Br. J. Pharmac. (1982), **76**, 003–031

EIGHTH GADDUM MEMORIAL LECTURE
UNIVERSITY OF LONDON INSTITUTE OF EDUCATION
DECEMBER 1980

BIOLOGICAL IMPORTANCE OF PROSTACYCLIN

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Introduction

Throughout Sir John Henry Gaddum's highly productive scientific life, two main interests are clearly discernible. One is the study of pharmacologically active substances obtained from tissues and the other is bioassay. Amongst his many contributions to science are, the discovery with von Euler of substance P (von Euler & Gaddum, 1931), with Chang, the demonstration that the sympathetic chain is rich in a substance with the physiological properties of acetylcholine (Chang & Gaddum, 1933) and with Feldberg the demonstration of the release of acetylcholine from stimulated superior cervical ganglion (Feldberg & Gaddum, 1934). The identification of these substances was made mainly by studying their biological activity *in vivo* preparations but often also by studying their effect on isolated pieces of smooth muscle. By the appropriate combination of several biological techniques it was possible to differentiate between adrenaline and noradrenaline (Gaddum, Peart & Vogt, 1949). Indeed, Gaddum maintained that the strongest evidence of identification depends on parallel pharmacological assays. Twenty years ago he also recognized that: 'New chemical methods have been described in recent years which are a great improvement on older methods, and there is little doubt that as time goes on biological methods of assay will be less used, but they are still important and chemical methods will only acquire universal confidence if they are shown to give the same results as biological methods' (Gaddum, 1959). It is difficult to think of a more appropriate sentence to introduce this VIIIth Gaddum Memorial Lecture.

Gaddum never worked on fatty acid metabolism, but most of the important developments in the area of prostaglandin research, including their discovery (Goldblatt, 1933; von Euler, 1934), were made by bioassay procedures.. Bioassay has provided crucial information on the role of the lungs in the removal of prostaglandins (Ferreira & Vane, 1967), the participation of prostaglandins in the inflammatory process (Willis, 1969), the contribution of prostaglandins to the autoregulation and maintenance of blood flow in the kidney (Lonigro, Itskovitz, Crowshaw & McGiff, 1973), the inhibitory effect of aspirin-like drugs on the biosynthesis of prostaglandins (Vane, 1971), the mediation of pyrogen fever by prostaglandins (Feldberg, Gupta, Milton & Wendlandt, 1973), and the release of rabbit aorta-contracting substances (RCS – now identified as thromboxane A_2) (Piper & Vane, 1969) from lungs during anaphylaxis. The discovery of prostacyclin arose from a series of observations made in the course of bioassay studies of extracts obtained from tissues, mainly platelets and vascular tissues (Moncada, Gryglewski, Bunting & Vane, 1976a). Later developments, using a combination of pharmacological and other studies, have led in the very short time of 6 years to the production of a drug suitable for use in human therapy. We have been fortunate to follow all the processes from the initial observations to the first clinical application, and this is surely a most rewarding experience for a pharmacologist.

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 (C18:2ω6). Arachidonic acid is liberated from membrane phospholipids by the action of phospholipases, activated by changes in their chemical environment (Vonkeman & van Dorp, 1968; Flower & Blackwell, 1976). Simple mechanical stimulation can result in generation of prostaglan-

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dins, as shown in lung (Piper & Vane, 1971), spleen (Ferreira & Vane, 1967; Gryglewski & Vane, 1972), and platelets (Salzman, Lindon & Rodvien, 1976; see also Flower, 1978). 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 (Christ & van Dorp, 1972).

Much of the earlier work concentrated on prostaglandins E_2 and $F_{2\alpha}$ as these possess potent and diverse biological activities and were available in pure form. Since 1973, important discoveries have been made about the unstable intermediates in arachidonic acid metabolism (Figure 1). These include the prostaglandin endoperoxides (PGG_2 and PGH_2), thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) and have potent

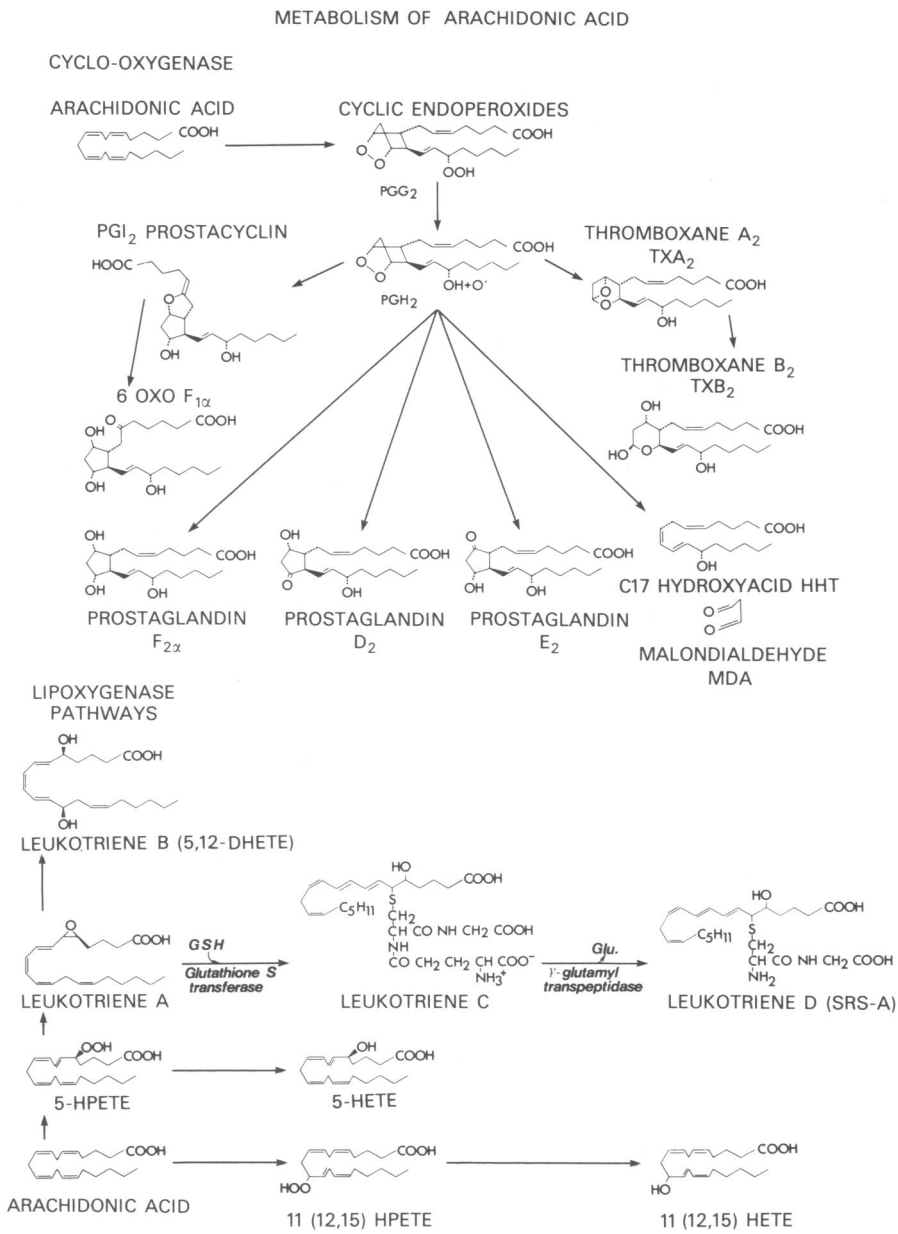

Figure 1 Metabolic pathways of arachidonic acid.

biological activities. Investigations into the sites of production of these compounds and their differing, in some cases opposing, biological activities have led to the development of new concepts in vascular homeostasis.

Bioassay procedures have played an important part in these developments. Indeed, it is unlikely that any of the unstable products of arachidonic acid metabolism would have been discovered or their biological effects recognized without bioassay techniques. Addition to the now classical bioassay superfusion technique developed by Vane (1964, 1969) of new bioassay tissues such as the rabbit coeliac and mesenteric artery strips (Bunting, Moncada & Vane, 1976a) and the bovine coronary artery (Kulkarni, Roberts & Needleman, 1976) and the adaptation of the technique to the fast assay of samples from biological fluids (Moncada, Ferreira & Vane, 1978) greatly contributed to the furtherance of this work and created the conditions for the discovery of prostacyclin. This final step highlighted one of the main advantages of bioassay which is that it measures biological activity (a seemingly tautologous statement) and by so doing creates an unusually high opportunity for serendipity (Figure 2).

Metabolic pathways

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 cyclo-oxygenase forms the prostaglandin endoperoxide PGG_2 (Hamberg, Svensson & Samuelsson, 1974a). This is converted to PGH_2 which then isomerizes enzymatically or non-enzymatically to the stable substances PGE_2, $PGF_{2\alpha}$, and PGD_2 (see 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 enzymatically into two other unstable products, prostacyclin and TXA_2. Unlike PGE_2, D_2 or $F_{2\alpha}$, these products are not formed by chemical breakdown of PGH_2. The cyclo-oxygenase is inhibited by aspirin-like drugs leading to the hypothesis (Vane, 1971) that the therapeutic as well as the shared side-effects of aspirin-like drugs are related to inhibition of prostaglandin biosynthesis. Since then, a great deal of evidence has accumulated in favour of this proposal (see Higgs, Moncada & Vane, 1980).

The lipoxygenase leads to the formation of eicosanoids which are non-cyclized hydroxy acids; 11 and 15-hydroxyeicosatetraenoic acid may be formed as the result of an incomplete cyclo-oxygenase reaction, but in 1974 a separate lipoxygenase was discovered in platelets which synthesizes 12-HETE (Hamberg & Samuelsson, 1974a; Nugteren, 1975). The synthesis of 5-HETE by polymorphonuclear leukocytes (PMNs) has also been described (Borgeat, Hamberg & Samuelsson, 1976). Formation of these mono-hydroxy 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; Murphy, Hammarström & Samuelsson, 1979). Leukotriene A_4 is a 5,6 epoxide of arachidonic acid which can be converted to the 5,12-dihydroxy acid (5,12 DHETE; leukotriene B_4; LTB_4). Alternatively, the addition of glutathione to the epoxide by glutathione-S-transferase results in the formation of LTC_4. The removal of glutamate from LTC_4 by γ-glutamyl transpeptidase gives LTD_4 (Figure 1) which is further metabolized to LTE_4 with the loss of glycine (Samuelsson & Hammarström, 1980; for review see Samuelsson, Hammarström, Murphy & Borgeat, 1980). These lipid-peptide structures account for the biological activity of slow reacting substances (SRSs) detected in immediate hypersensitivity reactions (Austen, 1978). LTD_4 may be identical to the SRS produced in anaphylactic responses (SRS-A; Morris, Taylor, Piper, Samhoun & Tippins, 1980) but it is more likely that SRS-A is a mixture of leukotrienes. SRS-A is thought to contribute to the bronchoconstriction seen in anaphylactic respiratory diseases of man.

The enzyme cyclo-oxygenase (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 (Hamberg & Samuelsson, 1974a; Hamberg *et al.*, 1974a; Nugteren, 1975; Herman, Claeys, Moncada & Vane, 1978).

Thromboxane synthetase

In 1974, Willis & Kuhn showed that short term incubations of arachidonic acid with sheep vesicular gland generated an unstable principle which induced platelet aggregation. They called this principle, whose generation was inhibited by aspirin, labile aggregation stimulating substance or 'LASS'. They went on to describe the isolation, purification and some biological properties of LASS which made it indistinguishable from PGH_2 (Willis, Vane, Kuhn, Scott & Petrin, 1974). Hamberg and co-workers showed at approximately the same time that purified

preparations of PGG_2 were strong inducers of platelet aggregation and that during aggregation induced by other agents like thrombin, prostaglandin endoperoxides were generated (Hamberg, Svensson, Wakabayashi & Samuelsson, 1974b). A similar report was made by Smith, Ingerman, Kocsis & Silver (1974).

Both prostaglandin endoperoxides, PGG_2 and

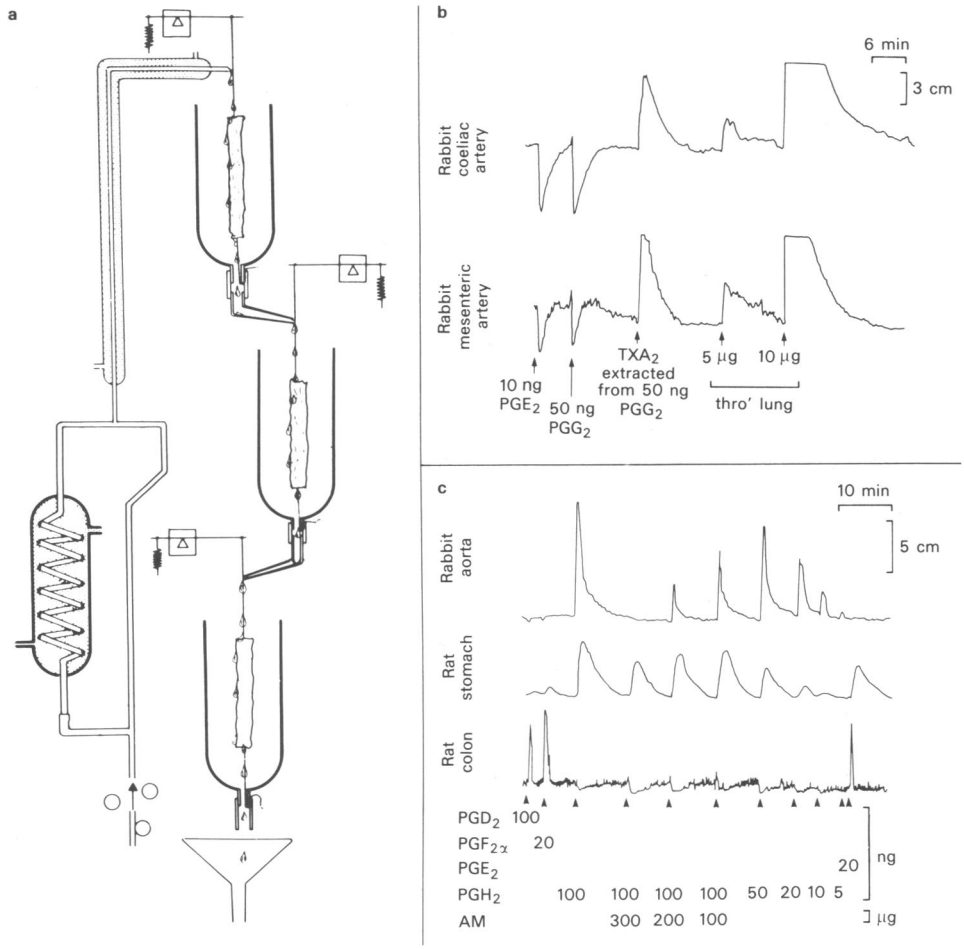

Figure 2 (a) This is a diagrammatic representation of the cascade bioassay technique (see Vane, 1964; 1969). The choice of bioassay tissues depends on the substance to be studied. In (b) a rabbit coeliac and a mesenteric artery are used for the bioassay of a prostaglandin endoperoxide (PGG_2) and thromboxane A_2. The cascade in this case was installed below a pair of isolated lungs of the guinea-pig (for details see Piper & Vane, 1969; Bunting *et al.*, 1976a). Prostaglandin E_2 (PGE_2) induces relaxation of both tissues; the prostaglandin endoperoxide (PGG_2) induces a short lasting contraction followed by relaxation. However, thromboxane A_2 (TXA_2) generated from the incubation of PGG_2 and platelet microsomes contracted both tissues. Challenging the guinea-pig lungs with different doses of arachidonic acid (AA) led to the release of a substance with a similar activity to TXA_2 on the assay tissues. (c) Shows an assay using a rabbit aortic strip, a rat stomach and a rat colon. These were used during the original experiments which led to the discovery of prostacyclin. Prostaglandin D_2 (PGD_2) contracts the rat colon with little activity on the other tissues. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) contracts the rat colon and has a slight effect on the other tissues. The prostaglandin endoperoxide (PGH_2) contracts the rabbit aorta and the rat stomach strip but has little activity on the rat colon. The next three responses are to the product of incubations of 100 ng of PGH_2 with different concentrations of aortic microsomes (AM). This shows the production of a substance which contracts the rat stomach strip and relaxes the rat colon, a profile unlike the prostaglandin endoperoxide, $PGF_{2\alpha}$, PGD_2 and PGE_2 which is shown at the end of the tracing to contract the rat stomach strip and the rat colon (for details see Moncada *et al.*, 1976a; Gryglewski *et al.*, 1976).

PGH_2, were shown by Hamberg *et al.* (1974b) to induce platelet aggregation. This was accompanied by the formation of an unstable vasoconstrictor substance identified as thromboxane A_2 (Hamberg, Svensson & Samuelsson, 1975). Thromboxane A_2 has a chemical half life of 30 s at body pH and temperature. The activity of RCS, described first by Piper & Vane (1969), can be accounted for by TXA_2 (see later and Figure 2). The enzyme thromboxane synthetase which generates TXA_2 from prostaglandin endoperoxides was first localized in the high speed particulate fraction of human and horse blood platelets (Needleman, Moncada, Bunting, Vane, Hamberg & Samuelsson, 1976; Moncada, Needleman, Bunting & Vane, 1976b). The enzyme has been solubilized and separated from the cyclo-oxygenase (Diczfalusy, Falardeau and Hammarström, 1977; Hammarström & Falardeau, 1977; Yoshimoto, Yamamoto, Okuma & Hayaishi, 1977) and detailed studies of human and bovine platelet thromboxane synthetase have been published (Ho, Walters & Sullivan, 1976; White & Glassman, 1976; Sun, 1977).

The involvement of the cyclic endoperoxides and thromboxane A_2 in platelet aggregation provided for the first time an explanation of the fact that aspirin-like drugs inhibit the second phase of platelet aggregation *in vitro*. Previously, it had been impossible to explain why inhibitors of prostaglandin biosynthesis also inhibited platelet aggregation, for PGE_2 and $PGF_{2\alpha}$ have little or no proaggregatory activity.

Other cells capable of synthesizing TXA_2 include rabbit and human polymorphonuclear leukocytes (Higgs, Bunting, Moncada & Vane, 1976; Goldstein, Malmsten, Kaplan, Kindahl, Samuelsson & Weissman, 1977), mouse, rat and guinea-pig macrophages (Brune, Glatt, Kalin & Peskar, 1978; Murota, Kawamura & Morita, 1978) and human lung fibroblasts (Hopkins, Sun & Gorman, 1978). Tissues capable of generating TXA_2 include rabbit and cat spleen (Nijkamp, Moncada, White & Vane, 1977), rabbit iris and conjunctiva (Bhattacherjee, Kulkarni & Eakins, 1979), guinea-pig lung (Hamberg & Samuelsson, 1974b), and rabbit and rat kidney (Zenser, Herman, Gorman & Davis, 1977; Morrison, Nishikawa & Needleman, 1978) although the exact location or cellular type possessing TXA_2-synthetase in these tissues is not known. In 1976 Tuvemo and colleagues showed that human umbilical artery produces TXA_2 (Tuvemo, Strandberg, Hamberg & Samuelsson, 1976). Since then, several reports of production of TXA_2 by vascular tissue have appeared. TXA_2 is formed by the rabbit pulmonary artery (Salzman, Salmon & Moncada, 1980) and two reports suggest that it may be produced by vascular endothelial cells in culture (Ali, Barrett & Eling, 1980; Ingerman-Wojenski, Silver, Smith & Macarak, 1981). Interestingly, it has been shown (Morrison *et al.*, 1978) that kidneys made hydronephrotic begin to synthesize TXA_2, suggesting the possibility that tissue damage unmasks in some way the ability to form this substance. Whether this also happens in other tissues remains to be investigated. It should, however, also be considered that in some instances the production of TXA_2 by tissues is due to the presence of platelets or migratory cells.

Although MDA and HHT can be formed non-enzymatically by spontaneous degradation of PGH_2 (Hammarström & Falardeau, 1977), the biosynthetic pathways of TXA_2 and HHT are linked, for a purified enzyme that synthesizes TXA_2 also catalyses HHT formation (Diczfalusy *et al.*, 1977; Yoshimoto *et al.*, 1977).

Prostacyclin synthetase

Prostacyclin is generated by blood vessel microsomes or fresh vascular tissue from the prostaglandin endoperoxide, PGH_2 (Moncada *et al.*, 1976a; Bunting, Gryglewski, Moncada & Vane, 1976b). It is chemically unstable with a half life of 2–3 min breaking down to 6-oxo-$PGF_{1\alpha}$. Prostacyclin synthetase has a broad pH optimum, and catalyses the rapid conversion of saturating concentrations of PGH_2 at 37°C (Salmon, Smith, Flower, Moncada & Vane, 1978). Wlodawer & Hammarström (1979) demonstrated that prostacyclin synthetase in porcine aorta microsomes was stimulated by the high-speed supernatant and that the soluble factor was non-dialysable and resistant to boiling. Various tissues have been shown to generate prostacyclin or its stable breakdown product 6-oxo-$PGF_{1\alpha}$ (for review see Moncada & Vane, 1979).

Discovery of prostacyclin

In October 1975 in collaboration with S. Bunting, R. Gryglewski and J.R. Vane, we looked for the presence of thromboxane synthetase in vascular tissue.

Firstly we wanted to find whether thromboxane generation in the vasculature would synergize with platelet TXA_2 and give us a better understanding of the haemostatic plug formation, especially of the immediate vasoconstriction which follows the cutting of a small vessel. Secondly, the suggestion that platelets and vascular tissue shared some antigenic properties (Morrison & Baldini, 1969), led us to believe that the two structures could share some enzymic proteins.

However, the work showed in its early stages that TXA_2 was not formed. Using the cascade bioassay technique (Vane, 1964) and the enhancement of endoperoxide-induced rabbit aorta contracting ac-

tivity as criteria for TXA_2 formation (Moncada *et al.*, 1976b; Needleman *et al.*, 1976), we showed that, although TXA_2 was not formed, the endoperoxide precursor was consumed enzymatically into an unknown product not identifiable by the standard bioassay tissues (see Figure 2 for bioassay of PGI_2). The substance resulting from this enzymatic activity was labile and relaxed the coeliac and mesenteric arteries of the rabbit. We began to refer to it as PGX (Moncada *et al.*, 1976a). Later, we tested PGX as an inhibitor of platelet aggregation and demonstrated that it was many times more active than PGE_1 or PGD_2 (Gryglewski, Bunting, Moncada, Flower & Vane, 1976).

PGX relaxed vascular strips *in vitro*, caused vasodilatation, was the most potent inhibitor of platelet aggregation yet discovered and possessed antithrombotic properties. Furthermore, it was the major metabolite of arachidonic acid in vascular tissue (for review see Moncada & Vane, 1979). PGX was shown to be the unstable intermediate in the formation of 6-oxo-$PGF_{1\alpha}$, a compound described at about that time by Pace-Asciak (1976) as a product of prostaglandin endoperoxides in the rat stomach. The structure of PGX was elucidated in a collaboration between scientists from our laboratory and scientists from the Upjohn Company at Kalamazoo (Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada & Vane, 1976). PGX was then renamed prostacyclin with the abbreviation of PGI_2.

Generation by the vessel wall

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 tested (for these and other actions of prostacyclin on the cardiovascular system, see Moncada & Vane, 1979). Not much is known about the microcirculation but Goehlert, Ng Ying Kin & Wolfe (1981) 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 towards the adventitia (Moncada, Herman, Higgs & Vane, 1977). Production of prostacyclin by cultured cells from vessel walls also shows that endothelial cells are the most active producers of prostacyclin (Weksler, Knapp & Jaffe, 1977; MacIntyre, Pearson & Gordon, 1978); moreover, this production persists after numerous subcultures *in vitro* (Christofinis, Moncada, Bunting & Vane, 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.*, 1976a). Later it was shown that fresh vascular tissue could use both precursors although the endoperoxides are much better substrates (Bunting *et al.*, 1976b). 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.*, 1976b; Gryglewski *et al.*, 1976; Bunting, Moncada & Vane, 1977). 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 (Gryglewski *et al.*, 1976; Moncada *et al.*, 1976a; Salmon *et al.*, 1978). 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 co-operation between platelet and vessel wall (Moncada & Vane, 1978). Several observations support this conclusion. Incubation of platelet-rich plasma (PRP) with fresh, indomethacin-treated arterial tissue leads to an increase in platelet cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (Best, Martin, Russell & Preston, 1977) 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 (Gryglewski *et al.*, 1976). Futhermore, Tansik, Namm & White (1978) 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 PGH_2 to prostacyclin (Marcus, Weksler & Jaffe, 1978).

However, the hypothesis was challenged by Needleman and associates (Needleman, Bronson, Wyche, Sivakoff & Nicolaou, 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 was necessary for the endoperoxide to be utilized by prostacyclin synthetase. Needleman, Wyche & Raz (1979) and Hornstra, Haddeman & Don (1979) 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 (Marcus, Broekman, Weksler, Jaffe, Safier,

Ullman & Tack-Goldman, 1981). Too high a platelet concentration induces a platelet to platelet interaction which 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 'co-operation'. This proposal has still to be verified *in vivo* and as will be seen later, the development of anti-thrombotic compounds based on selective inhibition of TXA_2 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 TXA_2 (Higgs *et al.*, 1976; Goldstein *et al.*, 1977; Davidson, Ford-Hutchinson, Smith & Walker, 1978), could interact with the vessel wall to promote formation of prostacyclin. Moreover, leukocytes themselves generate prostacyclin in whole blood, especially in the presence of thromboxane synthetase inhibitors (Flower & Cardinal, 1979). Thus, prostacyclin might regulate white cell behaviour and help control white cell activity during the inflammatory response (Weksler *et al.*, 1977; Higgs, Moncada & Vane, 1978a).

Using fresh human vascular tissue, we did not find any difference between the production of prostacyclin *in vitro* by veins and arteries (Moncada, Higgs & Vane, 1977). No difference in prostaglandin production by veins and arteries had previously been detected in bovine vessels (Terragno, Crowshaw, Terragno & McGiff, 1975). However, arteries have been shown to produce more prostacyclin than veins in rabbits, rats and dogs (Skidgel & Printz, 1978; Buchanan, Dejana, Cazenave, Mustard & Hirsch, 1979; Eldor, Hoover, Pett, Gay, Alonso & Weksler, 1981a). In addition, cultured cells obtained from human pulmonary arteries produce more prostacyclin than those obtained from pulmonary veins (Johnson, 1980). 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 (Eldor *et al.*, 1981a). 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 (Sinzinger, Silberbauer, Winter & Auerswald, 1978).

Prostacyclin is the most potent endogenous inhibitor of platelet aggregation yet discovered. It is 30 to 40 times more potent than PGE_1 (Moncada *et al.*, 1976a) 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, Moncada & Vane, 1977) and given systemically to the rabbit it prevents electrically-induced thrombus formation in the carotid artery and increases bleeding time (Ubatuba, Moncada & Vane, 1979). The duration of these effects *in vivo* is short; they disappear within 30 min of administration. Prostacyclin disaggregates platelets *in vitro* (Moncada *et al.*, 1976a; Ubatuba *et al.*, 1979), in extracorporeal circuits where platelet clumps have formed on collagen strips (Gryglewski, Korbut & Ocetkiewicz, 1978), and in the circulation of man (Szczeklik, Gryglewski, Nizankowski, Musial, Pieton & Mruk, 1978a). Moreover, it inhibits thrombus formation in a coronary artery model in the dog when given locally or systemically (Aiken, Gorman & Shebuski, 1979) and protects against sudden death (thought to be due to platelet clumping) induced by intravenous arachidonic acid in rabbits (Bayer, Blass & Förster, 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 3 min (Dusting, Moncada & Vane, 1977; 1978a). It has recently been reported that prostacyclin has an extended stability in plasma and in blood (Gimeno, Sterin-Borda, Borda, Lazzari & Gimeno, 1980) and that this may be associated with binding to albumin or with metabolism to 6-oxo-PGE_1 (Blasko, Nemesanszky, Szabo, Stadler & Palos, 1980). The relevance of these observations to the actual biological activity remains unclear. Alkaline pH increases the stability of prostacyclin (Johnson *et al.*, 1976; Cho & Allen, 1978) so that at pH 10.5 at 25°C, it has a half-life of 100 h. It is stabilized as a pharmaceutical preparation by freeze drying and can be reconstituted for use in man in an alkaline glycine buffer.

Mechanism of action

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

Prostacyclin is not only more potent than PGE_1 in elevating cyclic AMP but the elevation persists

longer. The elevation induced by PGE_1 starts falling after 30 s, while prostacyclin stimulation is not maximal until after 30 s and is maintained for 2 min, after which it gradually wanes over 30 min (Gorman *et al.*, 1977). Prostacyclin is also a strong direct stimulator of adenylate cyclase in isolated membrane preparations (Gorman *et al.*, 1977).

Prostacyclin, PGE_1 and PGD_2 increase adenylate cyclase activity by acting on two distinct receptors on the platelet membrane (Whittle, Moncada & Vane, 1978; Miller & Gorman, 1979). PGE_1 and prostacyclin act on one, whereas PGD_2 acts on another. These and other results suggest that the previously recognized PGE_1 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 TXA_2 it enhances Ca^{2+} sequestration (Kazer-Glanzman, Jakabova, George & Luscher, 1977). Moreover, inhibitory effects on platelet phospholipase (Lapetina, Schmitges, Chandrabose & Cuatrecasas, 1977; Minkes, Stanford, Chi, Roth, Raz, Needleman & Majerus, 1977) and platelet cyclo-oxygenase have been described (Malmsten, Granström & Samuelsson, 1976). All these effects are related to its ability to increase cyclic AMP in platelets. Moreover, prostacyclin inhibits endoperoxide-induced aggregation suggesting additional sites of action, still undefined, but dependent on the cyclic AMP effect (Minkes *et al.*, 1977). 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 cyclic AMP levels in cells other than platelets. These include cultured human fibroblasts (Gorman, Hamilton & Hopkins, 1979), human fat cell ghosts (Kather & Simon, 1979), guinea-pig lung homogenates (Macdermot & Barnes, 1980) and polymorphonuclear leukocytes (Boxer, Allen, Schmidt, Yoder & Baehner, 1980). Thus, there is the possibility that in these cells an interaction with the thromboxane system could lead to a similar regulation of cell behaviour to that observed in platelets, suggesting that the PGI_2/TXA_2 system has a wider biological significance. Indeed, prostacyclin inhibits white cell adherence to the vessel wall (Higgs *et al.*, 1978; Higgs, 1982) to nylon fibres and to endothelial monolayers *in vitro* (Boxer *et al.*, 1980). It has recently been shown (Schafer, Gimbrone & Handin, 1980; Hopkins & Gorman, 1981) that prostacyclin increases cyclic AMP 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 prostacyclin (Willems, van Aken & van Mourik, 1980) and respond to it by changing their deformability (Kovacs & O'Grady, 1982). This again suggests a wider biological role for prostacyclin.

Prostacyclin and platelet/vascular interactions

The anti-aggregating 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 that have no activity on the ADPase system, abolish most if not all of the antiaggregatory activity of vascular endothelial cells (Bunting *et al.*, 1977). Similar results were obtained with an antiserum that cross-reacts with and neutralizes prostacyclin *in vitro* (Bunting, Moncada, Reed, Salmon & Vane, 1978). Endothelial cells pretreated with this antiserum lose the ability to inhibit ADP-induced aggregation (Bunting *et al.*, 1977; Christofinis *et al.*, 1979).

However, it has not been clear to what extent PGI_2 generation is responsible for the thromboresistant properties of vascular endothelium. Dejana and co-workers studied the effect of inhibition of PGI_2 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 (Dejana, Cazenave, Groves, Kinlough-Rathbone, Richardson, Packham & Mustard, 1980).

Similar results have been obtained by Curwen and colleagues using a different preparation (Curwen, Gimbrone & Handin, 1980). In their hands neither treatment of vascular endothelium with aspirin or indomethacin nor increasing PGI_2 production by arachidonic acid affected basal platelet adherence. However, in transformed vascular endothelial cells (obtained after viral infection) there was very little PGI_2 generation and platelet adherence was greatly increased. This could be partially reversed by adding exogenous PGI_2. On the other hand, Czervionke and co-workers, using washed preparations of labelled 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-$PGF_{1\alpha}$ formation from 107 nM to <3 nM and could be reversed by addition of 25 nM of exogenous PGI_2 (Czervionke, Smith, Fry & Hoak, 1979). *In vivo*, and *in vitro*, Baumgartner & Muggli (1974) and Tschopp & Baumgartner (1981) 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 PGI_2 from rats to guinea-pigs. Moreover, there was a negative correlation between the ability of the vascular tissue of a species to produce PGI_2 and the degree of platelet adherence-aggregation that was observed after interaction of the de-endothelialized vascular tissue with the animals' blood *in vivo* (Tschopp & Baumgartner, 1981).

The fact that prostacyclin inhibits platelet aggregation (platelet-platelet interaction) at much lower concentrations than those needed to inhibit adhesion (platelet-collagen interaction) (Higgs, Moncada, Vane, Caen, Michel & Tobelem, 1978b), 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 & Turitto (1979) have observed some degree of inhibition of platelet subendothelium 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 de-endothelialization of the aortae 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 $50-100\,ng\,kg^{-1}\,min^{-1}$. Only concentrations of $650-850\,ng\,kg^{-1}\,min^{-1}$ could inhibit this platelet adhesion (Adelman, Stemerman, Mennell & Handin, 1981).

Using a new method to measure prostacyclin generated by the luminal surface of a vessel, Eldor and colleagues have demonstrated that the vascular endothelium is the only source of PGI_2 generated in the luminal surface of a rabbit aorta (Eldor, Falcone, Hajjar, Minick & Weksler, 1981b). Moreover, after balloon catheter de-endothelialization the capacity for generation of PGI_2 is abolished and only recovers slowly over a period of 70 days, concomitant with the appearance of neo-intimal cells on the vessel surface. The authors also observed in the de-endothelialized areas a 'carpet of platelets' which slowly disappeared during the time of re-endothelialization.

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 Moncada & Vane, 1979). Whether the passage of vascular cells, some of which would be larger than 10 μm in diameter, passing through capillaries of 5 μm or less is enough to stimulate PGI_2 synthesis is still to be investigated.

Stimulation of prostacyclin production

MacIntyre *et al.* (1978) observed that prostacyclin production by cultures of vascular endothelial cells was enhanced by platelet-poor plasma. Later, Defreyn, Vergara Dayden, Machin & Vermylen (1980) confirmed the presence of a factor in plasma which stimulates prostacyclin production and showed that platelet-poor plasma from 6 out of 7 patients with chronic uraemia caused greater stimulation of prostacyclin production by cultured endothelial cells than platelet-poor plasma from control volunteers. They suggested that increased levels of circulating prostacyclin could well contribute to the bleeding tendency frequently encountered in uraemic patients. Conversely, deficiency of a plasma factor regulating prostacyclin has been reported in haemolytic-uraemic syndrome which is associated with widespread thrombotic occlusions (Remuzzi, Misiani, Marchesi, Livio, Mecca, De Gaetano & Donati, 1978).

Thrombin, calcium ionophore A23187 and trypsin all stimulate prostacyclin production by cultured endothelial cells (Weksler, Ley & Jaffe, 1978a). It is interesting that all of these substances cause platelet aggregation and probably act by stimulating phospholipases which liberate free arachidonic acid from cellular phospholipids. The action of thrombin in stimulating prostacyclin production is indeed inhibited by mepacrine, a phospholipase inhibitor (Weksler, Ley & Jaffe, 1978b). Generation of thrombin and subsequent stimulation of prostacyclin formation at a site of vascular damage and haemostatic plug formation may be a controlling factor limiting thrombus size (Weksler *et al.*, 1978a). Trypsin, but not chymotrypsin, stimulates prostacyclin production by cultured cells, an effect inhibited by soybean trypsin inhibitor (Weksler *et al.*, 1978a). Release of trypsin-like enzymes by both platelets and white cells has been proposed as a mechanism by which prostacyclin production by the vessel wall might be regulated (Weksler, Reinus & Eldor, 1981). Investigating the prostacyclin-stimulating activity of serum, Coughlin and co-workers concluded that at least 80% of the activity was derived from platelets (Coughlin, Moskowitz, Zeiter, Antoniades & Levine, 1980). The platelet-dependent activity was non-dialysable, stable at 56°C, and at least partially stable at 100°C. Because these properties were similar to those of platelet-derived growth factor (PDGF) and PDGF had been proposed as the substance present in serum capable of activating phospholipase A_2 in Swiss mouse 3T3 fibroblasts (Shier, 1980) they tested PDGF for its ability to stimulate prostacyclin production in bovine cell cultures. PDGF reversibly stimulated prostacyclin production and at a concentration of 8.0 ng/ml stimulated synthesis by 74 fold in aortic endothelial cells, 84 fold in smooth muscle and

15 fold in capillary endothelial cell cultures. The mitogenic activity of PDGF was only observed with smooth muscle cells indicating dissociation between the two activities (Coughlin *et al.*, 1980). Control of prostacyclin production is possible, therefore, by a variety of agents acting locally at the level of the cell-vessel wall interaction but the precise mechanisms acting at any time have yet to be clarified.

Stimulation of prostacyclin release from the vessel wall by an antithrombotic compound Bay g 6575 has been reported (Vermylen, Chamone & Verstraete, 1979) and confirmed (Carreras, Chamone, Klerckx & Vermylen, 1980). Bendrofluazide used to treat patients with essential hypertension caused significant increases in circulating levels of 6-oxo-$PGF_{1\alpha}$ suggesting a possible contribution of prostacyclin to the reduction in peripheral resistance seen with thiazide treatment (Webster, Dollery & Hensby, 1980). Similarly, the vasodilator nitroglycerine in clinically attainable concentrations enhances prostacyclin production by cultured human endothelial cells (Levin, Jaffe, Weksler & Tack-Goldman, 1981). Finally, local or circulating vasoactive substances like bradykinin and angiotensin II also induce prostacyclin release *in vivo* and *in vitro* (Gryglewski, 1979; Swies, Radomski & Gryglewski, 1979; Mullane & Moncada, 1980; Dusting & Mullins, 1980).

Is prostacyclin acting as a circulating substance?

Using dogs, we showed by direct bioassay in circulating blood that prostacyclin escapes the pulmonary inactivation process (Dusting *et al.*, 1977) which normally removes 95% or more of PGE_2 and $PGF_{2\alpha}$ in a single circulation *in vivo*. Thus, prostacyclin can recirculate (Dusting *et al.*, 1978a). Furthermore, infused arachidonic acid is converted into prostacyclin in passage across the lungs *in vivo* (Dusting, Moncada & Vane, 1978b). Therefore, prostacyclin generated in the lung or elsewhere would not be confined to a local site of action, and is potentially a circulating hormone.

Gryglewski *et al.* (1978) modified the blood bathed organ technique (Vane, 1969) to measure continuously platelet aggregation on to tendon strips bathed in the circulating blood of anaesthetized cats, and showed that arterial blood contained higher concentrations of an antiaggregatory substance than mixed venous blood. They concluded that the arterial/venous difference was due to prostacyclin released from the lungs. 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; 1978a). Moncada, Korbut, Bunting & Vane (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 PGI_2 (PGI_1), which cross reacts with prostacyclin. Moreover, the PGI_2-like, disaggregatory substance in arterial blood is increased during hyperventilation of the lungs, or during pulmonary embolism induced by intravenous injection of air (Gryglewski, 1979). Respiratory stimulants, such as almitrine, also increase the release of prostacyclin by the lungs (Gryglewski, 1980). Vasoconstriction of rat isolated lungs also led to increased prostacyclin release (Voelkel, Gerber, McMurtry, Nies & Reeves, 1981).

Some reservations about extending the concept that prostacyclin is normally a circulating hormone should be mentioned. Firstly, in the studies with anaesthetized cats and rabbits, blood was drawn through an extracorporeal circuit with a peristaltic pump. Under such conditions, the circulating blood volume would be reduced slightly and this may lead to a stimulation of the renin-angiotensin system, which in turn could stimulate prostacyclin release (see below). Secondly, it is now well recognized that surgical procedures in anaesthetized small animals can exaggerate the contribution of prostaglandins to renal homeostasis (Terragno, Terragno & McGiff, 1977) and by analogy, the same may be true for the lungs. Thirdly, in these extracorporeal experiments, platelet emboli dislodged from the tendon strips return to the animal in the venous blood. The trapping of platelet emboli in the lungs may be another 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. Recent studies of human platelet aggregation performed within 3 min of withdrawal of arterial or venous blood (Steer, MacIntyre, Levine & Salzman, 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 bioassay tissues sensitive to prostacyclin (Neri Serneri, Masotti, Poggesi & Galanti, 1980). The level rose by several ng/ml with relief of ischaemia and was reduced by pretreatment with indomethacin.

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 in arterial blood were obtained (Hensby, Barnes, Dollery & Dargie, 1979a). Although these

levels are lower than those measured by bioassay they are still much higher than those obtained by measuring the daily turnover in urine of a metabolite of prostacyclin (Oates, Falardeau, FitzGerald, Branch & Brash, 1981). However, recent estimates of prostacyclin production using a more sensitive and specific assay have shown that the 6-oxo-$PGF_{1\alpha}$ level in normal, healthy individuals was two orders of magnitude lower than previously estimated by mass spectrometry (Blair, Barrow, Waddell, Lewis & Dollery, 1982). Recent work by Haslam & McClenaghan (1981) has also shown extremely low levels of circulating prostacyclin in rabbits.

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. Further work is necessary to establish clearly the routes of catabolism of both prostacyclin and 6-oxo-$PGF_{1\alpha}$ in the human circulation and to determine whether there is an effective level of circulating prostacyclin in normal man at rest or during exercise. There are several indications that detectable levels of prostacyclin or a prostacyclin-like activity in concentrations above those necessary to inhibit platelet aggregation occur in dogs during pregnancy (Gerber, Payne, Murphy & Nies, 1981) and in humans suffering from Bartter's syndrome (Gullner, Cerletti, Bartter, Smith & Gill, 1979).

Aspirin, haemostasis and thrombosis

Aspirin binds covalently to the active site of cyclo-oxygenase and therefore inhibits the enzyme in platelets for their entire lifespan because platelets are unable to synthesize new protein (Steiner, 1970). 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 favour 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 re-examine the use of aspirin as an anti-thrombotic compound. Two important considerations have emerged in relation to aspirin. (1) Inhibition of the vascular cyclo-oxygenase, unlike the platelet cyclo-oxygenase, may persist for a much shorter period because of the generation of new enzyme, and (2) The platelet cyclo-oxygenase seems to be more sensitive *in vitro* and *in vivo* than the vessel wall cyclo-oxygenase to the inhibitory action of aspirin (Burch, Baenziger, Stanford & Majerus, 1978) although reports to the contrary have been published (Jaffe & Weksler, 1979).

In addition, 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 (Amezcua, Parsons & Moncada, 1978; Korbut & Moncada, 1978). 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 (O'Grady & Moncada, 1978; Rajah, Penny & Kester, 1978) but not by others (Godal, Eika, Dybdahl, Daae & Larsen, 1979). This discrepancy may be due to the fact that the volunteers in the study by O'Grady & Moncada (1978) were in a younger age-group than those in the study by Godal *et al.* (1979). Subsequently, Jorgensen, Dyerberg, Olesen & Stoffersen (1980) 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 28 to 32 years but not in the age group 66 to 70 years. These findings are consistent with a decrease in prostacyclin production with age (see later).

Amezcua and co-workers showed that following a single high oral dose of aspirin (3.9 g) the bleeding time is unchanged 2 h after ingestion, although marked inhibition of platelet function and thromboxane A_2 release is demonstrable. Twenty-four and 72 h after aspirin, the bleeding time increased substantially, returning to normal levels one week after treatment (Amezcua, O'Grady, Salmon & Moncada, 1979). This suggests that shortly after high-dose aspirin the production of prostacyclin and thromboxane A_2 was blocked. As the recovery of the endothelial cyclo-oxygenase proceeded faster than the release of new platelets, the ratio of prostacyclin to thromboxane A_2 was changed in favour of prostacyclin at 24 and 72 h after treatment, thus explaining the increased bleeding time and its slow recovery over one week. Aspirin 0.3 g daily and 0.6 g twice daily administered for seven days both prolonged the bleeding time measured 2 h after the first and last doses (Treacher, Warlow & McPherson, 1978). However, at the higher dose the bleeding time was significantly shorter after the last as compared to the first aspirin dose and this could reflect inhibition of prostacyclin generation by chronic aspirin dosing. Experimental evidence indicates that aspirin treatment increases thrombus formation in injured veins

and in the microcirculation (Arfors, Bergqvist & Tangen, 1975; Bourgain, 1978; Kelton, Hirsch, Carter and Buchanan, 1978; Rosenblum & El-Sabban, 1978).

During the past three to four years, an attempt has been made to find a low dose of aspirin that will achieve inhibition of TXA_2 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 (Burch *et al.*, 1978); a single dose of 2 mg/kg is enough to inhibit completely the generation of MDA, measured 2 h after oral ingestion, while between 3 and 3.5 mg/kg is needed to inhibit ADP-, adrenaline- or collagen-induced aggregation (Masotti, Poggesi, Galanti, Abbate & Neri Serneri, 1979); and finally that a dose of 100 mg of aspirin reduces TXB_2 release by more than 90% in serum 2 h after oral administration and by 100% after 3 to 4 h (Patrono, Ciabattoni, Pinca, Pugliese Castrucci, De Salvo, Satta & Peskar, 1980). These last authors also found that a clear dose-effect on the generation of TXB_2 can only be obtained with doses below 2 mg/kg of aspirin. They concluded that a single oral dose of 2 mg/kg produces a maximal inhibition of TXB_2 generation.

These data suggest that the dose of aspirin needed to obtain its full effect on ADP-, adrenaline- 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 shown to have an anti-thrombotic effect in arteriovenous shunts in uraemic patients (Harter, Burch, Majerus, Stanford, Delmez, Anderson & Weerts, 1979).

However, recent studies in animals and in man suggest that the separation in the dose which inhibits platelet aggregation and prostacyclin formation might not be so great. Masotti and his colleagues (1979) using a bioassay method, calculated that 4.5 mg/kg was needed to produce a 50% inhibition of PGI_2 synthesis 2 h after oral ingestion; Preston, Whipps, Jackson, French, Wyld & Stoddard (1978a) (taking biopsies of superficial veins) found that 2 h after 150 or 300 mg of aspirin, 81–100% inhibition of prostacyclin synthesis had occurred; Pareti, D'Angelo, Mannucci & Smith (1980) found inhibition of PGI_2 synthesis in superficial veins 2 h after the ingestion of 150 mg of aspirin and finally, Hanley, Cockbill, Bevan & Heptinstall (1981) found about 50% inhibition of synthesis in varicose veins of patients 14 h after ingestion of aspirin at as low a dose as 81 mg. This has led to the suggestion that aspirin could be given at longer intervals (every 2–3 days) in doses which will produce either no inhibition of the vascular synthesis of PGI_2 or a very short reversible inhibition (for data and reviews see Korbut & Moncada, 1978; Moncada & Korbut, 1978; Amezcua *et al.*, 1979).

Patrono *et al.* (1980) have shown that TXB_2 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 h schedule. Unfortunately, they did not study prostacyclin generation. However, another study cited previously (Hanley *et al.*, 1981) 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 cyclo-oxygenase. Masotti *et al.* (1979), for example, demonstrated more than 50% recovery of PGI_2 production by 24 h even after ingestion of doses as high as 10 mg/kg (it is important to note that this was a bioassay method and the PGI_2-like activity was generated during arm ischaemia), while other studies report no more than 25 to 50% recovery of PGI_2 formation in human vascular biopsies 8–24 h after oral doses of aspirin ranging from 150–300 mg. With these data, all that can be said is that the problem of the recovery of PGI_2 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 TXA_2 formation. This was originally shown by Hoogendijk & Ten Cate (1980) who demonstrated an increased inhibition in platelet MDA production by platelets after dosing volunteers with 40 mg aspirin per day for ten 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 (Hoogendijk & Ten Cate, 1980; O'Brien, 1980; Hanley *et al.*, 1981). This would suggest 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 (Preston, Greaves & Jackson, 1981b) which suggests that even a dosage of 40 mg per day for three days has a cumulative effect on the production of prostacyclin by the vessel wall.

All these data indicate that it will be extremely difficult to find a suitable dose or schedule of aspirin in humans that will achieve selective and long lasting inhibition of TXA_2. However, it is likely that even if this is achieved it will not actually prove to be a better anti-thrombotic drug than has already been shown in the few trials in which it has been slightly efficacious (for review see Packham & Mustard, 1980). The reason is that platelet aggregation is a complex mechanism which takes place via different 'pathways', the generation and actions of TXA_2 being only one of them (the other two identified at present are the ADP and thrombin pathways).

Only the stimuli which induce platelet aggregation

via the release of TXA_2 will be affected by aspirin treatment, the others being largely unaffected (for review, see Packham & Mustard, 1977). 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.

Interestingly, in patients with rheumatoid arthritis who consume large doses of aspirin, a retrospective study over an observation period of 10 years showed that the incidence of myocardial infarction, sudden death, angina pectoris or cerebral infarction was not significantly different from that in a sex- and age-matched group in the general population (Linos, Worthington, O'Fallon, Fuster, Whisnant & Kurland, 1978). There is, however, one report of lower incidence of myocardial infarction in rheumatoid patients taking large doses of aspirin (Wood, 1972).

Thromboxane synthetase inhibitors

We have suggested that a selective inhibitor of thromboxane synthetase should prove to be a superior anti-thrombotic agent to aspirin by allowing prostacyclin formation by vessel walls or other cells either from their own endoperoxides or from those released from platelets (for review see Moncada & Vane, 1979). 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 (Nijkamp *et al.*, 1977). 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 blood cell must have done so (Blackwell, Flower, Russell-Smith, Salmon, Thorogood & Vane, 1978; Flower & Cardinal, 1979). Injection of heterologous blood into anaesthetized cats causes hypotension, respiratory distress and frequently death and this is accompanied by a sharp rise in blood levels of TXB_2 (Bunting, Castro, Salmon & Moncada, unpublished observation). Pretreatment with either a thromboxane synthetase inhibitor (1-methyl-cyclo-octyl imidazole) or a cyclo-oxygenase inhibitor such as aspirin prevented death. However, after inhibition of thromboxane synthetase the blood levels of 6-oxo-$PGF_{1\alpha}$ rose about five times more than after the shock in control animals, suggesting diversion of the platelet prostaglandin endoperoxides away from TXA_2 production towards prostacyclin production.

Other work on selective thromboxane synthetase inhibitors is beginning to appear (Feuerstein & Ramwell, 1981) including the first publications on administration of one of these compounds to man (Tyler, Saxton & Parry, 1981; Vermylen, Carreras, Schaeren, Defreyn, Machin & Verstraete, 1981). In two studies (Aiken, personal communication; Yarger, Schocken, Harris, McRae, Boyd, Best, Kennedy, Gill & Schrader, 1980) it has now been shown that TXA_2 synthetase inhibitors have a superior anti-thrombotic action to aspirin and perhaps more importantly, that the anti-thrombotic action of these compounds can be blocked by previous treatment with aspirin. This suggests that part of the activity of these compounds depends on the 'boosting' of a functional prostacyclin synthetase by endoperoxides released by platelets.

Prostacyclin, thromboxane A_2 in disease

A number of diseases have now been related to an imbalance in the prostacyclin–TXA_2 system. Platelets from patients with arterial thrombosis, deep venous thrombosis, or recurrent venous thrombosis produce more prostaglandin endoperoxides and TXA_2 than normal and have a shortened survival time (Lagarde & Dechavanne, 1977). Platelets from rabbits made atherosclerotic by dietary manipulation (Shimamoto, Kobayashi, Takahashi, Takashima, Sakamoto & Morooka, 1978) and from patients who have survived myocardial infarction (Szczeklik, Gryglewski, Musial, Grodzinska, Serwonska & Marcinkiewicz, 1978b) are abnormally sensitive to aggregating agents and produce more TXA_2 than controls. Elevated TXB_2 levels have been demonstrated in the blood of patients with Prinzmetal's angina (Lewy, Smith, Silver, Saia, Walinsky & Wiener, 1979) and vasotonic angina (Robertson, Robertson, Roberts, Maas, FitzGerald, Friesinger & Oates, 1981). Hirsh, Hillis, Campbell, Firth & Willerson (1981) 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 (Harrison, Reece & Johnson, 1978; Johnson, Reece & Harrison, 1978); these effects are reversed by chronic insulin treatment (Harrison *et al.*, 1978). Prostacyclin production by blood vessels from patients with diabetes is depressed (Johnson, Harrison, Raftery & Elder, 1979) and circulating levels of 6-oxo-$PGF_{1\alpha}$

are reduced in diabetic patients with proliferative retinopathy (Dollery, Friedman, Hensby, Kohner, Lewis, Porta & Webster, 1979). Davis, Brown, Finch, Mitchell & Turner (1981) have confirmed that vessels taken from diabetic man produced less prostacyclin than normal. 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 thrombo-emboli, and a deficiency in prostacyclin production may be responsible for the increased platelet consumption which occurs in TTP (Remuzzi *et al.*, 1978; Remuzzi, Rossi, Misiani, Marchesi, Mecca, de Gaetano & Donati, 1980; Cocchetto, Cook, Cato & Niedel, 1981). This deficiency is postulated to be secondary to a lack of a 'plasma factor' which normally stimulates prostacyclin production (MacIntyre *et al.*, 1978). 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 (Hensby, Lewis, Hilgard, Mufti, Hows & Webster, 1979b).

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 (Hokama, 1978) but there is no experimental evidence to support this hypothesis. Prostacyclin production is significantly lower in umbilical and placental vessels from pre-eclamptic patients that in those from normally pregnant women (Remuzzi, Marchesi, Mecca, Misiani, Rossi, Donati & de Gaetano, 1980).

An increased prostacyclin production, resulting from an accumulation of the 'plasma factor' which stimulates prostacyclin synthesis, has been suggested to explain the haemostatic defect in uraemic patients (Remuzzi, Cavenaghi, Mecca, Donati & de Gaetano, 1977). Patients with Bartter's syndrome excrete about four times as much 6-oxo-$PGF_{1\alpha}$ in the urine as controls (Gullner *et al.*, 1979). This has led to the suggestion that overproduction of prostacyclin mediates both the hyper-reninaemia and the hyporesponsiveness to pressor agents observed in these patients (Gullner *et al.*, 1979). Finally, enhanced prostacyclin production by blood vessels of spontaneously hypertensive rats has been demonstrated (Pace-Asciak, Carrara, Rangaraj & Nicolaou, 1978). However, Grose, Lebel & Gbeassor, (1980) 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 causes arrhythmias (Coker, Parratt, Ledingham & Zeitlin, 1981) and also vasoconstriction induced by TXA_2 in the gastric mucosa of the dog produces gastric ulceration (Whittle, Kauffman & Moncada, 1981).

Prostacyclin and atherosclerosis

High concentrations of lipid peroxides have been demonstrated in advanced atherosclerotic lesions (Glavind, Hartmann, Clemmesen, Jessen & Dam, 1952). Lipid peroxidation induced by free radical formation is known to occur in vitamin E deficiency, the ageing process and perhaps also in hyperlipidaemia accompanying atherosclerosis (Slater, 1972). It has been shown (Bunting *et al.*, 1976b; Gryglewski *et al.*, 1976; Moncada, Gryglewski, Bunting & Vane, 1976c) 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 (Salmon *et al.*, 1978). 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_1 (Mickel & Horbar, 1974). D'Angelo, Villa, Mysliwiec, Donati & de Gaetano (1978) 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 (Sinzinger, Feigl & Silberbauer, 1979). This suggests that the early 'fatty streak' may be a biochemically critical stage of the atherosclerotic process. Bourgain, Six & Andries (1980), 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 (Larrue, Rigaud, Daret, Demond, Durand & Bricaud, 1980). In addition, it has been shown in rats that a vitamin E deficient diet leads to an increase in peroxide levels in the aortae and to a decrease in prostacyclin production *in vitro* (Okuma, Takayama & Uchino, 1980). 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 (Marks, 1962; Boyd & Marks, 1963; Haeger, 1968) had in fact a biochemical rationale. It is important to point out that it has been shown *in vitro* that human diploid fibroblasts which produce prostacyclin lose the ability to do so during ageing while the other arachidonic acid metabolites like PGE_2, $PGF_{2\alpha}$ and thromboxane A_2 increase (Murota, Mitsui & Kawamura, 1979). In addition aortic smooth muscle cells obtained from old rats produce less PGI_2 in culture than those obtained from young animals (Chang, Murota, Nakao & Orimo, 1980). This is due to a specific decrease in the prostacyclin synthetase activity since the cyclo-oxygenase activity was similar in both groups. Similar results have been obtained with fresh swine arteries (Kent, Kitchell, Shand & Whorton, 1981) and *in vitro* using porcine smooth muscle and endothelial cells it has been observed that during subculture the ability to generate PGI_2 decreases while PGE_2 formation increases (Ager, Gordon, Moncada, Pearson, Salmon & Trevethick, 1982). 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 ischaemic heart disease (Medalie, Kahn, Neufeld, Riss & Gouldbourt, 1973; Streja, Steiner & Kwiterovich, 1978; Kannel, Castelli & Gordon, 1979) whereas high density lipoprotein (HDL) is thought to protect against the disease (Streja *et al.*, 1978; Kannel *et al.*, 1979). Nordoy, Svensson, Wiebe & Hoak (1978) were the first to show that LDL reduced the release of a prostacyclin-like substance by human endothelial cells. Beitz & Förster (1980) extended these observations by showing that LDL inhibited whereas HDL stimulated prostacyclin synthesis. A mixture of low LDL and high HDL also stimulated prostacyclin synthesis. Gryglewski & Szczeklik (1981) have confirmed that LDL inhibits prostacyclin synthesis. They also analysed lipoproteins taken from a group of hyperlipidaemics 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 which inhibits prostacyclin synthesis.

Cell proliferation *in vitro* is inhibited by substances which stimulate cyclic AMP formation (Pastan, Johnson & Anderson, 1975). Cell growth in tissue culture (Johnson, Pastan, Peery, Otten & Willingham, 1972; Santoro, Philpott & Jaffe, 1976), including vascular smooth muscle cell culture (Huttner, Gwebu, Panganamala, Milo, Cornwell, Sharma & Geer, 1977), is inhibited by PGE_1. Possibly prostacyclin has a role in the regulation of cell growth in the vascular wall. Smooth muscle proliferation in atherosclerotic plaques (Bendit, 1977) might be a consequence of inhibition of prostacyclin generation by lipid peroxides.

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 PGG_1 and thromboxane A_1 are not proaggregatory and PGE_1 is anti-aggregatory (Willis, Comai, Kuhn & Paulsrud, 1974). However, feeding rabbits with sufficient dihomo-γ-linolenic acid to elevate its content in tissues does not alter the platelet sensitivity to ADP (Oelz, Seyberth, Knapp, Sweetman & Oates, 1976). Since the discovery of prostacyclin it has become apparent that this is not the most rational approach to dietary manipulation, since prostaglandin 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 anti-aggregating substance (Gryglewski, Salmon, Ubatuba, Weatherley, Moncada & Vane, 1979). Synthetic Δ^{17} prostacyclin or PGI_3 is as potent an anti-aggregating agent as prostacyclin. In contrast, thromboxane A_3 has a weaker pro-aggregating activity than TXA_2 (Raz, Minkes & Needleman, 1977; Gryglewski *et al.*, 1979). The fatty acid available for prostaglandin biosynthesis in Greenland Eskimos is mainly EPA, unlike that in Caucasians which is mainly arachidonic acid (Dyerberg, Bang, Stoffersen, Moncada & Vane, 1978). These differences may explain why Eskimos have a low incidence of acute myocardial infarction, low blood cholesterol levels and an increased tendency to bleed (Dyerberg *et al.*, 1978). This prolonged bleeding time is related to a reduction in *ex vivo* platelet aggregability (Dyerberg & Bang, 1979). 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 (Bang & Dyerberg, 1972).

Eicosapentaenoic acid inhibits platelet aggregation in platelet rich plasma stimulated by ADP, collagen, arachidonic acid, and a synthetic analogue of PGH_2 (Gryglewski *et al.*, 1979). Also, EPA inhibits aggregation in aspirin- and imidazole-treated platelets (Dyerberg & Jorgensen, 1980) and inhibits thrombin-induced aggregation (Jakubowski & Ardlie, 1979). 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 antithrombotic effect either by competing with remaining arachidonic acid for cyclo-oxygenase and lipoxygenase (Culp, Titus & Lands, 1979; Needleman, Raz, Minkes, Ferrendelli & Sprecher, 1979) or by being converted to the less pro-aggregatory PGH_3 and TXA_3 (Gryglewski *et al.*, 1979). Studying seven Caucasians who had been on a mackerel diet for 1 week, Seiss and colleagues (1980) showed a reduced sensitivity of platelets to collagen, associated with a reduced ability to produce thromboxane B_2, which was dependent on the ratio of C20:5/C20:4 in platelet phospholipids (Seiss, Roth, Scherer, Kurzmann, Bohlig & Weber, 1980). ADP-induced aggregation was significantly reduced in some subjects and platelet aggregation to exogenously added arachidonic acid was unchanged, indicating normal cyclo-oxygenase activity. Similarly, Sanders, Naismith, Haines & Vickers (1980) 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 six 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 anti-thrombin III and blood pressure levels in the volunteers (Sanders, Vickers & Haines, 1981). In accordance with these results Brox and co-workers 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 TXB_2 during *ex vivo* platelet aggregation induced by collagen (Brox, Kille, Gunnes & Nordoy, 1981). So far it is clear that EPA feeding leads to a decrease in platelet aggregability and a reduction in TXB_2 formation during *ex vivo* platelet aggregation. What is not clear at the moment is whether changes in the production of PGI_2 are also present. Some studies in rats (Ten Hoor, De Dekere, Haddeman, Hornstra & Quadt, 1980) suggest that there is a reduction in the production of PGI_2 without formation of PGI_3, however, others (Hirai, personal communication) suggest that there is an increase in PGI_2-like activity generated by vascular tissue *in vitro*.

Under normal peroxide levels *in vivo*, eicosapentaenoic acid is a poor substrate for cyclo-oxygenase but increasing peroxide tone in an incubate containing purified cyclo-oxygenase enzyme increases the conversion of eicosapentaenoic acid considerably (Culp *et al.*, 1979). Incubation of platelet rich plasma with EPA does not induce the generation of a thromboxane-like material; indeed it prevents the formation of thromboxane A_2 induced by arachidonic acid or by collagen (Gryglewski *et al.*, 1979). Conversely, in human umbilical vasculature, Dyerberg & Jorgensen (1980) 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 PGH_3 to Δ^{17}-6-keto-$PGF_{1\alpha}$ (Smith, Weatherley, Salmon, Ubatuba, Gryglewski & Moncada, 1979) but formation of this metabolite or Δ^{17} 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 (Black, Culp, Madison, Randall & Lands, 1979; Culp, Lands, Luccesi, Pitt & Romson, 1980). Brain infarct volume after experimentally-induced cerebral ischaemia and the neurological deficit were less in cats fed fish oil than in a corresponding control group (Black *et al.*, 1979). In dogs fed fish oil, thrombosis and subsequent infarct size (3% compared to 25% in control group) induced by electrical stimulation were reduced, with less than 30% ectopic beats after 19 h compared with 80% at the same time in the control group (Culp *et al.*, 1980).

The prolonged bleeding time in Eskimos is reduced after aspirin ingestion (Dyerberg & Bang, 1979) suggesting a decreased thromboxane synthesizing capacity coupled with normal or possibly elevated prostacyclin production. Overall, then, the present evidence suggests that it is well worth while continuing to study the effects of EPA in man.

Prostacyclin in man

Prostacyclin has a potent effect on platelets in man. During intravenous infusion of prostacyclin in healthy volunteers at doses ranging from 2–16 $ng\,kg^{-1}\,min^{-1}$ there is a dose-related inhibition of platelet aggregation and this effect persists for as long as 100 min after stopping the infusion (Szczeklik *et al.*, 1978a; O'Grady, Warrington, Moti, Bunting, Flower, Fowle, Higgs & Moncada, 1979). Prostacyclin also disperses circulating platelet aggregates (Szczeklik *et al.*, 1978a).

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 are not altered by prostacyclin (Szczeklik *et al.*, 1978a; O'Grady *et al.*, 1979). Prostacyclin causes arteriolar vasodilatation, increases in skin temperature and produces facial flushing at doses of 2–5 $ng\,kg^{-1}\,min^{-1}$ (Szczeklik *et al.*, 1978a; O'Grady *et al.*, 1979). Facial flushing invariably occurred 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 studies with prostacyclin can be rendered double blind.

The cardiovascular effects of prostacyclin are shorter-lived than those on platelets and disappear within 15 min of the end of infusion (O'Grady, Warrington, Moti, Bunting, Flower, Fowle, Higgs & Moncada, 1980). Plasma renin activity and renal blood flow increase during prostacyclin infusion in man (FitzGerald, Friedman, Miyamori, O'Grady & Lewis, 1979).

Headache has been reported by several subjects when doses greater than 8 ng kg^{-1} min^{-1} are administered (Szczeklik *et al.*, 1978a; O'Grady *et al.*, 1979; FitzGerald *et al.*, 1979). 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 for the effects of prostacyclin on human gastrointestinal tissue have not been studied.

Ill-defined sensations of unease and restlessness have been experienced by subjects receiving higher doses (8–20 ng kg^{-1} min^{-1}) of prostacyclin (Szczeklik *et al.*, 1978a; O'Grady *et al.*, 1979; Chierchia, Ciabattoni, Cinotti, Maseri, Patrono, Pugliese, Distante, Simonetti & Bernini, 1979). When a relatively high dose of prostacyclin (50 ng kg^{-1} min^{-1}) was administered to two subjects (Szczeklik *et al.*, 1978a) both experienced sudden weakness with pallor and nausea, systolic and diastolic blood pressure fell and bradycardia occurred.

Therapeutic potential of prostacyclin

The circulation of blood through extracorporeal systems brings blood into contact with artifical surfaces which cannot generate prostacyclin. In the course of such procedures, thrombocytopenia and loss of platelet haemostatic function occur and make an important contribution to the bleeding problems following charcoal haemoperfusion and prolonged cardiopulmonary bypass in man (for review see Vane, Bunting & Moncada, 1982). Formation of microemboli during cardiopulmonary bypass may also contribute to cerebral complications which sometimes follow this procedure (Patterson & Kessler, 1969). In animals subjected to experimental renal dialysis (Woods, Ash, Weston, Bunting, Moncada & Vane, 1978), charcoal haemoperfusion (Bunting, Moncada, Vane, Woods & Weston, 1979) and cardiopulmonary bypass (Longmore, Bennett, Gueirrara, Smith, Bunting, Reed, Moncada, Read & Vane, 1979), infusion of prostacyclin during the procedure prevented this platelet damage and thrombocytopenia, thus increasing the biocompatibility of the procedure. Platelet aggregates in the blood returning to the animals were also reduced by infusion of prostacyclin. These findings have been confirmed in patients with fulminant hepatic failure undergoing charcoal haemoperfusion (Gimson, Hughes, Mellon, Woods, Langley, Canalese, Williams & Weston, 1980). Prostacyclin infusion prevented the fall in platelet count and elevation of β-thromboglobulin seen in the control patients. In addition, two of the control patients developed marked hypotension during the procedure, in one associated with a marked rise in Swank Screen filtration pressure, while this did not occur in the prostacyclin-treated patients. A study of the influence of serial haemoperfusion with prostacyclin on the survival rate of patients with fulminant hepatic failure is now in progress (Gimson, Canalese, Hughes, Langley, Mellon & Williams, 1981). Published double blind clinical trials of prostacyclin in cardiopulmonary bypass show a preservation of platelet number and function, with a reduction in the blood loss. The heparin-sparing effect of prostacyclin was confirmed and the vasodilator effects were not troublesome (for review see Vane *et al.*, 1982).

The observation that prostacyclin potentiates the effects of heparin (Bunting *et al.*, 1979) led to further studies on this interaction. These demonstrated that prostacyclin has a small indirect anticoagulant effect. Indeed, platelets stimulated by low doses of aggregating agents accelerate clotting by providing a surface upon which coagulation factors can combine and react more efficiently (see Marcus, 1978). Prostacyclin, by preventing platelet activation, inhibits the shortening of clotting time produced when either kaolin or collagen is incubated with platelet-rich plasma (Bunting & Moncada, 1980; Bunting, Simmons & Moncada, 1981). Platelets release antiheparin activity, which reduces the anticoagulant effect of heparin *in vitro*. Prostacyclin, by inhibiting this release and by preventing the development of procoagulant activity, can enhance the action of heparin by as much as 100% (Bunting & Moncada, 1980; Bunting *et al.*, 1981). In addition, heparin has been shown to inhibit the activation of the adenylate cyclase induced by PGI_2 (Eldor & Weksler, 1979), thus making the platelets less susceptible to PGI_2. The authors suggest that some of the adverse effects of heparin therapy might be related in part to this antagonism.

Heparin therapy in some patients is complicated by thrombocytopenia and thromboembolic episodes (Cimo, Moake, Weinger, Ben-Menachem & Khalil, 1979; Hussey, Bernhard, McLean & Fobian, 1979) and *in vitro* can cause platelet aggregation and potentiate aggregation to other aggregating agents (Salzman, Rosenberg, Smith, Lindon & Favreau, 1980). Perhaps because the stimulus to coagulation is

milder, we showed that during haemodialysis in dogs, prostacyclin could be used alone to prevent platelet loss and coagulation. Heparin was not needed. This surprising result has now been confirmed in 10 patients by Zusman, Rubin, Cato, Cocchetto, Crow & Tolkoff-Rubin (1981). Prostacyclin was infused intravenously for 10 min before dialysis and into the arterial line during dialysis. The rate of infusion was adjusted to prevent prostacyclin-induced hypotension. They concluded that prostacyclin can safely replace heparin as the sole antithrombotic agent during haemodialysis and that it may be more advantageous if anti-coagulant is contraindicated. This work has also been confirmed by Turney, Dodd & Weston (1981). A combination of heparin and prostacyclin might, however, prove beneficial in some clinical situations in which herapin therapy is necessary (Bunting *et al.*, 1981).

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 intra-arterial infusion of prostacyclin in five patients with advanced atherosclerotic lower limb peripheral vascular disease (Szczeklik, Nizankowski, Skawinski, Szczeklik, Gluszko & Gryglewski, 1979). Rest pain disappeared, previously refractory ulcers healed and the muscle blood flow, as measured by Xenon^{133} clearance, was significantly increased for at least six weeks after prostacyclin infusion. This group has now reported on 55 patients with advanced peripheral arterial disease of the lower extremities (Szczeklik & Gryglewski, 1981). In summary, 42% of patients treated showed a persistent, long-lasting improvement. In 40% of patients the improvement lasted no longer than 2 months, while in the remaining 18% of patients the results were virtually negative. The authors believe that these figures do not represent the true efficacy of prostacyclin therapy in advanced peripheral arterial disease and that successful treatment largely depends upon choice of patients, localization of the vascular lesions, and the advancement of the disease (Szczeklik & Gryglewski, 1981). Careful trials are therefore required to resolve this problem. Other reports also suggest that prostacyclin may have beneficial effects in peripheral artery disease (Olsson, 1980; Pardy, Lewis & Eastcott, 1980). 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 h (Zygulska-Mach, Kostka-Trabka, Niton & Gryglewski, 1980).

Prostacyclin has been successfully used in cases of pulmonary hypertension (Watkins, Peterson, Crone, Shannon, & Levine, 1980; Szczeklik, Szczeklik & Nizankowski, 1980). Single case studies have suggested that prostacyclin may be useful in the treatment of patent ductus arteriosus (Lock, Olley, Coceani, Swyer & Rowe, 1979) and pre-eclamptic toxaemia (Fidler, Bennett, de Swiet, Ellis & Lewis, 1980).

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^{-1} min^{-1} for 10 min had no deleterious effects (Bergman, Daly, Atkinson, Rothman, Richardson, Jackson & Jewitt, 1981). 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 241s. They concluded that acute administration of prostacyclin was beneficial in angina, having effects similar to short acting nitrates. Hall & Dewar (1981) concluded from their study of five patients with coronary artery disease that prostacyclin can safely be infused directly into diseased coronary arteries and Szczeklik & Gryglewski (1981) found a beneficial effect of intravenous prostacyclin infusions in patients with unstable angina.

A prostacyclin deficiency has been reported in thrombotic thrombocytopenic purpura (Hensby *et al.*, 1979b). However, infusion of prostacyclin into two patients with TTP did not produce an increase in circulating platelet count (Hensby *et al.*, 1979b; Budd, Bukowski, Lucas, Cato & Cocchetto, 1980). On the other hand, FitzGerald, Roberts, Maas, Brash & Oates (1981) 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.

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 pre-eclamptic toxaemia (Fidler, Ellis, Bennett, de Swiet & Lewis, 1981), haemolytic uraemic syndrome (Webster, Borysiewicz, Rees & Lewis, 1981), peptic ulceration (Whittle *et al.*, 1981), the thrombotic complications associated with transplant rejection (Mundy, Bewick, Moncada & Vane, 1980), the prevention of tumour metastasis (Hohn, Cicone & Skoff, 1981) and the treatment of pulmonary embolism (Utsunomiya, Krausz, Valeri, Shepro & Hechtman, 1980). Finally, there is increasing evidence that prostacyclin might have a general, yet undefined mechanism of cell protection. Indeed, in

some experiments of myocardial infarction it has been reported that prostacyclin reduces infarct size (Jugdutt, Hutchins, Bulkley & Becker, 1979) and also decreases oxygen demand (Ribeiro, Brandon, Hopkins, Reduto, Taylor & Miller, 1981) and the release of cathepsin D and creatine phosphokinase from infarcted areas (Ohlendorf, Perzborn & Schrör, 1981). In other studies on the effects of prostacyclin on lung injury in sheep, Demling, Smith, Gunther, Gee & Flynn (1981) found that prostacyclin protected the lungs against injury induced by endotoxin. A beneficial effect of prostacyclin has also been reported in endotoxin shock in the dog (Fletcher & Ramwell, 1980) and in the cat (Lefer, Tabas & Smith, 1980) where it improves splanchnic blood flow and reduces the formation and release of lysosomal hydrolases (Cathepsin D). These studies suggest a potential wider therapeutic role of prostacyclin in cell or tissue preservation. What is clear now is that our knowledge of the causes and treatment of cardiovascular diseases will be improved by the availability of prostacyclin.

Prostacyclin and the future development of anti-thrombotic therapy

Until recently, anti-thrombotic substances fell into four categories: (a) Drugs which affect the cyclic AMP levels in platelets. These include dipyridamole which inhibits the platelet phosphodiesterase, and the stimulators of adenylate cyclase such as PGE_1, PGD_2, (b) Drugs that affect arachidonic acid metabolism at the cyclo-oxygenase level, such as aspirin or sulphinpyrazone, (c) Drugs that inhibit thrombin formation and its action, like the oral anticoagulants or heparin and (d) a miscellaneous group of agents which include ticlopidine, propranolol and clofibrate (for review see Packham & Mustard, 1980). The clinical experience with these substances, although sometimes satisfactory in certain types of thrombosis, has not led to a comprehensive understanding or approach to treatment. There are two main reasons for this: firstly, not enough is known about the interaction of the three systems involved in the process, namely the platelets, the vessel wall, and the coagulation cascade, and secondly, there have been no drugs which strongly affect platelet aggregation without affecting the other systems. The discovery of prostacyclin has presented several possibilities for the development of antithrombotic therapy which at least merit investigation, namely, the use of selective thromboxane synthetase inhibitors, the use of anti-oxidants, dietary manipulation with fatty acids like eicosapentaenoic acid, the stimulation of prostacyclin production by the vessel wall, the development of phosphodiesterase inhibitors that might be acting by potentiating endogenous prostacyclin and finally the direct substitution of prostacyclin by synthetic prostacyclin or a chemical analogue. Several of these possibilities are being explored at present as well as some combinations of them.

As far as direct substitution of prostacyclin is concerned, a great effort is being made in an attempt to obtain a stable compound with fewer cardiovascular effects than prostacyclin itself (for review see Whittle, Moncada & Vane, 1981). If this is achieved, orally active compounds will be available in the future. From a theoretical point of view a more direct approach to antithrombotic therapy is to control platelet cyclic AMP; increasing platelet cyclic AMP 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 cyclic AMP, prostacyclin, or an analogue, alone or in combination with a phosphodiesterase inhibitor, should provide a more comprehensive approach to the control of platelet aggregation *in vivo*.

In addition, it is important to realize that increasing concentrations of prostacyclin produce an increasing inhibition of platelet activation progressing from inhibition of platelet aggregation to inhibition of adhesion. This property, which is unique amongst the known anti-platelet agents, will allow us to study the physiology and pathophysiology of platelet-vessel wall interactions and as a consequence develop a more rational anti-thrombotic therapy.

I gratefully acknowledge the help given in the preparation of this manuscript by Ann Higgs and Gillian Henderson.

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(*Received January 26, 1982.*)