus arteriosus (Coceani *et al.* 1978). Exceptions to this include the porcine coronary arteries (Dusting *et al.* 1977*c*), some strips of rat venous tissue, and isolated human saphenous vein (Levy 1978), which are weakly contracted by prostacyclin. Whether these same effects are induced in the corresponding circulations in the intact animals or man has not been studied. In the human umbilical arterial strip, prostacyclin induces a dose-dependent relaxation at low concentrations (less than 1 μM) and a dose-dependent contraction at higher concentrations (more than 10 μM) (Pomerantz *et al.* 1978). As mentioned earlier, prostacyclin, and not PGE_2, is the main metabolite of arachidonic acid in isolated vascular tissue, and this has led to an intense reassessment of the effects and role of arachidonic acid and its metabolites in vascular tissue and the cardiovascular system (for review see Dusting *et al.* 1979).

In their early experiments, Gryglewski *et al.* (1976) observed that a fatty acid peroxide, 15-hydroperoxyarachidonic acid, strongly and selectively inhibited prostacyclin synthetase, the enzyme responsible for the formation of prostacyclin from endoperoxides in vessel microsomes ($i.c._{50}$ = 0.5 μg/ml). Other fatty acid hydroperoxides and their methyl esters also inhibit this enzyme (Moncada & Vane 1978; Salmon *et al.* 1978). Tranylcypromine, which is a well known inhibitor of enzymes not related to the metabolic pathway of arachidonic acid, is a somewhat weaker inhibitor of prostacyclin synthetase ($i.c._{50}$ = 160 μg/ml) than are the fatty acid hydroperoxides (Gryglewski *et al.* 1976). Unfortunately, hydroperoxides of fatty acids are not useful tools for examining the role of endogenous prostacyclin biosynthesis *in vivo* (Dusting *et al.* 1978*c*), probably because they are rapidly reduced by enzymes such as glutathione peroxidase (Christopherson 1968). Other substances that inhibit prostacyclin synthetase in blood vessel microsomes include an analogue of prostaglandin endoperoxide (9,11-diaza- and 9,11-epoxy-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

imino-prosta-5,12-dienoic acid) (Fitzpatrick *et al.* 1978), and a hydroperoxy derivative of indole (Terashita *et al.* 1979).

Prostaglandin endoperoxides are at the crossroads of arachidonic acid metabolism, for they are precursors of substances with opposing biological properties. On the one hand, TXA_2 produced by the platelets contracts large blood vessels and induces platelet aggregation; on the other prostacyclin produced by the vessel wall is a strong vasodilator and the most potent inhibitor of platelet aggregation known. Each substance has opposing effects on cAMP concentrations in platelets (Moncada & Vane 1979*b*), thereby giving a balanced control mechanism which will therefore affect thrombus and haemostatic plug formation. Selective inhibition of the formation of TXA_2 should lead to an increased bleeding time and inhibition of thrombus formation, whereas inhibition of prostacyclin formation should be propitious for a 'pro-thrombotic state'. The amount of control exerted by this system can be tested, for selective inhibitors of each pathway have been described (see above and Moncada & Vane (1978) and Nijkamp *et al.* (1977)).

The use of aspirin as a pharmacological tool to investigate the interaction between these two substances has been fruitful. Aspirin is active against platelet cyclo-oxygenase *in vivo* and *in vitro*. Moreover, this effect is long lasting because aspirin acetylates the active site of the enzyme leading to irreversible inhibition (Roth & Majerus 1975; Roth & Siok 1978). Because platelets are unable to synthesize new protein (Marcus 1978), they cannot replace the cyclo-oxygenase. The inhibition will therefore only be overcome by new platelets coming into the circulation after the block of cyclo-oxygenase in megakaryocytes has worn off (Burch *et al.* 1978). Interestingly, the cyclo-oxygenase of vessel walls seems less sensitive to aspirin than that of platelets (Baenziger *et al.* 1977). Indeed, it has been suggested that indomethacin as well as aspirin may have restricted access to the cyclo-oxygenase that generates prostacyclin in the lung during stimulation by angiotensin (Dusting 1981*a*, *b*). Thus, the secretion of prostacyclin into the circulation may be partly resistant to inhibition after single doses of these anti-inflammatory drugs.

Studies in rabbits and cats also suggest that administration of low doses of aspirin reduce the formation of TXA_2 more than that of prostacyclin (Amezcua *et al.* 1978; Korbut & Moncada 1978). Infusions of arachidonic acid in untreated animals had an antithrombotic effect and increased bleeding time. These effects were potentiated by small doses of aspirin (up to 10 mg/kg) and blocked by larger doses (20–200 mg/kg), which presumably inhibit formation of both prostacyclin and TXA_2.

Endothelial cells recover from aspirin inhibition more rapidly than do platelets in rabbits and rats (Kelton *et al.* 1978; Villa *et al.* 1979). Endothelial cells probably recover their ability to synthesize prostacyclin by regeneration of cyclo-oxygenase (Czervionke *et al.* 1978; Kelton *et al.* 1978), because recovery can be prevented by the protein synthesis inhibitor cycloheximide (Czervionke *et al.* 1979).

Until the discovery of prostacyclin, the use of aspirin as an antithrombotic agent based on its effects on platelets seemed logical (Majerus 1976), although the results of clinical trials were inconclusive (Verstraete 1976). Now, however, the situation needs further clarification, especially with respect to the optimal dose of aspirin. Aspirin in large doses (200 mg/kg) increases thrombus formation in a model of venous thrombosis in the rabbit (Kelton *et al.* 1978), and *in vitro* treatment of endothelial cells with aspirin enhances thrombin-induced platelet adherence to them (Czervionke *et al.* 1978). In addition, there is an inverse correlation between the amount

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of prostacyclin produced by the tissue on the one hand, and platelet adhesion or aggregation on the other. Moreover, aspirin treatment of arterial tissue *in vitro* increases its thrombogenicity (Baumgartner & Tschopp 1979).

In man, O'Grady & Moncada (1978) showed that a small single dose of aspirin (0.3 g) increased bleeding time 2 h after ingestion, whereas a large dose (3.9 g) had no effect. Some workers have confirmed these results (Rajah *et al.* 1978), but others have been unable to do so (Godal *et al.* 1979). The variability might be linked to the differences in methodology or to the age of the subjects. Indeed, Jorgensen *et al.* (1979, 1980) showed that the cutaneous bleeding time in man decreases with age and the response to aspirin varies according to the age, being prolonged in young male volunteers and not in older subject. Moreover, platelet aggregation and TXA_2 formation are blocked 2 h after a single dose of aspirin (3.9 g). The bleeding time is unchanged at that time, but 24 and 72 h after aspirin it is increased and slowly recovers towards pretreatment levels over a period of 168 h, in a manner that mirrors the recovery of TXA_2 formation and platelet aggregability (Amezcua *et al.* 1979). An extension of the concept comes from the demonstration that tranylcypromine, an inhibitor of prostacyclin formation, enhances platelet aggregation in an experimental model of thrombosis in the microcirculation of the brain of the mouse (Rosenblum & El-Sabban 1978).

All these results show that the prostacyclin–thromboxane balance is an important mechanism of control of platelet aggregability *in vivo*. Clearly, manipulation of this control mechanism might lead to prothrombotic or antithrombotic states of clinical relevance. In this context it is interesting that Mustard's group has shown that hydrocortisone treatment of normal or thrombocytopenic rats blocks prostacyclin formation in the vessel wall and decreases the bleeding time (Blajchman *et al.* 1979), a result that would be expected because steroids prevent activation of phospholipase (Flower 1978), and should thereby inhibit the vascular release of prostacyclin induced by substances that release endogenous arachidonic acid, such as angiotension.

Attempts to measure, in man, TXB_2 and prostacyclin or 6-oxo-$PGF_{1\alpha}$ after different aspirin dose schedules have confirmed the higher sensitivity of platelet cyclo-oxygenase to aspirin. Masotti *et al.* (1979) found that aspirin at 3.0–3.5 mg/kg gave, in a sample removed 2 h later, a 50 % inhibition of *ex vivo* platelet aggregation by several agents, while 5.0 mg/kg was needed for 50 % inhibition of prostacyclin formation as measured by cascade superfusion bioassay. It has also recently been demonstrated that a single daily dose of aspirin (160 mg) reduced significantly (by 40 %) the incidence of thrombosis over a 5 month observation period in artificial arterio-venous shunts in patients (Harter *et al.* 1979).

From all these results it is clear that a selective inhibitor of thromboxane formation should now be tested for antithrombotic efficacy (Moncada & Vane 1977, 1978), because theoretically this provides an advantage over aspirin in allowing prostacyclin formation by vessel walls or other cells either from their own endoperoxides or from those released by platelets. This should be the main criterion for determining a 'superior' mechanism of action over a small dose of aspirin. Studies *in vivo* are not yet available, but Needleman *et al.* (1979) made the observation that when platelets were treated with a thromboxane synthetase inhibitor *in vitro*, endoperoxides were available for utilization by the vessel wall. Interestingly, in the presence of a thromboxane synthetase inhibitor, arachidonic acid or collagen added to blood *in vitro* lead to the formation of 6-oxo-$PGF_{1\alpha}$ rather than TXB_2. Platelets cannot synthesize prostacyclin, so some other cell in the blood must have done so (Blackwell *et al.* 1978; Flower & Cardinal 1979).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

These results support our suggestion that thromboxane synthetase inhibitors might have a superior antithrombotic effect to simple cyclo-oxygenase inhibitors (Moncada & Vane 1977, 1978). It is important to realize at this stage, however, that all these observations have been made *in vitro*, and that *in vivo* experiments are necessary to clarify further the nature of the interaction between platelets and normal or damaged vessel walls.

Whether other drugs exert their antithrombotic effect by acting on the prostacyclin–thromboxane system is not yet known, but studies with the use of sulphinpyrazone in cultured endothelial cells (Gordon & Pearson 1978) and ticlopidine given orally to rats (Ashida & Abiko 1978) suggest that these compounds have little or no effect on prostacyclin formation at concentrations at which they affect platelet behaviour. A compound that might stimulate prostacyclin formation in humans after oral ingestion has also been described (Vermylen *et al.* 1979).

Selective inhibition of prostacyclin formation by lipid peroxides could also lead to a condition in which platelet aggregation is increased; this could play a role in the development of atherosclerosis. Indeed, lipid peroxidation takes place as a non-enzymic reaction (Harman & Piette 1966), and it is known to occur in certain pathological conditions (Slater 1972). Hence, lipid peroxides present in these conditions could be shifting the balance of the system in favour of TXA_2 and predisposing to thrombus formation.

The role of lipid peroxides in the development of atherosclerosis has been debated for almost 30 years, since Glavind *et al.* (1952) described the presence of lipid peroxides in human atherosclerotic aortae. They found the peroxide content in diseased arteries to be directly proportional to the severity of the atherosclerosis. Subsequent investigations by Woodford *et al.* (1965) suggested that Glavind's findings were based on artefacts, ascribing the presence of lipid peroxides to their formation during the preparative procedure. Despite this, the presence of conjugated diene hydroperoxides in lipids of human atheroma has again been reported (Fukazumi 1965; Fukazumi & Iwata 1963), and lipid peroxides have been found in atherosclerotic rabbit aortae (Iwakami 1965) subjected to an extraction procedure that avoids lipid peroxidation *in vitro*. Some authors (Brooks *et al.* 1971; Harland *et al.* 1971) favour the suggestion that lipid peroxides do accumulate in atherosclerotic plaques, whether or not these peroxides act by inhibiting prostacyclin formation and as a consequence reduce the arteries' defence mechanism. This theory is of interest especially since other substances related to atherosclerosis such as the cholesterol carriers, the low-density lipoproteins (LDLs), also inhibit prostacyclin formation in endothelial cell cultures (Nordoy *et al.* 1978).

Gryglewski and coworkers (Dembinska *et al.* 1977) have found that there is a substantial reduction in prostacyclin formation in the vascular tissue of rabbits made atherosclerotic, and more recently there has been a report that human tissue obtained from atherosclerotic plaque does not produce prostacyclin, whereas tissue obtained from a normal vessel does (D'Angelo *et al.* 1978). Sinzinger *et al.* (1979) have also shown that different types of atherosclerotic lesions ranging from fatty streaks to complicated lesions all produced much less prostacyclin than normal arteries. Nordoy *et al.* (1978) have shown that low-density lipoproteins inhibit prostacyclin formation. Gryglewski *et al.* (unpublished observations) have recently confirmed this link by their finding that LDLs contain high concentrations of lipid peroxides. High-density lipoproteins (HDLs), on the other hand, prevent the inhibitory effect of LDLs on prostacyclin formation. From epidemiological studies there is a positive correlation between the plasma concentration of LDLs and the risk of developing clinical coronary heart disease (Medalie *et al.* 1973), but a stronger, inverse relation has recently been demonstrated between HDL–cholesterol

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

levels and coronary heart disease (Havel 1979). Since the mechanisms relating changes in plasma lipoproteins to increased tendency for thrombosis have not yet been adequately defined, the interaction of lipoproteins with prostacyclin biosynthesis promises to be an exciting area for further study.

Before the discovery of prostacyclin, it was suggested that the use of dietary dihomo-γ-linolenic acid, the precursor of the monoenoic series of prostaglandins, could be an approach to the prevention of thrombosis, because PGG_1 and TXA_1 are not proaggregating and PGE_1 is anti-aggregating (Willis *et al.* 1974). Other reports tend to agree with this proposal (Sim & McCraw 1977), but there is some doubt, because feeding rabbits with dihomo-γ-linolenic acid leads to an increase in the tissue content of this acid without change in platelet responsiveness, at least to ADP (Oelz *et al.* 1976). The main criticism of all this work, including that of human platelets (Kernoff *et al.* 1977), is that the conclusions are based on studies performed *in vitro* in which platelets are studied as isolated cells without contact with vessel walls.

It is now evident that the use of dihomo-γ-linolenic acid in an attempt to direct the synthetic machinery of the platelets is not the most rational approach for the prevention of thrombosis. This is because the endoperoxides PGG_1 and PGH_1 are not substrates for prostacyclin synthetase; indeed, they or their precursor might adversely affect the prostacyclin protective mechanism. Eicosapentaenoic acid (C20:5ω3), the precursor of the trienoic prostaglandins, could, however, act as a precursor for an antiaggregating agent, Δ-^{17}prostacyclin (PGI_3), and it is known that C20:5ω3 is itself a weak anti-aggregating agent. TXA_3, if generated, is a weaker proaggregating agent than TXA_2 (Gryglewski *et al.* 1979). Thus, the use of this fatty acid could afford a dietary protection against thrombosis. Indeed, it has been suggested that the low incidence of myocardial infarction in Eskimos and their increased tendency to bleed could be due to the high eicosapentaenoic acid and low arachidonate content of their diet and consequently of their tissue lipid (Dyerberg *et al.* 1978). In Greenland Eskimos, there is an elevated content of C20:5ω3 (compared with Danes) in the platelet lipids and a prolonged bleeding time. Furthermore, their platelets are resistant to aggregation (Dyerberg & Bang 1979). In a recent study in which thrombosis and subsequent infarction were induced in dogs, dietary supplementation with fish oil resulted in a more normal electrocardiogram pattern and a reduced infarction size compared with the control group (Culp *et al.* 1980). The understanding of the role of fatty acids and their oxidized products in thrombosis and/or atherosclerosis is, however, at an early stage, and much experimental and clinical work is needed before the full picture emerges (see also Dyerberg, this symposium).

β-Thromboglobulin is a small protein related to platelet factor IV and is stored in the α-granules of platelets and released with other granular constituents during aggregation or adherence of the platelets to a damaged vessel wall (Moore *et al.* 1975). Hope *et al.* (1979) demonstrated that β-thromboglobulin inhibits formation of prostacyclin by bovine aortic endothelial cells in culture, at concentrations that are exceeded locally during platelet aggregation and release. Platelet factor IV does not have this action (Hope *et al.* 1979). This phenomenon may be an important component of the process of thrombosis, but the precise mechanism of inhibition has not been determined.

MacIntyre *et al.* (1978) have found in cell-free plasma a factor that stimulates prostacyclin production by pig aortic endothelial cells. Thrombin, trypsin and a calcium ionophore have also been shown to stimulate prostacyclin formation in human endothelial cells (Weksler *et al.* 1978). The mechanism of action and significance of these factors in regulating prostacyclin bio-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

synthesis has yet to be established. Finally, unidentified factors that inhibit prostacyclin formation have been found in renal cortex (Terragno *et al.* 1978) and in a microsomal fraction of rat placenta (Harrowing & Williams 1979). Both these inhibitors appear to act at the cyclo-oxygenase level and they may be related to a similar endogenous cyclo-oxygenase inhibitor found in plasma (Saeed *et al.* 1977). More work is necessary to define the significance and function of these factors.

Increased production of prostaglandin endoperoxides or TXB_2 *in vitro* by platelets has been found in blood from patients with arterial thrombosis, deep venous thrombosis or recurrent thrombosis; these conditions are associated with a shortened platelet survival time (Lagarde & Dechavanne 1977). In addition, increased sensitivity of platelets to aggregating agents and increased release of TXB_2 has been described in rabbits made atherosclerotic by diet (Shimamoto *et al.* 1978) and in patients who survived myocardial infarction (Szczeklik *et al.* 1978*a*). An increased level of TXB_2 in blood has been observed in patients with Prinzmetal's angina (Lewy *et al.* 1979). Moreover, platelets from rats made diabetic release more TXB_2 than platelets from normal rats (Harrison *et al.* 1978; Johnson *et al.* 1978).

Changes in prostacyclin production associated with disease have also been postulated. An increased production in uraemic patients has been suggested to explain their haemostatic defect (Remuzzi *et al.* 1977). On the other hand, a lack of prostacyclin production has been suggested in patients with idiopathic thrombocytopaenic purpura (Remuzzi *et al.* 1978), and a recent report suggests the absence of detectable levels of 6-oxo-$PGF_{1\alpha}$ in humans suffering from this condition (Hensby *et al.* 1979*b*). Both diseases may be linked by the accumulation during uraemia or the lack of production during idiopathic thrombocytopaenic purpura of a 'plasma factor' that stimulates prostacyclin synthesis (MacIntyre *et al.* 1978). A lower release of prostacyclin by the blood vessels of rats made diabetic has also been described (Harrison *et al.* 1978; Johnson *et al.* 1978): this decreased production can be corrected by chronic insulin treatment (Harrison *et al.* 1978). Prostacyclin production by blood vessels from patients with diabetes is also lower than normal (Johnson *et al.* 1979), and circulating levels of 6-oxo-$PGF_{1\alpha}$ are reduced in diabetic patients with proliferative retinopathy (Dollery *et al.* 1979).

Pace-Asciak *et al.* (1978) demonstrated that aortae from spontaneously hypertensive rats of the Japanese strain generate more prostacyclin than aortae from normotensive rats when incubated with exogenous arachidonic acid *in vitro*. Furthermore, Armstrong *et al.* (1976) found that prostaglandin endoperoxide (PGH_2) has a greater hypotensive effect in genetically hypotensive rats of the New Zealand strain than in normotensive controls, whereas PGE_2 had a similar hypotensive action in the two strains. These results indicate that PGH_2 may be more readily converted to prostacyclin in hypertensive rats, and it has been suggested that the greater formation of prostacyclin in blood vessels represents an adaptive mechanism to the elevated arterial pressure (Pace-Asciak *et al.* 1978). However, chronic treatment with indomethacin or aspirin does not alter arterial pressure in spontaneously hypertensive rats (Antonaccio *et al.* 1979; DiNicolantonio *et al.* 1981), although it does markedly reduce the vasodepressor action of intravenous arachidonic acid (DiNicolantonio *et al.* 1981).

It is interesting that plasma exchange in patients suffering from hypertension as a complication of haemolytic uraemic syndrome restored a 'prostacyclin stimulating factor', and led to improved control of blood pressure (Remuzzi *et al.* 1978). Moreover, others have reported that plasma exchange has an antihypertensive effect in patients with glomerulonephritis and essential hypertension (Whitworth *et al.* 1978). These observations suggest that essential hypertension in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

man may be associated with impairment, rather than enhancement, of prostacyclin formation in the vasculature. Clearly, more work is necessary to define any role of prostacyclin in the experimental models of hypertension in the rat, and to substantiate the relevance of development of hypertension in the rat to essential hypertension in man.

Intra-arterial thrombus formation and haemostatic plug formation have been described in general terms as equivalent phenomena (Mustard & Packham 1975). It is, however, possible that the relative importance of prostacyclin and TXA_2 in these conditions is different, because prostacyclin, at least under some conditions, is an unstable circulating hormone (Gryglewski *et al.* 1978*a*; Moncada *et al.* 1978) as well as a locally generated one. Its role in controlling intra-arterial thrombus formation might be more important than that of TXA_2, which seems to be generated only after strong interaction between aggregating platelets or by their interaction with vessel wall materials.

As far as aspirin is concerned, more information is needed on the rate of recovery of the endothelial cyclo-oxygenase *in vivo* after single doses of aspirin. Equally important is the assessment of any cumulative effect of a multiple-dose régime on platelet and endothelial cyclo-oxygenase, to establish the optimal interval of administration. The demonstration of the ability of aspirin to prevent thromboembolism in some circumstances but not in others (Jobin 1978; Verstraete 1976) may suggest a qualitative or quantitative difference in the underlying pathophysiology. Further clinical trials should be conducted in which aspirin is given at low doses either alone or combined with phosphodiesterase inhibitors such as dipyridamole. Ideally, a selective inhibitor of thromboxane synthetase should be developed to be used alone or in combination with phosphodiesterase inhibitors (Moncada & Vane 1978).

A more direct approach to antithrombotic therapy, however, would be to control platelet cAMP; increasing platelet cAMP inhibits most forms of aggregation whether or not they are dependent on arachidonic acid metabolic products. Since prostacyclin is the most powerful substance known in both preventing aggregation and increasing platelet cAMP (Gorman *et al.* 1977; Tateson *et al.* 1977), prostacyclin or an analogue, alone or in combination with a phosphodiesterase inhibitor, should be a more comprehensive approach to the control of platelet aggregation *in vivo*. Alternatively, drugs that stimulate endogenous prostacyclin production (Vermylen *et al.* 1979) could be developed. Several of these possibilities are at present being explored.

Prostacyclin or chemical analogues may find a use as a 'hormone replacement' therapy in conditions such as atherosclerosis, acute myocardial infarction or 'crescendo angina' and other states in which excessive platelet aggregation may take place in the circulation or in specific areas such as in organ transplants. Moreover, we have suggested its use in extracorporeal circulations such as cardiopulmonary bypass and renal dialysis (Moncada & Vane 1979*a*). In these systems the main problems are platelet loss with the formation of micro-aggregates which, when returning to the patient in bypass, are responsible for the cerebral and renal impairment observed after operation (Abel *et al.* 1976; Branthwaite 1972). In addition, there are side effects associated with the chronic use of heparin, especially the development of osteoporosis (Griffith *et al.* 1965).

Several anti-platelet drugs have been proposed to deal with these two problems and some have been used with moderate success. PGE_1 has been reported to be beneficial during cardiopulmonary bypass in dogs (Balanowski *et al.* 1977). However, prostaglandins of the E type induce

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

diarrhoea (Main & Whittle 1975), an effect not shared by prostacyclin (Robert *et al.* 1979; Ubatuba *et al.* 1979). Therefore, prostacyclin is not only more potent but more specific in achieving platelet protection. Prostacyclin has proved beneficial in several systems of extracorporeal circulation in experimental animals, including renal dialysis, cardiopulmonary bypass and charcoal haemoperfusion (Bunting *et al.* 1979; Coppe *et al.* 1979; Longmore *et al.* 1979; Woods *et al.* 1978). In one of these systems (renal dialysis), prostacyclin can replace heparin altogether (Woods *et al.* 1978). In charcoal haemoperfusion, heparin is also necessary since charcoal seems to activate the clotting cascade directly (Bunting *et al.* 1979).

Prostacyclin has potent effects on platelets and on the cardiovascular system in man (Szczeklik *et al.* 1978*b*). During infusion of prostacyclin in healthy volunteers for 60 min at rates ranging from 2 to 16 ng kg^{-1} min^{-1} there was a dose-related inhibition of platelet aggregation measured in platelet-rich plasma and in whole blood (O'Grady *et al.* 1979). Similar inhibition of platelet aggregation was seen when the responses were measured 15 or 45 min after starting the infusion. At infusions of 8 ng kg^{-1} min^{-1}, partial inhibition of aggregation was demonstrable for up to 105 min after the end of infusion, and this persistence of effect on platelets has recently been confirmed (Chierchia *et al.* 1979). Template bleeding time was not significantly increased though Szczeklik *et al.* (1978*b*) found an approximate doubling of bleeding time in response to prostacyclin at 20 ng kg^{-1} min^{-1}.

Prostacyclin disperses circulating platelet aggregates (Szczeklik *et al.* 1978*b*). Significant inhibition of platelet aggregation induced by ADP was seen (FitzGerald *et al.* 1979) when prostacyclin was administered under blind conditions at rates of 4 and 8 ng kg^{-1} min^{-1}. Other haematological variables such as platelet count, platelet factor 3 concentration, accelerated partial thromboplastin time, prothrombin time, euglobin clot lysis time, concentration of fibrinogen degradation products and blood glucose were not affected by prostacyclin (O'Grady *et al.* 1979; Szczeklik *et al.* 1978*b*).

It was originally suggested (Szczeklik *et al.* 1978*b*) that prostacyclin had direct positive chronotropic and inotropic effects in man. However, in a double blind controlled study with the use of prostacyclin up to 4 ng kg^{-1} min^{-1} an increase in heart rate accompanied by decrease in diastolic blood pressure, pre-ejection period and QS_2 index was observed (Warrington & O'Grady 1980). Systolic blood pressure, left ventricular ejection time index and the normalized first derivative of the apex cardiogram were unaltered by prostacyclin. These findings were consistent with an arteriolar vasodilator effect of prostacyclin, which would be expected to lower diastolic and mean blood pressure and thus reflexly increase heart rate and contractility.

When heart rate was increased by more than 10 % over control values during prostacyclin infusion, peripheral temperature measured at the great toe increased by 1–6 K (O'Grady *et al.* 1979). Increases in skin temperature as well as facial flushing were also observed at rates of 2–5 ng kg^{-1} min^{-1} (Szczeklik *et al.* 1978*b*). Facial flushing invariably occurs at doses above 4 ng kg^{-1} min^{-1} when an increase in heart rate of more than 10 % is recorded (O'Grady *et al.* 1979). This flushing limits the extent to which double blind studies with prostacyclin can be performed.

The cardiovascular effects of prostacyclin are shorter-lived than those on platelets and disappear within 15 min of the end of infusion (O'Grady *et al.* 1979). Plasma renin activity rises significantly during prostacyclin infusion but plasma noradrenalin and plasma aldosterone levels did not change significantly (FitzGerald *et al.* 1979).

Renal blood flow measured by using ^{125}I-hippuran increased in response to an infusion of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

prostacyclin (6 ng kg^{-1} min^{-1}) that caused a small reduction in diastolic blood pressure, while the glomerular filtration rate measured by using ^{51}Cr-EDTA remained unchanged (J. Henry & J. O'Grady, unpublished).

Headache has been reported when doses greater than 8 ng kg^{-1} min^{-1} are administered (FitzGerald *et al.* 1979; O'Grady *et al.* 1979; Szczeklik *et al.* 1978*b*). Colicky central abdominal discomfort has been less frequently experienced but was reproducible in one subject (O'Grady *et al.* 1979). The precise mechanism of these gastrointestinal effects is unclear. It may be that they reflect the contraction of human gastrointestinal smooth muscle by prostacyclin; they may also be vagally mediated or represent secondary effects of prostacyclin or of its metabolic products.

Ill-defined sensations of unease and restlessness have been experienced by subjects receiving higher infusion rates of prostacyclin (Chierchia *et al.* 1979; O'Grady *et al.* 1979; Szczeklik *et al.* 1978*b*). In two subjects, the administration of prostacyclin at the rate of 50 ng kg^{-1} min^{-1} (Szczeklik *et al.* 1978*b*) caused sudden weakness with pallor and nausea, a fall in systolic and diastolic blood pressure and bradycardia. It is possible that this effect is mediated by a vagal reflex, which has been observed in dogs (Chapple *et al.* 1978*a*, *b*).

Following reports that PGE_1 has been used successfully in the treatment of peripheral vascular disease (Carlson & Olsson 1976), prostacyclin has been shown to have a similar effect, producing a long-lasting increase in muscle blood flow, disappearance of ischaemic pain and healing of trophic ulcers after an intra-arterial infusion to the affected limb for 3 days (Szczeklik *et al.* 1979). In a subsequent trial in 30 patients, symptoms were alleviated in 22 patients; this improvement was sustained for up to 15 months in 12 of them (Szczeklik *et al.* 1980; see also Gryglewski *et al.*, this symposium).

Recently, the first report (Gimson *et al.* 1980) on the use of prostacyclin during charcoal haemoperfusion in humans has demonstrated that there is a protection against platelet loss and activation (assessed by the prevention of the release into the plasma of β-thromboglobulin). These are basically the results obtained in clinical trials with prostacyclin in cardiopulmonary bypass operations (Bunting *et al.* 1981; Walker *et al.* 1980; see also Longmore, this symposium). Many other uses of prostacyclin are yet to be explored in clinical conditions. One of them is its use in transplant surgery, where in animals, prostacyclin added to the washing solution normally used to flush the donor kidney before transplant improved the efficacy (Munday *et al.* 1981). Prostacyclin also protected against hyperacute kidney rejection in a dog model (Munday *et al.* 1980). Results in these and other areas will certainly be produced in the near future.

References (Moncada & Vane)

Abel, R. M., Buckley, M. J., Austen, W. G., Barnett, G. O., Beck, C. H. & Fischer, J. E. 1976 Etiology, incidence and prognosis of a prospective analysis of 500 consecutive patients. *J. thorac. cardiovasc. Surg.* **71**, 323–333.

Aiken, J. W. 1979 See Discussion following 'Is the lung an endocrine organ that secretes prostacyclin?', by R. J. Gryglewski. In *Prostacyclin* (ed. J. R. Vane & S. Bergström), p. 287. New York: Raven Press.

Aiken, J. W., Gorman, R. R. & Shebuski, R. J. 1979 Prevention of blockage of partially obstructed coronary arteries with prostacyclin correlates with inhibition of platelet aggregation. *Prostaglandins* **17**, 483–494.

Aiken, J. W. & Vane, J. R. 1973 Intrarenal prostaglandin release attenuates the renal vascoconstrictor activity of angiotensin. *J. Pharmac. exp. Ther.* **184**, 678–687.

Amezcua, J.-L., O'Grady, J., Salmon, J. A. & Moncada, S. 1979 Prolonged paradoxical effect of aspirin on platelet behaviour and bleeding time in man. *Thromb. Res.* **16**, 69–79.

Amezcua, J.-L., Parsons, M. & Moncada, S. 1978 Unstable metabolites of arachidonic acid, aspirin and the formation of the haemostatic plug. *Thromb. Res.* **13**, 477–488.

Antonaccio, M. J., Harris, D., Goldenberg, H., High, J. P. & Rubin, B. 1979 The effects of captopril, propranolol and indomethacin on blood pressure and plasma renin activity in spontaneously hypertensive and normotensive rats. *Proc. Soc. exp. Biol. Med.* **162**, 429–433.

Armstrong, J. M., Boura, A. L. A., Hamberg, M. & Samuelsson, B. 1976 A comparison of the vasodepressor effects of the cyclic endoperoxides PGG_2 and PGH_2 with those of PGD_2 and PGE_2 in hypertensive and normotensive rats. *Eur. J. Pharmac.* **39**, 251–258.

Armstrong, J. M., Chapple, D. J., Dusting, G. J., Hughes, R., Moncada, S. & Vane, J. R. 1977 Cardiovascular actions of prostacyclin (PGI_2) in chloralose anaesthetized dogs. *Br. J. Pharmac.* **61**, 136P.

Armstrong, J. M., Lattimer, N., Moncada, S. & Vane, J. R. 1978 Comparison of the vasodepressor effects of prostacyclin and 6-oxo-prostaglandin $F_{1\alpha}$ with those of prostaglandin E_2 in rats and rabbits. *Br. J. Pharmac.* **62**, 125–130.

Ashida, S.-I. & Abiko, Y. 1978 Effect of ticlopidine and acetylsalicylic acid on generation of prostaglandin I_2-like substance in rat arterial tissue. *Thromb. Res.* **13**, 901–908.

Baenziger, N. L., Dillender, M. J. & Majerus, P. 1977 Cultured human skin fibroblasts and arterial cells produce a labile platelet-inhibitory prostaglandin. *Biochem. biophys. Res. Commun.* **78**, 294–301.

Balanowski, P. J. P., Bauer, J., Machiedo, G. & Neville, W. E. 1977 Prostaglandin influence on pulmonary intravascular leukocytic aggregation during cardiopulmonary bypass. *J. thorac. cardiovasc. Surg.* **73**, 221–224.

Baumgartner, H. R. & Tschopp, Th.B. 1979 Platelet interaction with aortic sub-endothelium (S.E.) in vitro. Locally produced PGI_2 inhibits adhesion and formation of mural thrombi in flowing blood. In *Thrombosis and haemostasis abstracts* (VII Int. Congress on Thrombosis and Haemostasis), p. 6.

Bayer, B. L., Blass, K. E. & Förster, W. 1979 Antiaggregatory effect of prostacyclin (PGI_2) *in vivo*. *Br. J. Pharmac.* **66**, 10–12.

Best, L. C., Martin, T. J., Russell, R. G. G. & Preston, F. E. 1977 Prostacyclin increases cyclic AMP levels and adenylate cyclase activity in platelets. *Nature, Lond.* **267**, 850–851.

Blackwell, G. J., Flower, R. J., Russell-Smith, N., Salmon, J. A., Thorogood, P. B. & Vane, J. R. 1978 I-*n*-Butylimidazole: a potent and selective inhibitor of 'thromboxane synthetase'. *Br. J. Pharmac.* **64**, 436P.

Blajchman, M.A., Senyi, A. F., Hirsh, J., Surya, Y., Buchanan, M. & Mustard, J. F. 1979 Shortening of the bleeding time in rabbits by hydrocortisone caused by inhibition of prostacyclin generation by the vessel wall. *J. clin. Invest.* **63**, 1026–1035.

Blasko, G., Nemesanszky, E., Szabo, G., Stadier, I. & Palos, L. A. 1980. The effects of PGI_2 and PGI_2 analogues with increased stability on platelet cAMP and aggregation. *Thromb. Res.* **17**, 673–681.

Blumberg, A. L., Denny, S. E., Marshall, G. R. & Needleman, P. 1977 Blood vessel hormone interactions: angiotensin, bradykinin and prostaglandins. *Am. J. Physiol.* **232**, H303–310.

Born, G. V. R. 1962 Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature, Lond.* **194**, 927–929.

Boullin, D. J., Bunting, S., Blaso, W. P., Hunt, T. M. & Moncada, S. 1979 Response of human and baboon arteries to prostaglandin endoperoxides and biologically generated and synthetic prostacyclin: their relevance to cerebral arterial spasm in man. *Br. J. clin. Pharmac.* **7**, 139–147.

Branthwaite, M. A. 1972 Neurological damage related to open heart surgery. *Thorax* **27**, 748–753.

Brooks, C. J. W., Steel, G., Gilbert, J. D. & Harland, W. A. 1971 Lipids of human atheroma. 4. Characteristics of a new group of polar sterol esters from human atherosclerotic plaques. *Atherosclerosis* **13**, 223–237.

Bunting, S., Gryglewski, R., Moncada, S. & Vane, J. R. 1976 Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. *Prostaglandins* **12**, 897–913.

Bunting, S., Moncada, S., Reed, P., Salmon, J. A. & Vane, J. R. 1978 An antiserum to 5,6-dihydro prostacyclin (PGI_1) which also binds prostacyclin. *Prostaglandins* **15**, 565–574.

Bunting, S., Moncada, S. & Vane, J. R. 1977 Antithrombotic properties of vascular endothelium. *Lancet* ii, 1075–1076.

Bunting, S., Moncada, S., Vane, J. R., Woods, H. F. & Weston, M. J. 1979 Prostacyclin improves hemocompatability during charcoal hemoperfusion. In *Prostacyclin* (ed. J. R. Vane & S. Bergström), pp. 361–369. New York: Raven Press.

Bunting, S., O'Grady, J., Moncada, S., Vane, J. R., Fabiani, J. N., Terrier, E. & Dubost, C. 1981 The use of prostacyclin in cardiopulmonary bypass. (In preparation.)

Burch, J. W., Stanford, N. & Majerus, P. W. 1978 Inhibition of platelet prostaglandin synthetase by oral aspirin. *J. clin. Invest.* **61**, 314–319.

Carlson, L. A. & Olsson, A. G. 1976 Intravenous prostaglandin E_1 in severe peripheral vascular disease. *Lancet* ii, p. 810.

Chapple, D. J., Dusting, G. J., Hughes, R. & Vane, J. R. 1978*a* A vagal reflex contributes to the hypotensive effect of prostacyclin in anaesthetized dogs. *J. Physiol., Lond.* **281**, 43–44P.

Chapple, D. J., Dusting, G. J., Hughes, R. & Vane, J. R. 1978*b* Some direct and reflex cardiovascular actions of prostacyclin (PGI_2) and PGE_2 in anaesthetized dogs. *Br. J. Pharmac.* **68**, 437–447.

Chierchia, S., Ciabattoni, G., Cinotti, G., Maseri, A., Patrono, C., Pulgiese, F., Distante, A., Simonetti, I. & Bernini, W. 1979 Haemodynamic and antiaggregatory effects of prostacyclin (PGI_2) in the healthy man. *Circulation* **59–60**, suppl. II, p. 83.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Cho, M. J. & Allen, M. A. 1978 Chemical stability of prostacyclin (PGI_2) in aqueous solutions. *Prostaglandins* **25**, 943–954.

Christofinis, G. J., Moncada, S., Bunting, S. & Vane, J. R. 1979 Prostacyclin (PGI_2) release by rabbit aorta and human umbilical vein endothelial cells after prolonged subculture. In *Prostacyclin* (ed. J. R. Vane & S. Bergström), pp. 77–84. New York: Raven Press.

Christopherson, B. O. 1968 Formation of monohydroxypolenic fatty acids from lipid peroxides by a glutathione peroxidase. *Biochim. biophys. Acta* **164**, 35.

Coceani, F., Bishai, I., White, E., Bodach, E. & Olley, P. M. 1978 Action of prostaglandins, endoperoxides and thromboxanes on the lamb ductus arteriosus. *Am. J. Physiol.* **234**, H117–H122.

Coppe, D., Wonders, T., Snider, M. & Salzman, E. W. 1979 Preservation of platelet number and function during extracorporeal membrane oxygenation (ECMO) by regional infusion of prostacyclin. In *Prostacyclin* (ed. J. R. Vane & S. Bergström), pp. 371–383. New York: Raven Press.

Culp, B. R., Lands, W. E. M., Lucchesi, B. R., Pitt, B. & Romson, J. 1980 The effect of dietary supplementation of fish oil on experimental myocardial infarction. *Prostaglandins* **20**, 1021–1031.

Czervionke, R. L., Hoak, J. C. & Fry, G. L. 1978 Effect of aspirin on thrombin-induced adherence of platelets to cultured cells from the blood vessel walls. *J. clin. Invest.* **62**, 847–857.

Czervionke, R. L., Smith, J. B., Fry, G. L. & Hoak, J. C. 1979 Inhibition of prostacyclin by treatment of endothelium with aspirin. *J. clin. Invest.* **63**, 1089–1092.

D'Angelo, V., Villa, S., Myslieviec, M., Donati, M. B. & De Gaetano, G. 1978 Defective fibrinolytic and prostacyclin-like activity in human atheromatous plaques. *Thromb. Haemostas.* **39**, 535–536.

Davison, E. M., Ford-Hutchinson, A. W., Smith, M. J. H. & Walker, J. R. 1978 The release of thromboxane B_2 by rabbit peritoneal polymorphonuclear leukocytes. *Br. J. Pharmac.* **63**, 407P.

Dembinska-Kiec, A., Gryglewska, T., Zmuda, A. & Gryglewski, R. J. 1977 The generation of prostacyclin by arteries and by the coronary vascular bed is reduced in experimental atherosclerosis in rabbit. *Prostaglandins* **14**, 1025–1034.

DiNicolantonio, R., Dusting, G. J., Hutchinson, J. S. & Mendelsohn, F. A. O. 1981 Failure of aspirin to modify the hypotensive action of captopril in spontaneously hypertensive rats. *Clin. exp. Pharmac. Physiol.* (Submitted for publication.)

Dollery, C. T., Friedman, L. A., Hensby, C. N., Kohner, E., Lewis, P. J., Porta, M. & Webster, J. 1979 Circulating prostacyclin may be reduced in diabetes. *Lancet* ii, 1365.

Dusting, G. J. 1981*a* Prostacyclin released by angiotensins from lungs and isolated vascular tissue. In *Proceedings of the 28th International Congress of Physiological Sciences*. Budapest: Publishing House of the Hungarian Academy of Sciences. (In the press.)

Dusting, G. J. 1981*b* On angiotensin-induced release of a prostacyclin (PGI_2)-like substance from the lung. *J. Cardiovasc. Pharmac.* (In the press.)

Dusting, G. J., Moncada, S., Mullane, K. M. & Vane, J. R. 1978*a* Implications of prostacyclin (PGI_2) generation for modulation of vascular tone. *Clin. Sci. molec. Med.* **55**, 195s–198s.

Dusting, G. J., Moncada, S. & Vane, J. R. 1977*a* Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries by arachidonic acid. *Prostaglandins* **13**, 3–15.

Dusting, G. J., Moncada, S. & Vane, J. R. 1977*b* Disappearance of prostacyclin in the circulation of the dog. *Br. J. Pharmac.* **62**, 414–514P.

Dusting, G. J., Moncada, S. & Vane, J. R. 1977*c* Prostacyclin is a weak contractor of coronary arteries in the pig. *Eur. J. Pharmac.* **45**, 301–304.

Dusting, G. J., Moncada, S. & Vane, J. R. 1978*b* Recirculation of prostacyclin (PGI_2) in the dog. *Br. J. Pharmac.* **64**, 315–320.

Dusting, G. J., Moncada, S. & Vane, J. R. 1978*c* Vascular actions of arachidonic acid and its metabolites in perfused mesenteric and femoral beds of the dog. *Eur. J. Pharmac.* **49**, 65–72.

Dusting, G. J., Moncada, S. & Vane, J. R. 1979 Prostaglandins, their intermediates and precursors: cardiovascular roles and regulatory mechanisms in normal and abnormal circulatory systems. *Proc. Cardiovasc. Dis.* **21**, 405–430.

Dusting, G. J. & Mullins, E. M. 1980 Stimulation by angiotensin of prostacyclin biosynthesis in rats and dogs. *Clin. exp. Pharmac. Physiol.* **7**, 545–550.

Dusting, G. J., Mullins, E. M. & Nolan, R. D. 1981 Prostacyclin release accompanying angiotensin conversion in rat mesenteric vasculature. (Submitted for publication.)

Dyerberg, J. & Bang, H. O. 1979 Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. *Lancet* ii, 433–435.

Dyerberg, J., Bang, H. O., Stofferson, E., Moncada, S. & Vane, J. R. 1978 Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* ii, 117–119.

Eakins, K. E., Rajadhyaksha, V. & Schroer, R. 1976 Prostaglandin antagonism by sodium *p*-benzyl-4-(1-oxo-2(4-chlorobenzyl)-3-phenylpropyl)phenyl phosphonate (N-0164). *Br. J. Pharmac.* **58**, 333–339.

Ferreira, S. H., Moncada, S. & Vane, J. R. 1973 Prostaglandins and the mechanism of analgesia produced by aspirin-like drugs. *Br. J. Pharmac.* **49**, 86–97.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FitzGerald, G. A., Friedman, L. A., Miyamori, I., O'Grady, J. & Lewis, P. J. 1979 A double blind placebo controlled crossover study of prostacyclin in man. *Life Sci.* **25**, 665–672.

Fitzpatrick, F. A., Bundy, G. L., Gorman, R. R. & Honohan, T. 1978 9,11-Epoxyiminoprosta-5,13-dienoic acid is a thromboxane A_2 antagonist in human platelets. *Nature, Lond.* **275**, 764–766.

Flower, R. J. 1978 Steroidal anti-inflammatory drugs as inhibitors of phospholipase A_2. In *Advances in prostaglandin and thromboxane research* (ed. R. Paoletti & B. Samuelsson), pp. 105–112. New York: Academic Press.

Flower, R. J. & Cardinal, D. C. 1979 Use of a novel platelet aggregometer to study the generation by, and actions of prostacyclin in whole blood. In *Prostacyclin* (ed. J. R. Vane & S. Bergström), pp. 211–220. New York: Raven Press.

Fukazumi, K. 1965 Lipids of the atherosclerotic artery. III. A hypothesis on the cause of atherosclerosis from the viewpoint of fat chemistry. *J. pharm. Soc. Japan* **14**, 119–122.

Fukazumi, K. & Iwata, Y. 1963 Lipids of atherosclerotic artery. II. Dialysis of lipids of abdominal aorta and lipids in lipid protein complexes existing in the aorta. *J. pharm. Soc. Japan* **12**, 93–97.

Gimbrone, M. A. & Alexander, R. W. 1975 Angiotensin II stimulation of prostaglandin production in cultured human vascular endothelium. *Science, N.Y.* **189**, 219–220.

Gimeno, M. F., Sterin-Borda, L., Borda, E. S., Lazzari, M. A. & Gimeno, A. L. 1980 Human plasma transforms prostacyclin (PGI_2) into a platelet antiaggregatory substance which contracts isolated bovine coronary arteries. *Prostaglandins* **19**, 907–916.

Gimson, A. E. S., Hughes, R. D., Mellon, P. J., Woods, H. F., Langley, P. G., Canalese, J., Williams, R. & Weston, M. J. 1980 Prostacyclin to prevent platelet activation during charcoal haemoperfusion in fulminant hepatic failure. *Lancet* i, 173–175.

Glavind, J., Hartmann, S., Clemmesen, J., Jessen, K. E. & Dam, H. 1952 Studies on the role of lipoperoxides in human pathology. II. The presence of peroxidized lipids in the atherosclerotic aorta. *Acta path. microbiol. scand.* **30**, 1.

Godal, H. C., Eika, C., Dybdahl, J. H., Daae, L. & Larsen, S. 1979 Aspirin and bleeding time. *Lancet* i, 1236.

Goldstein, I. M., Malmsten, C. L., Kaplan, Kindahl, H., Samuelsson, B. & Weissman, G. 1977 Thromboxane generation by stimulated human granulocytes: inhibition by glucocorticoids and superoxide dismutase. *Clin. Res.* **25**, 518A.

Gordon, J. L. & Pearson, J. D. 1978 Effects of sulphinpyrazone and aspirin on prostaglandin I_2 (prostacyclin) synthesis by endothelial cells. *Br. J. Pharmac.* **64**, 481–483.

Gorman, R. R., Bunting, S. & Miller, O. V. 1977 Modulation of human platelet adenylate cyclase by prostacyclin (PGX). *Prostaglandins* **13**, 377–388.

Gorman, R. R., Hamilton, R. D. & Hopkins, N. K. 1979 Stimulation of human foreskin fibroblast adenosine 3′5′-cyclic monophosphate levels by prostacyclin (prostaglandin I_2). *J. biol. Chem.* **254**, 1671–1676.

Griffith, G. C., Nichols, G., Asher, J. D. & Flanagan, B. 1965 Heparin osteoporosis. *J. Am. med. Ass.* **193**, 91–94.

Grodzinska, L. & Gryglewski, R. J. 1980 Angiotensin-induced release of prostacyclin from perfused organs. *Pharmac. Res. Commun.* **12**, 339–347.

Gryglewski, R. J. 1979 Prostacyclin is a circulatory hormone. *Biochem. Pharmac.* **28**, 3161–3166.

Gryglewski, R. J., Bunting, S., Moncada, S., Flower, R. J. & Vane, J. R. 1976 Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins* **12**, 685–714.

Gryglewski, R. J., Korbut, R. & Ocetkiewicz, A. C. 1978*a* Generation of prostacyclin by lungs *in vivo* and its release into the arterial circulation. *Nature, Lond.* **273**, 765–767.

Gryglewski, R. J., Korbut, R., Ocetkiewicz, A., Splawinski, J., Wojtaszek, B. & Swiens, J. 1978*b* Lungs as a generator of prostacyclin. Hypothesis on physiological significance. *Naunyn Schmiedebergs Arch. Pharmac.* **304**, 45–50.

Gryglewski, R. J., Korbut, R., Ocetkiewicz, A. C. & Stachwa, T. 1978*c* *In vivo* method for quantitation of anti-platelet potency of drugs. *Naunyn Schmiedebergs Arch. Pharmac.* **302**, 25–30.

Gryglewski, R. J., Salmon, J. A., Ubatuba, F. B., Weatherley, B. C., Moncada. S. & Vane, J. R. 1979 Effects of all *cis*-5,8,11,14,17-eicosapentaenoic acid and PGH_3 on platelet aggregation. *Prostaglandins* **18**, 453–478.

Hamberg, M. & Samuelsson, B. 1973 Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. *Proc. natn. Acad. Sci. U.S.A.* **70**, 899–903.

Hamberg, M., Svensson, J. & Samuelsson, B. 1975 Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994–2998.

Hamberg, M., Svensson, J., Wakabayashi, T. & Samuelsson, B. 1974 Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proc. natn. Acad. Sci. U.S.A.* **71**, 345–349.

Harland, W. A., Gilbert, J. D., Steel, G. & Brooks, C. J. W. 1971 Lipids of human atheroma. 5. The occurrence of a new group of polar sterol esters in various stages of human atherosclerosis. *Atherosclerosis* **13**, 239–246.

Harman, D. & Piette, L. H. 1966 Free radical theory of aging: free radical reaction in serum. *J. Geront.* **21**, 560–565.

Harrison, H. E., Reece, A. H. & Johnson, M. 1978 Decreased vascular prostacyclin in experimental diabetes. *Life Sci.* **23**, 351–356.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Harrowing, P. D. & Williams, K. I. 1979 Homogenates of rat placenta contain a factor(s) which inhibits uterine arachidonic acid metabolism. *Br. J. Pharmac.* **67**, 428P.

Harter, H. R., Burch, J. W., Majerus, P. W., Standford, N., Pelmes, J. A., Anderson, C. B. & Weerts, C. A. 1979 Prevention of thromboembolism in patients of haemodialysis by low dose aspirin. *New Engl. J. Med.* **301**, 577–579.

Havel, R. J. 1979 High density lipoproteins, cholesterol transport and coronary heart disease. *Circulation* **60**, 1–3.

Hensby, C. N., Barnes, P. J., Dollery, C. T. & Dargie, H. 1979*a* Production of 6-oxo-$PGF_{1\alpha}$ by human lung *in vivo*. *Lancet* ii, 1162–1163.

Hensby, C. N., Lewis, P. J., Hilgard, P., Mufti, G. J., Hows, J. & Webster, J. 1979*b* Prostacyclin deficiency in thrombotic thrombocytopenic purpura. *Lancet* ii, 748.

Heyns, A. du P., van den Berg, D. J., Potgieter, G. M. & Retief, F. P. 1974 The inhibition of platelet aggregation by an aorta intima extract. *Thromb. Diathes. haemorrh.* **32**, 417–431.

Higgs, E. A., Moncada, S., Vane, J. R., Caen, J. P., Michel, H. & Tobelem, G. 1978*a* Effect of prostacyclin (PGI_2) on platelet adhesion to rabbit arterial subendothelium. *Prostaglandins* **16**, 17–22.

Higgs, G. A., Bunting, S., Moncada, S. & Vane, J. R. 1976 Polymorphonuclear leukocytes produce thromboxane A_2-like activity during phagocytosis. *Prostaglandins* **12**, 749–757.

Higgs, G. A., Moncada, S. & Vane, J. R. 1977 Prostacyclin (PGI_2) inhibits the formation of platelet thrombi induced by adenosine diphosphate (ADP) *in vivo*. *Br. J. Pharmac.* **61**, 137P.

Higgs, G. A., Moncada, S. & Vane, J. R. 1978*b* Prostacyclin reduces the number of 'slow moving' leucocytes in hamster pouch cheek venules. *J. Physiol., Lond.* **280**, 55P–56P.

Hope, W., Martin, T. J., Chesterman, C. N. & Morgan, F. J. 1979 Human β-thromboglobulin inhibits PGI_2 production and binds to a specific site in bovine aortic endothelial cells. *Nature, Lond.* **228**, 210–212.

Hopkins, N. K., Sun, F. F. & Gorman, R. R. 1978 Thromboxane A_2 biosynthesis in human lung fibroblasts, WI 38. *Biochem. biophys. Res. Commun.* **85**, 827–836.

Hornstra, G., Haddeman, E. & Don, J. A. 1979 Blood platelets do not provide endoperoxides for vascular prostacyclin production. *Nature, Lond.* **279**, 66–68.

Iwakami, M. 1965 Peroxidase as a factor of atherosclerosis. *Nagoya J. med. Sci.* **28**, 50–66.

Jobin, F. 1978 *Semin. Thromb. Haemostas.* **4**, 199–240.

Johnson, M., Harrison, H. E., Raftery, A. T. & Elder, J. B. 1979 Vascular prostacyclin may be reduced in diabetes in man. *Lancet* i, 325–326.

Johnson, M., Reece, A. H. & Harrison, H. E. 1978 Decreased vascular prostacyclin in experimental diabetes. In *Abstracts of 7th International Congress of Pharmacology*, Paris, p. 342. Oxford: Pergamon Press.

Johnson, R. A., Morton, D. R., Kinner, J. H., Gorman, R. R., McGuire, J. C., Sun, F. F., Whittaker, N., Bunting, S., Salmon, J., Moncada, S. & Vane, J. R. 1976 The chemical structure of prostaglandin X (prostacyclin). *Prostaglandins* **12**, 915–928.

Jorgensen, K. A., Dyerberg, J., Olesen, A. S. & Stoffersen, E. 1980 Acetylsalicylic acid, bleeding time and age. *Thromb. Res.* **19**, 799–805.

Jorgensen, K. A., Olesen, A. S., Dyerberg, J. & Stoffersen, E. 1979 Aspirin and bleeding time: dependency of age. *Lancet* ii, 302.

Kazer-Glanzman, R., Jakabova, M., George, J. & Luscher, E. 1977 Stimulation of calcium uptake in platelet membrane vesicles by adenosine 3′,5′-cyclic monophosphate and protein kinase. *Biochim. biophys. Acta* **446**, 429–440.

Kelton, J. G., Hirsch, J., Carter, C. J. & Buchanan, M. R. 1978 Thrombogenic effect of high dose aspirin in rabbits: relationship to inhibition of vessel wall synthesis of prostaglandin I_2-like activity. *J. clin. Invest.* **62**, 892–895.

Kernoff, P. B. A., Willis, A. L., Stone, R. J., Davies, J. A. & McNicol, G. P. 1977 Antithrombotic potential of dihomo-γ-linolenic acid in man. *Br. med. J.* ii, 1441–1444.

Korbut, R. & Moncada, S. 1978 Prostacyclin (PGI_2) and thromboxane A_2 interaction *in vivo*. Regulation by aspirin and relationship with antithrombotic therapy. *Thromb. Res.* **13**, 489–500.

Lagarde, M. & Dechavanne, M. 1977 Increase of platelet prostaglandin cyclic endoperoxides in thrombosis. *Lancet* i, 88.

Lapetina, E. G., Schmitges, G. J., Chandrabose, K. & Cuatrecasas, P. 1977 Cyclic adenosine 3′,5′-monophosphate and prostacyclin inhibit membrane phospholipase activity in platelets. *Biochem. biophys. Res. Commun.* **76**, 828–835.

Levy, S. V. 1978 Contractile responses to prostacyclin (PGI_2) of isolated human saphenous and rat venous tissue. *Prostaglandins* **16**, 93–97.

Lewy, R. I., Smith, J. B., Silver, M. J., Wiener, L. & Walinsky, P. 1979 Detection of thromboxane A_2 in peripheral blood of patients with Prinzmetal's angina. *Prostaglandins Med.* **2**, 243–248.

Lieberman, G. E., Lewis, G. P. & Peters, T. J. 1977 A membrane-bound enzyme in rabbit aorta capable of inhibiting adenosine-diphosphate-induced platelet aggregation. *Lancet* ii, 330–332.

Longmore, D. B., Bennett, G., Gueirrara, S., Smith, M., Bunting, S., Reed, P., Moncada, S., Read, N. G. & Vane, J. R. 1979 Prostacyclin: a solution to some problems of extracorporeal circulation. *Lancet* i, 1001–1005.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

MacDonald, J. W. D. & Stuart, R. K. 1974 Interactions of prostaglandins E_1 and E_2 in regulation of cyclic AMP and aggregation in human platelets: evidence for a common prostaglandin receptor. *J. Lab. clin. Med.* **84**, 111–121.

MacIntyre, D. E., Pearson, J. D. & Gordon, J. L. 1978 Localisation and stimulation of prostacyclin production in vascular cells. *Nature, Lond.* **271**, 549–551.

Main, I. H. M. & Whittle, B. J. R. 1975 Potency and selectivity of methyl analogues of prostaglandin E_2 on rat gastrointestinal function. *Br. J. Pharmacol.* **54**, 309–317.

Majerus, P. W. 1976 Why apirin? *Circulation* **54**, 357–359.

Malmsten, C., Granstrom, E. & Samuelsson, B. 1976 Cyclic AMP inhibits synthesis of prostaglandin endoperoxide (PGG_2) in human platelets. *Biochem. biophys. Res. Commun.* **68**, 569–576.

Marcus, A. J. 1978 The role of lipids in platelet function with particular reference to the arachidonic acid pathway. *J. Lipid Res.* **19**, 793–826.

Marcus, A. J., Weksler, B. B. & Jaffe, E. A. 1978 Enzymatic conversion of prostaglandin endoperoxide H_2 and arachidonic acid to prostacyclin by cultured human endothelial cells. *J. biol. Chem.* **253**, 7138–7141.

Marcus, A. J., Weksler, B. B., Jaffe, E. A. & Broekman, M. J. 1979 Synthesis of prostacyclin $(PGI)_2$ from platelet-derived endoperoxides by cultured human endothelial cells. *Blood*, **54**, suppl. 1, abstr. 803, p. 290a.

Masotti, G., Galanti, G., Pogessi, L., Abbate, R. & Neri Serneri, G. G. 1979 Differential inhibition of prostacyclin production and platelet aggregation by aspirin. *Lancet* ii, 1213–1216.

McGiff, J. C., Crowshaw, K., Terragno, N. A. & Lonigro, A. J. 1970 Release of a prostaglandin E-like substance into renal venous blood in response to angiotensin II. *Circuln Res.* **26–27**, suppl. 1, I-121–I-130.

McGiff, J. C., Terragno, N. A., Malik, K. U. & Lonigro, A. J. 1972 Release of a prostaglandin E-like substance from canine kidney by bradykinin. Comparison with eledoisin. *Circuln Res.* **31**, 36–43.

Medalie, J. H., Kahn, H. A., Naufeld, H. N., Riss, E. & Gouldbourt, U. 1973 Five year myocardial infarction incidence. II. Association of single variables to age and birth place. *J. chron. Dis.* **26**, 329–349.

Miller, O. V. & Gorman, R. R. 1979 Evidence for distinct PGI_2 and PGD_2 receptors in human platelets. *J. Pharmacl. exp. Ther.* **210**, 134–140.

Minkes, S., Stanford, M., Chi, M., Roth, G., Raz, A., Needleman, P. & Majerus, P. 1977 Cyclic adenosine 3′5′-monophosphate inhibits the availability of arachidonate to prostaglandin synthetase in human platelet suspensions. *J. clin. Invest.* **59**, 449–454.

Moncada, S., Gryglewski, R. J., Bunting, S. & Vane, J. R. 1976*a* An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature, Lond.* **263**, 663–665.

Moncada, S., Gryglewski, R. J., Bunting, S. & Vane, J. R. 1976*b* A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (prostaglandin X) which prevents platelet aggregation. *Prostaglandins* **12**, 715–733.

Moncada, S., Herman, A. G., Higgs, E. A. & Vane, J. R. 1977 Differential formation of prostacyclin (PGX or PGI_2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. *Thromb. Res.* **11**, 323–324.

Moncada, S., Korbut, R., Bunting, S. & Vane, J. R. 1978 Prostacyclin is a circulating hormone. *Nature, Lond.* **273**, 767–768.

Moncada, S. & Vane, J. R. 1977 The discovery of prostacyclin – a fresh insight into arachidonic acid metabolism. In *Biochemical aspects of prostaglandins and thromboxanes* (ed. N. Kharasch & J. Fried), pp. 155–177. New York, San Francisco and London: Academic Press.

Moncada, S. & Vane, J. R. 1978 Unstable metabolites of arachidonic acid and their role in haemostasis and thrombosis. *Br. med. Bull.* **34**, 129–135.

Moncada, S. & Vane, J. R. 1979*a* Arachidonic acid metabolites and the interactions between platelets and blood vessel walls. *New Engl. J. Med.* **300**, 1142–1147.

Moncada, S. & Vane, J. R. 1979*b* The role of prostacyclin in vascular tissue. *Fedn Proc. Fedn Am. Socs. exp. Biol.* **38**, 62–66.

Moore, S., Pepper, D. S. & Cash, J. D. 1975 The isolation and characterization of a platelet-specific beta-globulin (β-thromboglobulin). *Biochim. biophys. Acta* **379**, 360–369.

Mullane, K. M., Dusting, G. J., Salmon, J. A., Moncada, S. & Vane, J. R. 1979 Biotransformation and cardiovascular effects of arachidonic acid in the dog. *Eur. J. Pharmac.* **54**, 217–228.

Mullane, K. M. & Moncada, S. 1980*a* Prostacyclin mediates the potentiated hypotensive effect of bradykinin following captopril treatment. *Eur. J. Pharmac.* **66**, 355–365.

Mullane, K. M. & Moncada, S. 1980*b* Prostacyclin release and the modulation of some vasoactive hormones. *Prostaglandins* **20**, 25–49.

Munday, B. R., Bewick, M., Moncada, S. & Vane, J. R. 1981 An experimental assessment of prostacyclin in the harvesting of kidneys for transplantation. *Transplantation.* (In the press.)

Munday, B. R., Bewick, M., Moncada, S. & Vane, J. R. 1980 Suppression of hyperacute renal allograft rejection in presensitized dogs with prostacyclin. *Prostaglandins* **19**, 595–603.

Mustard, J. F. & Packham, M. A. 1975 Platelets, thrombosis and drugs. *Drugs* **9**, 19–76.

Needleman, P. 1976 The synthesis and function of prostaglandins in the heart. *Fedn Proc. Fedn Am. Socs exp. Biol.* **35**, 2376–2381.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Needleman, P., Bronson, S. D., Wyche, A., Sivakoff, M. & Nicolaou, K. 1978 Cardiac and renal prostaglandin I_2. *J. clin. Invest.* **61**, 839–849.

Needleman, P., Marshall, G. R. & Sobel, B. E. 1975 Hormone interactions in the isolated rabbit heart: synthesis and coronary vasomotor effects of prostaglandins, angiotensin and bradykinin. *Circulation Res.* **37**, 802–808.

Needleman, P., Wyche, A. & Raz, A. 1979 Platelet and blood vessel arachidonate metabolism and interactions. *J. clin. Invest.* **63**, 345–349.

Neri Serneri, G. G., Masotti, G., Poggesi, L. & Galante, G. 1980 Release of prostacyclin into the bloodstream and its exhaustion in humans after local blood flow changes (ischaemia and venous stasis). *Thromb. Res.* **17**, 197–208.

Nijkamp, F. P., Moncada, S., White, H. L. & Vane, J. R. 1977 Diversion of prostaglandin endoperoxide metabolism by selective inhibition of thromboxane A_2 biosynthesis in lung, spleen or platelets. *Eur. J. Pharmac.* **44**, 179–187.

Nordoy, A., Svensson, B., Wiebe, D. & Hoak, J. C. 1978 Lipoproteins and the inhibitory effect of human endothelial cells on platelet function. *Circulation Res.* **43**, 527–534.

Nugteren, D. H. & Hazelhof, E. 1973 Isolation and properties of intermediates in prostaglandin biosynthesis. *Biochim. biophys. Acta* **326**, 448–461.

Oates, J. A., Falardeau, P., FitzGerald, G. A., Branch, R. A. & Brash, A. R. 1981 Quantification of urinary prostacyclin metabolites in man: estimates of the rate of secretion of prostacyclin into the general circulation. In *The clinical pharmacology of prostacyclin* (ed. P. J. Lewis & J. O'Grady). Raven Press. (In the press.)

Oelz, O., Seyberth, H. W., Knapp, H. R., Sweetman, B. J. & Oates, J. A. 1976 Effects of feeding ethyl-dihomo-γ-linolenate on prostaglandin biosynthesis and platelet aggregation in the rabbit. *Biochim. biophys. Acta* **431**, 268–277.

O'Grady, J. & Moncada, S. 1978 Aspirin: a paradoxical effect on bleeding time. *Lancet* ii, 780.

O'Grady, J., Warrington, S., Moti, M. J., Bunting, S., Flower, R. J., Fowle, A. S. E., Higgs, E. A. & Moncada, S. 1979 Effects of intravenous prostacyclin infusions in healthy volunteers – some preliminary observations. In *Prostacyclin* (ed. J. R. Vane & S. Bergström), pp. 409–417. New York: Raven Press.

Omini, C., Moncada, S. & Vane, J. R. 1977 The effects of prostacyclin (PGI_2) on tissues which detect prostaglandins (PG's). *Prostaglandins* **14**, 625–632.

Pace-Asciak, C. 1976 Isolation, structure and biosynthesis of 6-keto prostaglandin $F_{1\alpha}$ in the rat stomach. *J. Am. chem. Soc.* **98**, 2348–2349.

Pace-Asciak, C. R., Carrara, M. C., Rangaraj, G. & Nicolaou, K. G. 1978 Enhanced formation of PGI_2, a potent hypotensive substance, by aortic rings and homogenates of the spontaneously hypertensive rat. *Prostaglandins* **15**, 1005–1012.

Pomerantz, K., Sintetos, A. & Ramwell, P. 1978 The effect of prostacyclin on the human umbilical artery. *Prostaglandins* **15**, 1035–1044.

Rajah, S. M., Penny, S. & Kester, R. 1978 Aspirin and bleeding time. *Lancet* ii, 1104.

Remuzzi, G., Cavenaghi, A. E., Mecca, G., Donati, M. B. & De Gaetano, G. 1977 Prostacyclin (PGI_2) and bleeding time in uremic patients. *Thromb. Res.* **11**, 919–920.

Remuzzi, G., Misiani, R., Marchesi, D., Livio, M., Mecca, G., De Gaetano, G. & Donati, M. B. 1978 Haemolytic-uraemic syndrome: deficiency of plasma factor(s) regulating prostacyclin activity. *Lancet* ii, 871–872.

Robert, A., Hanchar, A. J., Lancaster, C. & Nezamis, J. E. 1979 Prostacyclin inhibits enteropooling and diarrhoea. In *Prostacyclin* (ed. J. R. Vane & S. Bergström), pp. 147–158. New York: Raven Press.

Rosenblum, W. I. & El-Sabban, F. 1978 Enhancement of platelet aggregation by tranylcypromine in mouse cerebral microvessels. *Circuln Res.* **43**, 238–241.

Roth, G. J. & Majerus, P. W. 1975 The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particular fraction protein. *J. clin. Invest.* **56**, 624–632.

Roth, G. J. & Siok, C. J. 1978 Acetylation of the NH_2-terminal serine of prostaglandin synthetase by aspirin. *J. biol. Chem.* **253**, 3782–3784.

Saba, S. R. & Mason, R. G. 1974 Studies of an activity from endothelial cells that inhibits platelet aggregation, serotonin release and clot retraction. *Thromb. Res.* **5**, 747–757.

Saeed, S. A., McDonald-Gibson, W. J., Cuthbert, J., Copas, J. L., Schneider, C., Gardiner, P. J., Butt, N. M. & Collier, H. O. J. 1977 Endogenous inhibitor of prostaglandin synthetase. *Nature, Lond.* **270**, 32–33.

Salmon, J. A., Smith, D. R., Flower, R. J., Moncada, S. & Vane, J. R. 1978 Further studies on the enzymatic conversion of prostaglandin endoperoxide into prostacyclin by porcine aorta microsomes. *Biochim. biophys. Acta* **523**, 250–262.

Shimamoto, T., Kobayashi, M., Takahashi, T., Takashima, Y., Sakamoto, M. & Morooka, S. 1978 An observation of thromboxane A_2 in arterial blood after cholesterol feeding in rabbits. *Jap. Heart J.* **19**, 748–753.

Sim, A. K. & McCraw, A. P. 1977 The activity of γ-linolenate and dihomo-γ-linolenate methyl esters *in vitro* and *in vivo* on blood platelet function in nonhumans. *Thromb. Res.* **10**, 385–397.

Sinzinger, H., Feigl, W. & Silberbauer, K. 1979 Prostacyclin generation in atherosclerotic arteries. *Lancet* ii, 469.

Slater, T. F. 1972 *Free radical mechanisms in tissue injury*. London: Pion Ltd.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Steer, M. L., MacIntyre, D. E., Levine, L. & Salzman, E. W. 1980 Is prostacyclin a physiologically important circulating antiplatelet agent? *Nature, Lond.* **283**, 124–125.

Szczeklik, A., Gryglewski, R. J., Musial, J., Grodzinska, L., Serwonska, M. & Marcinkiewicz, E. 1978*a* Thromboxane generation and platelet aggregation in survivals of myocardial infarction. *Thromb. Diathes. haemorrh.* **40**, 66–74.

Szczeklik, A., Gryglewski, R. J., Nizankowski, R. & Musial, J. 1978*b* Pulmonary and antiplatelet effects of intravenous and inhaled prostacyclin in man. *Prostaglandins* **16**, 654–660.

Szczeklik, A., Gryglewski, R. J., Nizankowski, R., Skawinski, S., Gluszko, P. & Korbut, R. 1980 Prostacyclin therapy in peripheral artery disease. *Thromb. Res.* **19**, 191–199.

Szczeklik, A., Nizankowski, R., Skawinski, S., Szczeklik, J., Gluszko, P. & Gryglewski, R. J. 1979 Successful therapy of advanced arteriosclerosis obliterans with prostacyclin. *Lancet* i, 1111–1114.

Tansik, R. L., Namm, D. H. & White, H. L. 1978 Synthesis of prostaglandin 6-keto-$F_{1\alpha}$ by cultured aortic smooth muscle cells and stimulation of its formation in a coupled system with platelet lysates. *Prostaglandins* **15**, 399–408.

Tateson, J. E., Moncada, S. & Vane, J. R. 1977 Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platetets. *Prostaglandins* **13**, 389–399.

Terashita, Z., Nishikawa, K., Terao, S., Nakagawa, M. & Hino, T. 1979 A specific prostaglandin I_2 synthetase inhibitor, 3-hydroperoxy-3-methyl-2-phenyl-3H Indole. *Biochem. biophys. Res. Commun.* **91**, 72–78.

Terragno, N. A., Terragno, D. A., Early, J. A., Roberts, M. A. & McGiff, J. C. 1978 Endogenous prostaglandin synthesis inhibitor in the renal cortex. Effects on production of prostacyclin by renal blood vessels. *Clin. Sci. molec. Med.* **55**, 199s–202s.

Terragno, N. A., Terragno, D. A. & McGiff, J. C. 1977 Contribution of prostaglandins to the renal circulation in conscious anaesthetized and laparatomized dogs. *Circuln Res.* **40**, 590–595.

Ubatuba, F. B., Moncada, S. & Vane, J. R. 1979 The effect of prostacyclin (PGI_2) on platelet behaviour, thrombosis formation *in vivo* and bleeding time. *Thromb. Diathes. haemorrh.* **41**, 425–434.

Vane, J. R. 1964 The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmac. Chemother.* **23**, 360–373.

Vane, J. R. & Ferreira, S. H. 1976 Interactions between bradykinin and prostaglandins. In *Chemistry and biology of the kallikrein-kinin system in health and disease* (*Fogarty International Center Proceedings*, no. 27) (ed. J. J. Pisano & K. F. Austen), pp. 255–266. Washington, D. C.: U.S. Government Printing Office.

Vane, J. R. & McGiff, J. C. 1975 Possible contributions of endogenous prostaglandins to the control of blood pressure. *Circuln Res.* **36–37**, suppl. 1, I-68–I-75.

Vargaftig, B. B. & Chignard, M. 1975 Substances that increase the cyclic AMP content prevent platelet aggregation and concurrent release of pharmacologically active substances evoked by arachidonic acid. *Agents Actions* **5**, 137–144.

Vermylen, J., Chamone, D. A. F. & Verstraete, M. 1979 Stimulation of prostacyclin release from vessel wall by BAYg6575, an antithrombotic compound. *Lancet* i, 518–520.

Verstraete, M. 1976 Are agents affecting platelet functions clinically useful? *Am. J. Med.* **81**, 897–914.

Villa, S., Livio, M. & De Gaetano, G. 1979 The inhibitor effect of aspirin on platelet and vascular prostaglandins in rats cannot be completely dissociated. *Br. J. Haemat.* **42**, 425–431.

Walker, I. D., Davidson, J. F., Faichney, A., Wheatley, D. & Davidson, K. 1980 Prostacyclin in cardiopulmonary bypass surgery. In *Abstracts*, 6*th Int. Congress on Thrombosis*, Monaco.

Warrington, S. & O'Grady, J. 1980 Cardiovascular effects of prostacyclin (PGI_2) in man. In *Advances in prostaglandin and thromboxane research* (ed. B. Samuelsson, P. W. Ramwell & R. Paoletti), vol. 7, pp. 619–624. New York: Raven Press.

Weiss, H. J. & Turitto, V. T. 1979 Prostacyclin (prostaglandin I_2, PGI_2) inhibits platelet adhesion and thrombus formation on subendothelium. *Blood* **53**, 244–250.

Weksler, B. B., Knapp, J. M. & Jaffe, E. A. 1977*a* Prostacyclin synthesized by cultured endothelial cells modulates polymorphonuclear leukocyte function. *Blood* **50**, 287.

Weksler, B. B., Ley, C. W. & Jaffe, E. W. 1978 Stimulation of endothelial cell prostacyclin production by thrombin, trypsin, and the ionophore A23187. *J. clin. Invest.* **62**, 923–930.

Weksler, B. B., Marcus, A. J. & Jaffe, E. A. 1977*b* Synthesis of prostaglandin I_2 (prostacyclin) by cultured human and bovine endothelial cells. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3922–3926.

Whittle, B. J. R., Moncada, S. & Vane, J. R. 1978 Comparison of the effects of prostacyclin (PGI_2), prostaglandin E_1 and D_2 on platelet aggregation in different species. *Prostaglandins* **16**, 373–388.

Whitworth, J. A., D'Apice, A. J. F., Kincaid-Smith, P., Shulkes, A. A. & Skinner, S. L. 1978 Antihypertensive effect of plasma exchange. *Lancet* i, 1205.

Willis, A. L., Comai, K., Kuhn, D. C. & Paulsrud, J. 1974 Dihomo-γ-linolenate suppresses platelet aggregation when administered *in vitro* or *in vivo*. *Prostaglandins* **8**, 509–519.

Woodford, F. P., Bottcher, C. J. F., Oette, K. & Ahrens, E. H., Jr 1965 The artifactual nature of lipid peroxides detected in extracts of human aorta. *J. Atheroscler. Res.* **5**, 311–316.

Woods, H. F., Ash, G., Weston, M. J., Bunting, S., Moncada, S. & Vane, J. R. 1978 Prostacyclin can replace heparin in haemodialysis in dogs. *Lancet* ii, 1075–1077.

Wynalda, M. A. & Fitzpatrick, F. A. 1980 Albumins stabilize prostaglandin I_2. *Prostaglandins* **20**, 853–861.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Discussion

ELSPETH B. SMITH (*Department of Chemical Pathology, University Medical Buildings, Foresterhill, Aberdeen, U.K.*). In the trial with low-dose aspirin [data not in published report] I was interested to see that there were two non-responders, both with the same initial. Were they members of the same family? Is there evidence of a familial factor in response to aspirin?

S. MONCADA. They were not members of the same family; there is no evidence of a familial factor in response to aspirin, although we have not looked at this in detail.

D. B. LONGMORE (*National Heart Hospital, London, U.K.*). In this very important paper, the possible role of prostacyclin in cross-species transplantation has been mentioned. In the mid-1960s at the National Heart Hospital, we attempted animal–man transplantation unsuccessfully. We used a pigs heart–lung as a supplementary organ system to boost the circulation in two patients whose cardiovascular system was inadequate to enable us to wean them off the heart–lung machine. There was total cessation of coronary flow within 4–6 minutes. We have recently tried pig–dog and dog–pig cross-species heart transplant experiments in our laboratories with prostacyclin. We have tried dose levels of PGI_2 varying between 8 and 15 ng kg^{-1} min^{-1}. We have achieved a lengthening of the time for vascular occlusion of up to 2 h. We wonder why, in spite of the PGI_2 infusion and the initially high flow rates through the transplanted organ, total occlusion of the vascular system should still occur relatively quickly. The addition of dipyridamole, a powerful coronary vasodilator in dogs, has not in our initial experiments made any difference to this acute intravascular clotting. PGI_2 is obviously helpful, but a survival time of an hour or two is of little value to the patient receiving a cross-species heart transplant. Has Dr Moncada any suggestions as to how we should try to extend the beneficial effect of PGI_2 in this difficult situation?

S. MONCADA. A lengthening of about 1 h in xenografts is already a great improvement since controls run for about 5–6 min. What we have to study now in detail is, first, whether prostacyclin is effective in other mechanisms apart from inhibiting platelet aggregation and second, whether by increasing prostacyclin doses we shall obtain a further prolongation in the time. This is, of course, difficult to do since the cardiovascular effects of prostacyclin prevent us from increasing the dose.

H. O. J. COLLIER (*Miles Laboratories Limited, U.K.*). May I call attention to a third factor that may affect the outcome of an interaction between prostacyclin and thromboxane A_2 at the platelet? Awareness of this factor derives from our observation that blood plasma or serum inhibits synthesis of the main prostaglandins from arachidonic acid *in vitro* (Saeed *et al.* 1977; Collier *et al.* 1980; Denning-Kendall *et al.* 1981). This ability of plasma can be expressed in its inhibition of arachidonate-induced aggregation of platelets. Thus, we have found that platelets suspended in plasma require about ten times more arachidonate to induce their aggregation than do washed platelets suspended in buffer (Collier & McDonald-Gibson 1980). This inhibitory effect on platelet aggregation can largely be attributed to plasma albumin, which is a potent inhibitor of prostaglandin synthesis (Collier & McDonald-Gibson 1980; Collier *et al.* 1981).

The authors have said that there is not enough prostacyclin in plasma to inhibit strongly

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

platelet aggregation in the circulating blood. If this is so, then the presence of free arachidonic acid in the blood, which might arise from its ingestion or from its liberation from store, would threaten to induce aggregation of circulating platelets, if another inhibitory mechanism did not operate. We have proposed that a hitherto unrecognized function of plasma albumin may be to make it harder for free arachidonic acid in the plasma to induce aggregation, since, in the presence of albumin, a greater amount of arachidonic acid, such as might be liberated at a damaged vessel wall, would be required to induce aggregation (Collier & McDonald-Gibson 1980).

References

Collier, H. O. J., Denning-Kendall, P. A., McDonald-Gibson, W. J. & Saeed, S. A. 1980 Plasma proteins that inhibit prostaglandin synthesis. In *Hemostasis, prostaglandins, and renal disease* (ed. G. Remuzzi, G. Mecca & G. de Gaetano), pp. 257–267. New York: Raven Press.

Collier, H. O. J., Denning-Kendall, P. A., McDonald-Gibson, W. J., Saeed, S. A., Brennecke, S. P. & Mitchell, M. D. 1981 Endogenous inhibitors of prostaglandin synthesis (EIPS) in blood plasma: possible identity and function. In *The role of chemical mediators in hemodynamic and metabolic failure in the critically-ill* (ed. R. McConn). New York: Raven Press. (In the press.)

Collier, H. O. J. & McDonald-Gibson, W. J. 1980 Plasma albumin inhibits arachidonate-induced aggregation of suspended platelets. *J. Physiol., Lond.* (In the press.)

Denning-Kendall, P. A., Saeed, S. A. & Collier, H. O. J. 1981 Comparison of the inhibition by human serum, albumin and haptoglobin of biosynthesis of various prostaglandins. *Biochem. Soc. Trans.* (In the press.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES