The discovery of prostacyclin has allowed new in-depth investigations of platelet vessel wall interactions and their relationship to pathological conditions like thrombosis and atherosclerosis. This paper reviews current research in prostacyclin and arterial wall biology.

Arachidonic Acid Metabolism

Arachidonic acid, the precursor of all bisenoic prostaglandins, is the most common fatty acid present in cellular phospholipids and can be obtained directly from the diet or by desaturation and chain elongation from dietary linoleic acid (C18:2 ω-6). Arachidonic acid is liberated from membrane phospholipids by the action of phospholipases, activated by changes in their chemical environment. Simple mechanical stimulation can result in generation of prostaglandins, as shown in many tissues (for review see reference 2). The enzymes that synthesize prostaglandins are present in most organs but some tissues, such as seminal vesicles, kidneys, and lungs, have a greater capacity for prostaglandin synthesis than others. The term “eicosanoids” is applied to all the 20-carbon derivatives, whereas “prostanoids” refers only to those with a prostanoic acid skeleton. Once released from the membrane phospholipids, arachidonic acid is metabolized by two enzymes. The cyclooxygenase forms the prostaglandin endoperoxide PGG₂. This is converted to PGH₂, which then isomerizes enzymically or nonenzymically to the stable substances PGE₂, PGF₂α, and PGD₂ (figure 1). A 17-carbon hydroxy acid called 12-hydroxy-5,8,10 heptadecatrienoic acid (HHT) is also formed, together with malondialdehyde (MDA). The prostaglandin endoperoxides are also transformed enzymically into two other unstable products, prostacyclin (strong vasodilator and inhibitor of platelet aggregation) and TXA₂ (vasoconstrictor and platelet aggregator). Unlike PGE₂, D₂, or F₂α, these products are not formed by chemical breakdown of PGH₂. The cyclooxygenase is inhibited by aspirin-like drugs leading to the hypothesis that the therapeutic, as well as the shared side-effects, of aspirin-like drugs are related to inhibition of prostaglandin biosynthesis. Since the publication of this data, a great deal of evidence has accumulated in favor of this proposal.

The lipoxygenase leads to the formation of eicosanoids, which are noncyclized hydroxy acids. 11 and 15-hydroxyecosatetraenoic acid (11 & 15-HETE) may be formed as the result of an incomplete cyclooxygenase reaction, but in 1974 a separate lipoxygenase was discovered in platelets that synthesizes 12-HETE. The synthesis of 5-HETE by polymorphonuclear leukocytes (PMNs) has also been described. Formation of these monohydroxy acids is preceded by abstraction of hydrogen from arachidonic acid and peroxidation at the appropriate position to give unstable hydroperoxy intermediates (HPETEs; figure 1).

The 5-lipoxygenase in leukocytes also gives rise to a family of compounds containing a conjugated triene structure, named “leukotrienes” (figure 1). Leukotriene A₄ is a 5,6 epoxide of arachidonic acid which can be converted to the 5,12-dihydroxy acid (5,12 DHETE; leukotriene B₄; LTB₄). Alternatively, the addition of glutathione to the epoxide by glutathione-S-transferase results in the formation of LTC₄. The removal of glutamate from LTC₄ by γ-glutamyl transpeptidase gives LTD₄ (figure 1) which is further metabolized to LTE₄, with the loss of glycine (for review see reference 11). These lipid-peptide structures account for the biological activity of slow-reacting substances (SRGs) detected in immediate hypersensitivity reactions.

The enzyme cyclooxygenase (sometimes called “prostaglandin synthetase”) is present in all cell types (except erythrocytes), whereas 5- or 12-lipoxygenases have so far been identified in platelets, lungs, white cells, blood vessels, and epicardium (for review see reference 2).
Prostacyclin

Prostacyclin is the main product of arachidonic acid in all arteries and veins so far tested. It is a strong hypotensive agent and a vasodilator of all vascular beds studied (for these and other actions of prostacyclin on the cardiovascular system, see reference 13). Not much is known about the microcirculation, but Goehlert and coworkers' have demonstrated that microvessels, mainly capillaries, isolated from rat cerebrum generate predominantly prostacyclin.

The ability of the large vessel wall to synthesize prostacyclin is greatest at the intimal surface and progressively decreases toward the adventitia. Production of prostacyclin by cultured cells from vessel walls also shows that endothelial cells are the most active producers of prostacyclin; moreover, this production persists after numerous subcultures in vitro.

Initially, it was demonstrated that vessel microsomes in the absence of cofactors could utilize pros-
taglandin endoperoxides, but not arachidonic acid, to synthesize prostacyclin. Later it was shown that fresh vascular tissue could utilize both precursors, although the endoperoxides are much better substrates. Moreover, vessel microsomes, fresh vascular rings, or endothelial cells treated with indomethacin could, when incubated with platelets, generate a prostacyclin-like antiaggregating activity. The release of this substance was inhibited by 15-hydroperoxy arachidonic acid (15-HPAA) and other fatty acid hydroperoxides, known to be selective inhibitors of prostacyclin formation. From all these data we concluded that the vessel wall can synthesize prostacyclin not only from its own endogenous precursors, but also from prostaglandin endoperoxides released by the platelets, thus suggesting a biochemical cooperation between platelet and vessel wall. Several observations support this conclusion. Incubation of platelet-rich plasma (PRP) with fresh, indomethacin-treated arterial tissue leads to an increase in platelet cyclic AMP (cAMP) which 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. Furthermore, Tansik and colleagues showed that lysed aortic smooth muscle cells could be supplied with prostaglandin endoperoxides by lysed human platelets to form prostacyclin. Undisturbed endothelial cell monolayers can also readily transform PGH₂ to prostacyclin.

However, the hypothesis was challenged by Needleman and associates who demonstrated that, while arachidonic acid was rapidly converted to prostacyclin by perfused rabbit hearts and kidneys, PGH₂ was not readily transformed. They concluded that some degree of vascular damage was necessary for the endoperoxide to be utilized by prostacyclin synthetase. Needleman and colleagues and Homstra and coworkers using vessel microsomes or fresh vascular tissue also concluded that endoperoxides from platelets cannot be utilized by other cells under their experimental conditions. However, Marcus and colleagues showed that feeding of endoperoxides to endothelial cells suspended in PRP takes place in vitro, but only when the platelet concentration is similar to that in normal blood. Too high a platelet concentration induces a platelet-to-platelet interaction that limits the platelet-endothelial cell reaction. It should be stressed, however, that the possibility of endoperoxides released from platelets being utilized by endothelial cells has not yet been tested in vivo. Adherence of the platelet to the vessel wall, known to be one of the first responses to injury, could well provide the close proximity that would be needed for such "cooperation." This proposal has still to be verified in vivo and, as will be seen later, the development of antithrombotic compounds based on selective inhibition of TXA₂ synthetase is likely to be successful only if this mechanism of transfer of endoperoxides from the platelet to the vessel wall takes place.

It is also possible that formed elements of blood such as white cells, which produce endoperoxides and TXA₂, could interact with the vessel wall to promote formation of prostacyclin. Thus, prostacyclin might regulate white cell behavior and help control white cell activity during the inflammatory response.

Using fresh human vascular tissue, we did not find any difference between the production of prostacyclin in vitro by veins and arteries. No difference in prostaglandin production by veins and arteries had previously been detected in bovine vessels. However, arteries have been shown to produce more prostacyclin than veins in rabbits, rats, and dogs. In addition, cultured cells obtained from human pulmonary arteries produce more prostacyclin than those obtained from pulmonary veins. In "arterialized" venous (carotid to jugular) grafts implanted in dogs for up to 6 weeks, the venous tissue, although becoming arterialized from a structural point of view, maintained a lower production of prostacyclin than the carotid artery. These observations suggest that there might indeed be a biochemical difference in the cells from both systems. There has also been a suggestion that the production of prostacyclin by dacron grafts in humans is similar to that observed in the nearby artery. At this stage, however, it is necessary to continue more detailed work in this area before the published differences can be definitely accepted.

Prostacyclin is unstable and is the most potent endogenous inhibitor of platelet aggregation yet discovered. It is 30 to 40 times more potent than PGE₁.

In vivo, prostacyclin applied locally in low concentrations inhibits thrombus formation due to ADP in the microcirculation of the hamster cheek pouch, and given systemically to the rabbit prevents electrically induced thrombus formation in the carotid artery and increases bleeding time. The duration of these effects in vivo is short; they disappear within 30 minutes of administration. Prostacyclin also disaggregates platelets in vitro and in vivo in experimental models and in humans (for review see reference 13).

**Mechanism of Action**

Prostacyclin inhibits platelet aggregation by stimulating adenylate cyclase, leading to an increase in cAMP levels in the platelets. In this respect prostacyclin is much more potent than either PGE₁ or PGD₂. 6-oxo-PGF₁α has weak antiaggregating activity and is almost devoid of activity on platelet cAMP.

Prostacyclin is not only more potent than PGE₁ in elevating cAMP but the elevation persists longer. The elevation induced by PGE₁ starts falling after 30 seconds, while prostacyclin stimulation is not maximal until after 30 seconds and is maintained for 2 minutes, after which it gradually wanes over 30 minutes. Prostacyclin is also a strong direct stimulator of adenylate cyclase in isolated membrane preparations.
Prostacyclin, PGE₁, and PGD₂ increase adenylate cyclase activity by acting on two distinct receptors on the platelet membrane.⁴⁶ ⁴⁷ PGE₁ and prostacyclin act on one, whereas PGD₂ acts on another. These and other results suggest that the previously recognized PGE₁ receptor in platelets is, in fact, the prostacyclin receptor.

There have not been many detailed studies on the mechanism of action of prostacyclin. In contrast to TXA₂, it enhances Ca²⁺ sequestration.⁴⁸ In addition, an inhibitory effect on platelet phospholipase⁴⁹ ⁵⁰ and platelet cyclooxygenase⁵¹ have been described. All these effects are related to its ability to increase cAMP in platelets. Moreover, prostacyclin inhibits endoperoxide-induced aggregation suggesting additional sites of action, still undefined, but dependent on the cAMP effect.⁵⁰ Prostacyclin, by inhibiting several steps in the activation of the arachidonic acid metabolic cascade, exerts an overall control of platelet aggregability in vivo.

Prostacyclin increases cAMP levels in cells other than platelets. These include cultured human fibroblasts,⁵² human fat cell ghosts,⁵³ guinea pig lung homogenates,⁵⁴ and polymorphonuclear leukocytes.⁵⁵ Thus, there is the possibility that in these cells an interaction with the thromboxane system could lead to a regulation of cell behavior similar to that observed in platelets, suggesting that the PGI₂/TXA₂ system has a wider biological significance. Indeed, prostacyclin inhibits white cell adherence to the vessel wall,⁵⁶ to nylon fibers, and to endothelial monolayers in vitro.⁵⁷ It has recently been shown⁵⁸ that prostacyclin increases cAMP in the endothelial cell itself and the authors have suggested that this may act as a negative feedback control for prostacyclin production by the endothelium. Red blood cells have receptors for prostacyclin⁵⁹ and respond to it by changing their deformability.⁶⁰

**Prostacyclin and Platelet-Vascular Interactions**

The antiaggregating activity of the vascular wall is mainly related to the release of prostacyclin, for 15-HPAA or 13-hydroperoxy linoleic acid (13-HPLA), two inhibitors of prostacyclin formation abolish most, if not all, of the antiaggregatory activity of vascular endothelial cells.⁶¹ Similar results were obtained with an antiserum that crossreacts with and neutralizes prostacyclin in vitro.⁶² Endothelial cells pretreated with this antiserum lose the ability to inhibit ADP-induced aggregation.¹⁸ ²²

It has not been clear, however, to what extent PGI₂ generation is responsible for the thromboresistant properties of vascular endothelium. Dejana and co-workers⁶³ studied the effect of inhibition of PGI₂ synthesis by aspirin on platelet adhesion to the endothelial lining of rabbit aorta in vivo and in vitro. They concluded that inhibition of prostacyclin production does not promote platelet adhesion. Similar results have been obtained by Curwen and colleagues⁶⁵ using a different preparation. In their hands neither treatment of vascular endothelium with aspirin or indomethacin nor increasing PGI₂ production by arachidonic acid affected basal platelet adherence. However, in transformed vascular endothelial cells (obtained after viral infection) there was very little PGI₂ generation and platelet adherence was greatly increased. This could be partially reversed by adding exogenous PGI₂. On the other hand, Czervionke and coworkers⁶⁴ using washed preparations of labeled human platelets did not observe an effect on platelet adhesion to human endothelial cultures. However, platelet adherence in the presence of thrombin increases from 4% to 44% after treatment with 1 mM aspirin. This increase was paralleled by a decrease in 6-oxo-PGF₁α formation from 107 nM to < 3 nM and could be reversed by addition of 25 nM of exogenous PGI₂.⁶⁶ In vivo and in vitro Baumgartner and Muggli⁶⁷ and Tschopp and Baumgartner⁶⁸ have shown that aspirin treatment does not enhance platelet adherence to the vascular wall. However, after the removal of the vascular endothelium, aspirin treatment enhances both adherence and aggregation. Interestingly, they studied vascular tissue from rats, rabbits, and guinea pigs and found a decreasing ability to generate PGI₂ from rats to guinea pigs. Moreover, there was a negative correlation between the ability of the vascular tissue of a species to produce PGI₂ and the degree of platelet adherence-aggregation that was observed after interaction of the deendothelialized vascular tissue with the animals' blood in vivo.⁶⁹

The fact that prostacyclin inhibits platelet aggregation (platelet-platelet interaction) at much lower concentrations than those needed to inhibit adhesion (platelet-collagen interaction)⁷⁰ suggests that prostacyclin allows platelets to stick to vascular tissue and to interact with it, while at the same time preventing or limiting thrombus formation. Weiss and Turitto⁷¹ have observed some degree of inhibition of platelet subendothelial 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.

More recently, it has been demonstrated that shortly after balloon deendothelialization of the aortas of rabbits there is a closely adherent layer of spread platelets. A small reduction of adherent platelets could be observed in animals receiving prostacyclin at 50–100 ng/kg/min. Only concentrations of 650–850 ng/kg/min could inhibit this platelet adhesion.⁷²

Using a new method to measure prostacyclin generated by the luminal surface of a vessel, Eldor and colleagues⁷³ have demonstrated that the vascular endothelium is the only source of PGI₂ generated in the luminal surface of a rabbit aorta. After balloon catheter deendothelialization, the capacity for generation of PGI₂ is abolished and only recovers slowly over a period of 70 days, concomitant with the ap-
pearance of neointimal cells on the vessel surface. The authors also observed in the deendothelialized areas a "carpet of platelets" which slowly disappeared during the time of reendothelialization.

All this work suggests that prostacyclin, although not responsible for all the thromboresistant properties of vascular endothelium, plays a very important part in the control of platelet aggregability especially in situations in which platelet reactivity might be enhanced due to local tissue damage. This would represent a pathological state; however, it is worth remembering that very mild tissue damage leads to prostaglandin and prostacyclin synthesis (for review, see reference 13). Whether the passage of vascular cells, some of which are larger than 10 μ in diameter, through capillaries of 5 μ or less is enough to stimulate PGI₂ synthesis is still to be investigated.

**Aspirin, Hemostasis, and Thrombosis**

Aspirin binds covalently to the active site of cyclooxygenase and therefore inhibits the enzyme in platelets for their entire lifespan because platelets are unable to synthesize new protein. Aspirin also has an effect on the platelet precursors in the marrow. Thus, a single therapeutic dose of aspirin will lead to a platelet defect that lasts for well over a week. This long-lasting effect is observed only with aspirin and not with other commonly used aspirin-like drugs, which have a shorter inhibitory effect. This consideration has encouraged clinical trials in which aspirin has been used to prevent thrombotic phenomena. So far, however, the evidence in favor of this clinical use of aspirin is not very satisfactory and the reasons are becoming clear as research progresses. A consequence of the discovery of prostacyclin has been the need to reexamine the use of aspirin as an antithrombotic compound. Two important considerations have emerged in relation to aspirin: 1) inhibition of the vascular cyclooxygenase, unlike the platelet cyclooxygenase, may persist for a much shorter period because of the generation of new enzyme; and 2) the platelet cyclooxygenase seems to be more sensitive in vitro and in vivo than the vessel wall cyclooxygenase to the inhibitory action of aspirin, although reports to the contrary have been published.

Aspirin has a biphasic effect on cutaneous bleeding time in rabbits and on the formation of platelet clumps in an extracorporeal system. Low doses increase the bleeding time and reduce platelet aggregates in an experimental model but with higher doses neither effect occurs. This dose-dependent effect has been attributed to the low dose affecting only platelets and allowing prostacyclin production to continue, while both systems are inhibited by high-dose aspirin. These results have been confirmed in human volunteers by some workers but not by others. This discrepancy may be due to the fact that the volunteers in the study by O'Grady and Moncada were in a younger age group than those in the study by Godal and colleagues. Subsequently, Jorgensen and coworkers showed that bleeding time in man decreased with age and confirmed that higher doses of aspirin produced a significantly shorter bleeding time than low doses in the age groups 18 to 22 and 26 to 32 years, but not in the age group 66 to 70 years. These findings might also suggest a decrease in prostacyclin production with age (see below).

During the past 3 to 4 years, an attempt has been made to find a low dose of aspirin that will achieve inhibition of TXA₂ formation in man without affecting prostacyclin production.

Some of these studies have demonstrated that a single dose of 80 mg of aspirin reduces the activity of prostaglandin synthetase by 85% and a dose of 300 mg completely abolishes it; a single dose of 2 mg/kg is enough to inhibit completely the generation of MDA, measured 2 hours after oral ingestion, while 3 to 3.5 mg/kg are needed to inhibit ADP-, adrenaline-, or collagen-induced aggregation; and finally that a dose of 100 mg of aspirin reduces TXB₂ release by more than 90% in serum 2 hours after oral administration. These last authors have also found that a clear dose-effect on the generation of TXB₂ can only be obtained with doses below 2 mg/kg of aspirin. These data suggest that the dose of aspirin needed to obtain its full effect on TXB₂, adenine-, or collagen-induced aggregation, as well as on the inhibition of related biochemical parameters, is between 2 and 4 mg/kg. A dose of aspirin of 160 mg/day has been suggested to have an antithrombotic effect in arteriovenous shunts in uremic patients.

Recent studies in animals and in man, however, suggest that the separation in the dose that inhibits platelet aggregation and prostacyclin formation might not be so great as originally thought. Masotti and his colleagues, using a bioassay method, calculated that 4.5 mg/kg were needed to produce a 50% inhibition of PGI₂ synthesis 2 hours after oral ingestion; Preston and coworkers, taking biopsies of superficial veins, found that 2 hours after 150 or 300 mg of aspirin, 81% to 100% inhibition of prostacyclin synthesis was present; Parette and colleagues found inhibition of PGI₂ synthesis in superficial veins 2 hours after the ingestion of 150 mg of aspirin; and finally, Hanley and coworkers found about 50% inhibition of synthesis in varicose veins of patients 14 hours after ingestion of aspirin in as low a dose as 81 mg. This has led to the suggestion that aspirin could be given at longer intervals (every 2 to 3 days) in doses that will produce either no inhibition of the vascular synthesis of PGI₂ or a very short reversible inhibition (for data and reviews see references 75, 86, 87).

Patrono and colleagues have shown that TXB₂ generation in serum ex vivo can be inhibited for as long as 6 weeks (the duration of the study) on a 200 mg every 72 hour schedule. Unfortunately, they did not study prostacyclin generation. However, another
study cited previously suggests that this dose might have some longer lasting effect on the prostacyclin system. Other studies indicate different times of recovery of the vessel wall cyclooxygenase. Masotti and colleagues, for example, demonstrated more than 50% recovery of PGJ2 production by 24 hours even after ingestion of doses as high as 10 mg/kg (it is important to note that this was a bioassay method and the PGJ2-like activity was generated during arm ischemia), while other studies report no more than 25% to 50% recovery of PGJ2 formation in human vascular biopsies 8 to 24 hours after oral doses of aspirin ranging from 150 to 300 mg. With this data, all that can be said is that the problem of the recovery of PGJ2 synthesis after aspirin is still largely unresolved and much more work is needed.

Finally, some investigators have tried to find a dose of aspirin that will not affect prostacyclin formation and will have a cumulative effect on platelets producing an important inhibition of TXA2 formation. This was originally shown by Hoogendijk and ten Cate who demonstrated an increased inhibition in platelet MDA production by platelets after dosing volunteers with 40 mg aspirin a day for 10 days (the inhibition increasing to 95% at Day 10). However, there is a great variation in the individual platelet response to a 40 mg dose. This suggests that every patient would have to be titrated for their optimal dose. However, a greater problem might exist, represented by the findings of a recent study that suggests that even a dosage of 40 mg a day for 3 days has a cumulative effect on the production of prostacyclin by the vessel wall.

The available data suggest that it will be extremely difficult to find a suitable dose or schedule of aspirin in man that will achieve selective and long-lasting inhibition of TXA2.

In addition, it is also likely that even if this is achieved it will not actually prove to be a better antithrombotic drug than it has already been shown in the few trials in which it has been slightly efficacious (for review see reference 91). The reason is that platelet aggregation is a complex mechanism that takes place via different "pathways," the generation and actions of TXA2 being only one of them (the other two identified at present are the ADP and thrombin pathways).

Only the stimuli that induce platelet aggregation via the release of TXA2 will be affected by aspirin treatment, the others being largely unaffected (for review, see reference 92). Since not enough is known about the pathophysiology of intravascular thrombosis, it is difficult to predict what to expect after aspirin treatment. It is highly likely that platelet aggregation during disseminated intravascular coagulation, venous thrombosis, and arterial thrombosis have different triggering mechanisms, and it is also possible that platelet aggregation on a fissure of an atherosclerotic plaque or during coronary vasospasm might depend on the activation of different pathways.

### Thromboxane Synthetase Inhibitors

Theoretically, a selective inhibitor of thromboxane synthetase should prove to be a superior antithrombotic agent to aspirin by allowing prostacyclin formation by vessel walls or other cells either from their endoperoxides or from those released from platelets (for review see reference 13). It was originally observed during in vitro studies that when platelets were treated with a thromboxane synthetase inhibitor, 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-PGF1α rather than TXB2. Platelets cannot synthesize prostacyclin, so some other blood cell must have done so. Injection of heterologous blood into anesthetized cats causes hypotension, respiratory distress, and frequently death, and this is accompanied by a sharp rise in blood levels of TXB2 (Bunting, Castro, Salmon, and Moncada, unpublished observations). Pretreatment with either a thromboxane synthetase inhibitor (1-methylcyclooctyl imidazole) or a cyclooxygenase inhibitor such as aspirin prevented death. However, after inhibition of thromboxane synthetase, the blood levels of 6-oxo-PGF1α rose about five times more than after the shock in control animals, suggesting diversion of the platelet prostaglandin endoperoxides away from TXA2 production toward prostacyclin production.

Other work on selective thromboxane synthetase inhibitors is beginning to appear, including the first publications on administration of one of these compounds to humans. It has now been shown that TXA2 synthetase inhibitors have a superior antithrombotic action to aspirin and, more important, that the antithrombotic action of these compounds can be blocked by previous treatment with aspirin. Thus, the activity of these compounds depends upon prostaglandin endoperoxides (released by activated platelets) boosting a "functional" prostacyclin synthetase in the vessel wall.

### Prostacyclin and TXA2 In Disease

A number of diseases have now been related to an imbalance in the prostacyclin-TXA2 system. Platelets from patients with arterial thrombosis, deep venous thrombosis, or recurrent venous thrombosis produce more PG endoperoxides and TXA2 than normal and have a shortened survival time. Platelets from rabbits made atherosclerotic by dietary manipulation and from patients who have survived myocardial infarction are abnormally sensitive to aggregating agents and produce more TXA2 than controls. Elevated TXB2 levels have been demonstrated in the blood of patients with Prinzmetal's an-
gina and vasotonic angina. Hirsh and colleagues also studied TXB \(_2\) levels in coronary sinus blood of patients with unstable angina. They concluded that local thromboxane \(A_2\) release is associated with recent episodes of angina but were unable to distinguish whether the release was cause or effect.

Platelets from rats made diabetic release more TXA \(_2\) than normal, whereas their blood vessels show a reduced production of prostacyclin; these effects are reversed by chronic insulin treatment. Prostacyclin production by blood vessels from patients with diabetes is depressed and circulating levels of 6-oxo-PGF \(_{1\alpha}\) are reduced in diabetic patients with proliferative retinopathy. Davis and colleagues have confirmed that vessels taken from diabetic patients produced less prostacyclin than normals. However, their results did not support an association between reduced prostacyclin production and diabetic retinopathy.

Thrombocytopenic purpura (TTP), like diabetes, is associated with formation of microvascular thromboemboli, and a deficiency in prostacyclin production may be responsible for the increased platelet consumption that occurs in TTP. This deficiency is postulated to be secondary to a lack of a "plasma factor" that normally stimulates prostacyclin production. A patient with TTP had an undetectable level of 6-oxo-PGF \(_{1\alpha}\) (<60 pg/ml), whereas the mean value in control subjects was 154 ± 48 pg/ml.

It has been postulated that a deficiency or lack of maturation of prostacyclin synthetase, when combined with elevated levels of endoperoxides and TXA \(_2\), may account for sudden infant death, but there is no experimental evidence to support this hypothesis. Prostacyclin production is significantly lower in umbilical and placental vessels from pre-eclamptic patients than in those from normally pregnant women. An increased prostacyclin production, resulting from an accumulation of the "plasma factor" that stimulates prostacyclin synthesis, has been suggested to explain the hemostatic defect in uremic patients. Patients with Bartter's syndrome excrete in the urine about four times as much 6-oxo-PGF \(_{1\alpha}\) as controls. This has led to the suggestion that overproduction of prostacyclin mediates both the hyperreninemia and the hyporesponsiveness to pressor agents observed in these patients. Finally, enhanced prostacyclin production by blood vessels of spontaneously hypertensive rats has been demonstrated. However, Grose and colleagues have described a diminished excretion of 6-oxo-PGF \(_{1\alpha}\) in the urine of patients with essential hypertension. This could reflect diminished prostacyclin production by the kidney itself, or less likely, by the body as a whole. Thromboxane \(A_2\) produced during ligation of the coronary artery of the dog produces arrhythmias; also, vasoconstriction induced by TXA \(_2\) in the gastric mucosa of the dog produces gastric ulceration. Finally, during transplant rejection there is an increased level of TXB \(_2\) which is excreted in the urine preceding the acute crisis.

**Prostacyclin and Atherosclerosis**

High concentrations of lipid peroxides have been demonstrated in advanced atherosclerotic lesions. Lipid peroxidation induced by free radical formation is known to occur in Vitamin E deficiency, the ageing process, and perhaps also in hyperlipidemia accompanying atherosclerosis. It has been shown that 15-HPAA, a lipid peroxide, is a potent (IC \(_{50}\) 0.48 µg/ml) and selective inhibitor of prostacyclin generation by vessel wall microsomes or by fresh vascular tissue. Other fatty acid peroxides and their methyl esters behave similarly. Accumulation of lipid peroxides in, for example, atheromatous plaques could predispose to thrombus formation by inhibiting generation of prostacyclin by the vessel wall without affecting thromboxane \(A_2\) production by platelets. Moreover, platelet aggregation is induced by 15-HPAA, and this aggregation is not inhibited by adenosine or PGE \(_2\). D'Angelo and coworkers reported that human atheromatous plaques from three patients were incapable of prostacyclin production. Prostacyclin generation by atherosclerotic arterial tissue has been shown to be significantly lower than from normal arterial tissue, but no difference was found between early and advanced atherosclerotic lesions. This suggests that the early "fatty streak" may be a biochemically critical stage of the atherosclerotic process. Bourgain and coworkers, using a model of thrombosis in vivo in the rat, demonstrated that application of 15-HPAA to the outside of mesenteric vessels increased the rate of thrombus formation in response to superfusion with ADP. Interestingly, smooth muscle cells obtained from atherosclerotic lesions and cultured in vitro consistently produce less prostacyclin than normal vascular smooth muscle cells. This effect persists after subculture. In addition, the vitamin E deficient diet leads to an increase in peroxide levels in the aorta and to a decrease in prostacyclin production in vitro. All these results, therefore, suggest that it would be worth exploring whether attempts to reduce lipid peroxide formation by inhibiting peroxidation influence the development of atherosclerosis and arterial thrombosis. Vitamin E acts as an antioxidant and perhaps its empirical use in arterial disease in the past had, in fact, a biochemical rationale. It is important to point out that it has been shown in vitro that human diploid fibroblasts that produce prostacyclin lose the ability to do so during ageing, while the other arachidonic acid metabolites like PGE \(_2\), PGF \(_2\), and thromboxane \(A_2\) increase. In addition, aortic smooth muscle cells obtained from old rats produce less PGI \(_2\) in culture than those obtained from young animals. This is due to a specific decrease in the production of prostacyclin.
prostacyclin synthetase activity since the cyclooxygenase activity was similar in both groups. Similar results have been obtained with fresh swine arteries,138 and investigators using bovine smooth muscle and endothelial cells in vitro have observed that during subculture the ability to generate PGI₂ decreases while PGE₂ formation increases.139 Whether these changes are due to a specific damage of the prostacyclin synthetase due to increased lipid peroxidation with age remains to be investigated.

Raised concentrations of low density lipoprotein (LDL) are regarded as one of the risk factors associated with ischemic heart disease.140-142 Whereas high density lipoprotein (HDL) is thought to protect against the disease.141,142 Nordoy and coworkers143 were the first to show that LDL reduced the release of a prostacyclin-like substance by human endothelial cells. Beitz and Förster144 extended these observations by showing that LDL inhibited, whereas HDL stimulated, prostacyclin synthesis. A mixture of low LDL and HDL also stimulated prostacyclin synthesis. Gryglewski and Szczeklik145 have confirmed that LDL inhibits prostacyclin synthesis. They also analysed lipoproteins taken from a group of hypertensive and found the LDL fraction (but not the HDL) contained lipid peroxides at a concentration several times higher than those in the total serum. Thus, the interesting possibility arises that it is the lipid peroxide associated with LDL that inhibits prostacyclin synthesis.

Cell proliferation in vitro is inhibited by substances that stimulate cyclic AMP formation.146 Cell growth in tissue culture,147 including vascular smooth muscle cell culture,148 is inhibited by PGE₂. Possibly prostacyclin has a role in the regulation of cell growth in the vascular wall. Smooth muscle proliferation in atherosclerotic plaques might be a consequence of inhibition of prostacyclin generation by lipid peroxidation.

**Modification of Fatty Acid Precursors**

Enrichment of the diet with dihomo-γ-linolenic acid, the precursor of monoenoic prostaglandins, has been suggested as a means of preventing thrombosis, since PGG₂ and thromboxane A₂ are not proaggregatory and PGE₂ is antiaggregatory.149 However, feeding rabbits with sufficient dihomo-γ-linolenic acid to elevate its content in tissues does not alter the platelet sensitivity to ADP.150 Since the discovery of prostacyclin it has become apparent that this is not the most rational approach to dietary manipulation, since PG endoperoxides of the '1' series cannot give rise to a prostacyclin. Eicosapentaenoic acid (EPA), on the other hand, gives rise to prostaglandins of the '3' series and when incubated with vascular tissue leads to the release of an antiaggregating substance.151 Synthetic Δ₁⁷ prostacyclin or PGI₂ is as potent an antiaggregating agent as prostacyclin. Thromboxane A₃, in contrast, has a weaker proaggregating activity than TXA₂.151 The fatty acid available for PG biosynthesis in Greenland Eskimos is mainly EPA, unlike that in Caucasians which is mainly arachidonic acid.152 These differences may explain why Eskimos have a low incidence of acute myocardial infarction, low blood cholesterol levels, and an increased tendency to bleed.153 This prolonged bleeding time is related to a reduction in ex vivo platelet aggregability.153 The plasma concentrations of cholesterol, triglyceride, low and very low density lipoprotein (VLDL) are low in Eskimos, whereas that of high density lipoprotein is high.154

Eicosapentaenoic acid inhibits platelet aggregation in platelet-rich plasma stimulated by ADP, collagen, arachidonic acid, and a synthetic analogue of PGH₂.155 Also, EPA inhibits aggregation in aspirin and imidazole-treated platelets156 and inhibits thrombin-induced aggregation.157 It is clear, therefore, that both prostaglandin-dependent and independent pathways of platelet aggregation are inhibited by EPA in vitro. In vivo, however, EPA would be incorporated into platelet phospholipids, to some extent replacing arachidonic acid and exerting an anti-thrombotic effect either by competing with remaining arachidonic acid for cyclooxygenase and lipoxygenase158,159 or by being converted to the less proaggregatory PGH₂ and TXA₂.151 Studying seven Caucasians who had been on a mackerel diet for 1 week, Seiss and colleagues160 showed a reduced sensitivity of platelets to collagen, associated with a reduced ability to produce thromboxane B₂, which was dependent on the ratio of C20:5/C20:4 in platelet phospholipids. ADP-induced aggregation was significantly reduced in some subjects and platelet aggregation to exogenously added arachidonic acid was unchanged, indicating normal cyclooxygenase activity. Similarly, Sanders and coworkers162 showed a significant increase in bleeding time of 40% in volunteers who had taken cod liver oil (equivalent to 1.8 g eicosapentaenoic acid) daily for 6 weeks. This was consistent with a decrease in arachidonic acid and an increase in eicosapentaenoic acid in the platelet phospholipids. This diet also led after 6 weeks to a reduction of antithrombin III and blood pressure levels in the volunteers.161 In accordance with these results, Brox and coworkers162 have shown that a supplement of 25 ml of cod liver oil to the diet of normal volunteers leads to a decreased platelet aggregability and a decrease in the formation of TXB₂ during ex vivo platelet aggregation induced by collagen. So far it is clear that EPA feeding leads to a decrease in platelet aggregability and a reduction in TXB₂ formation during ex vivo platelet aggregation. What is not clear is whether changes in the production of PGI₂ are also present. Some studies in rats163 suggest that there is a reduction in the production of PGI₂ without formation of PGI₃; however, others (Hirai, personal communication) suggest that there is an increase in PGI₂-like activity generated by vascular tissue in vitro.
A recent study on volunteers ingesting between 2–3 g of EPA daily demonstrated a decrease on platelet aggregability and an increase in bleeding time during the diet period. The fact that aspirin ingestion during the diet induced a further increase in bleeding time, led the authors to suggest that EPA might have other effects besides changing PGI₂ or TXA₂ synthesis.

Under normal peroxide levels in vivo, eicosapentaenoic acid is a poor substrate for the cyclooxygenase but increasing peroxide tone in an incubate containing purified cyclooxygenase enzyme increases the conversion of eicosapentaenoic acid considerably. Incubation of platelet-rich plasma with EPA does not induce the generation of a thromboxane-like material; indeed, it prevents the formation of thromboxane A₂ induced by arachidonic acid or by collagen. Conversely, in human umbilical vasculature, Dyerberg and Jorgensen demonstrated that EPA did not influence the conversion of arachidonic acid to prostacyclin but gave rise to additional synthesis of prostacyclin-like material. Aortic microsomes readily convert PGH₃ to Δ17-6-keto-PGF₁α but formation of this metabolite or Δ17 prostacyclin from exogenous or endogenous EPA in vivo has yet to be confirmed.

Fish oil fed to cats and dogs increased the amount of 20:5 (n = 3) fatty acids present in heart and liver of the cats and the platelets of the dogs. Brain infarct volume after experimentally-induced cerebral ischemia and the neurological deficit was less in cats fed fish oil than in a corresponding control group. In dogs fed fish oil, thrombosis and subsequent infarct size (3% compared to 25% in control group) induced by electrical stimulation was reduced with less than 30% ectopic beats after 19 hours compared to 80% in the control group at the same time.

The prolonged bleeding time in Eskimos is reduced after aspirin ingestion, suggesting a decreased thromboxane synthesizing capacity coupled with normal or possibly elevated prostacyclin production. Overall, then, the present evidence suggests that it is well worthwhile continuing to study the effects of EPA in man.

**Therapeutic Potential of Prostacyclin**

Clinical assessment of prostacyclin is still in its infancy, with many trials in progress. Open studies and individual case reports have been described where both the platelet inhibitory activity and vasodilator properties of prostacyclin have been utilized. The results in many cases are therefore preliminary, but nevertheless they point the way to conditions in which prostacyclin therapy may be useful. Szczeklik and colleagues have reported striking and prolonged benefits following intraarterial infusion of prostacyclin in five patients with advanced atherosclerotic lower limb peripheral vascular disease. Resting pain disappeared, previously refractory ulcers healed, and the muscle blood flow, as measured by xenon clearance, was significantly increased for at least 6 weeks after prostacyclin infusion. This group has now reported 55 patients with advanced peripheral arterial disease of the lower extremities. Their results indicate that successful treatment with prostacyclin depends upon the localization of the vascular lesions and on the advancement of the disease. Other reports also suggest that prostacyclin may have beneficial effects in peripheral artery disease and that these effects might be very prolonged. When prostacyclin was infused into three patients with sudden blockage of central retinal veins, improvement was observed in those two patients who were treated within the first 48 hours.

Prostacyclin has been successfully used in cases of pulmonary hypertension. One, an 8-year-old girl with severe idiopathic pulmonary artery hypertension, received a prostacyclin infusion of 8–44 ng/kg/min. Pulmonary vascular resistance was reduced during the period of infusion, and no adverse effects were reported. In a group of patients with pulmonary hypertension secondary to mitral valve stenosis, prostacyclin caused a dose-dependent pulmonary vasodilatation with no observed side effects. In both of these studies, prostacyclin was shown to be more effective than PGE₁.

Bergman and colleagues gave an intravenous infusion of prostacyclin to patients with coronary artery disease and showed that doses between 2 and 8 ng/kg/min for 10 minutes had no deleterious effects. Heart rate and cardiac index were increased and mean blood pressure, systemic and pulmonary resistance all fell. Mean atrial pacing time to angina rose from 142 to 241 seconds. They concluded that acute administration of prostacyclin was beneficial in angina, having effects similar to short-acting nitrates. Hall and Dewar concluded from their study of five patients with coronary artery disease that prostacyclin can safely be infused directly into diseased coronary arteries, and Szczeklik and Gryglewski found a beneficial effect of intravenous prostacyclin infusions in patients with unstable angina.

A prostacyclin deficiency has been reported in thrombotic thrombocytopenic purpura. However, infusion of prostacyclin into two patients with TTP did not produce an increase in circulating platelet count. On the other hand, FitzGerald and colleagues have reported an increase in platelet count and an improvement in the neurological status of one such patient during 18 days of prostacyclin infusion. They were sufficiently encouraged to conclude that the controlled evaluation of prostacyclin in TTP was warranted.

Prostacyclin has already been shown to prevent the platelet damage, formation of microemboli, and thrombocytopenia frequently associated with the circulation of blood through extracorporeal systems. Infusion of prostacyclin into animals subjected to
renal dialysis, charcoal hemoperfusion, and cardiopulmonary bypass increases the biocompatibility of the procedure and reduces the requirement for heparin, thus avoiding the complications of thrombocytopenia and thromboembolic episodes that sometimes accompany the use of heparin. Prostacyclin has now been used in patients undergoing cardiopulmonary bypass, charcoal hemoperfusion, and hemodialysis with the same beneficial results.

Clearly, there are many clinical conditions that may respond to prostacyclin treatment, and its place in therapeutics (or that of stable analogues) will be defined in the next few years. Some of these conditions are preeclamptic toxemia, hemolytic uremic syndrome, peptic ulceration, the thrombotic complications associated with transplant rejection, the treatment of thromboembolism, and, as suggested recently, the prevention of tumor metastasis.

Prostacyclin and the Future Development of Antithrombotic Therapy

Until recently, antithrombotic substances fell into four categories: 1) drugs that affect the cyclic AMP levels in platelets, such as diprydamole, which inhibits the platelet phosphodiesterase, and the stimulators of adenylate cyclase, such as PGE,


Hlggs GA, Moncada S, Vane JR. Prostaglandins in vivo. Increasing concentrations of prostacyclin produce an increasing inhibition of platelet activation progressing from inhibition of platelet aggregation to inhibition of adhesion. This will permit the study of the physiology and pathophysiology of platelet vessel wall interactions and as a consequence may lead to a more rational antithrombotic therapy.

As far as atherosclerosis is concerned, the availability of compounds with strong antiplatelet activity will allow an in-depth investigation into the role of platelets in the development of atherosclerosis. In any event, we can say that the discovery of prostacyclin, besides giving a new insight into platelet vessel wall interactions, has provided a tool to investigate pathological conditions like thrombosis and atherosclerosis and will in the future lead to the development of powerful antiplatelet agents.

A great effort is being made in an attempt to obtain a stable analogue with fewer cardiovascular effects than prostacyclin itself (for review see reference 197). If this is achieved, orally active compounds will be available in the future. From a theoretical point of view, a more comprehensive approach to the control of platelet aggregation is to increase 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 both in preventing aggregation and increasing platelet cAMP, prostacyclin, or an analogue, alone or in combination with a phosphodiesterase inhibitor should provide a better control of platelet aggregation in vivo. Increasing concentrations of prostacyclin produce an increasing inhibition of platelet activation progressing from inhibition of platelet aggregation to inhibition of adhesion. This will permit the study of the physiology and pathophysiology of platelet vessel wall interactions and as a consequence may lead to a more rational antithrombotic therapy.

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