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Pharmacology and Endogenous Roles of Prostaglandin Endoperoxides, Thromboxane A_2, and Prostacyclin

S. MONCADA AND J. R. VANE

The Wellcome Research Laboratories, Beckenham, Kent, England

I. Introduction

FORTY-FIVE years ago an endogenous "substance" with vasodepressor and smooth-muscle stimulating activity was newly described in accessory genital glands and human semen independently by Goldblatt (123) and von Euler (89), who called it "prostaglandin" (90). The activity turned out to be due to several different acidic lipids, and several years later Bergström and Sjövall (22) isolated in pure form prostaglandin E_1 (PGE_1) and prostaglandin $F_{1\alpha}$ and later several other stable prostaglandins (21, 324).

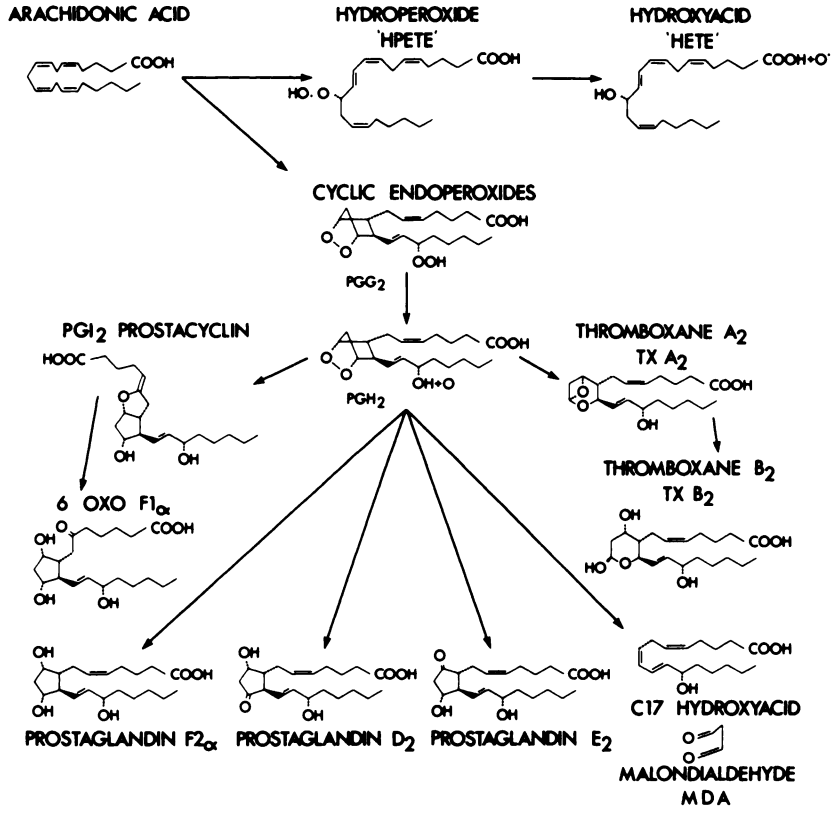
In the following years, two independent groups (20, 365) demonstrated that prostaglandins are biosynthesized from polyunsaturated fatty acids. These acids include dihomo-γ-linolenic acid (C20:3ω6), arachidonic acid (C20:4ω6), and eicosapentaenoic acid (C20:5ω3), which give rise to the mono-, bis-, or trienoic prostaglandins, respectively.

Arachidonic acid, the precursor of all bisenoic prostaglandins, is the most common

fatty acid precursor of prostaglandins in membrane phospholipids and can be obtained directly from the diet or by anabolic desaturation and chain elongation from dietary linoleic acid (C18:2ω6). Arachidonic acid is transported in blood, largely bound to albumin, and is incorporated as a structural component of phospholipids into cell membranes and other subcellular structures of all tissues of the body (303).

Arachidonic acid can be released from cell membranes by the action of phospholipases, which can be activated by changes in their chemical environment. Little is known at present about the activation of these enzymes (107, 375). However, simple mechanical stimulation can result in generation of prostaglandins, as shown in lung (297), spleen (142), and platelets (323). The reader is referred to some recent reviews (106, 225). The enzymes that synthesize prostaglandins are present in most organs so far studied, but some tissues, such as seminal vesicles, kidney, and lungs, have a greater capacity for prostaglandin synthesis than others (61).

Until recently it was thought that of the chemically identified metabolites of arachidonic acid, the only ones with substantial biological activity were PGE_2 and $PGF_{2\alpha}$. This and the availability of these compounds in pure form led to an intense study of their biological actions. However, since 1973, there have been important discoveries on the nature of the intermediates in arachidonic acid metabolism (fig. 1). These substances, which include the prostaglandin endoperoxides (PGG_2 and PGH_2), thromboxane A_2 (TXA_2), and prostacyclin (PGI_2), are unstable but have potent biological ac-



METABOLIC PATHWAYS OF ARACHIDONIC ACID

FIG. 1. Metabolic pathways of arachidonic acid.

tivities. Investigations into the roles of these substances have already led to important new concepts, which will be discussed in this review.

II. Arachidonic Acid Metabolism

Once released from the membrane phospholipids, arachidonic acid is metabolized by two types of enzyme. The term "eicosanoids" has been suggested by E. J. Corey to include all the 20-carbon derivatives whereas "prostanoids" refers only to those with a prostanoic acid skeleton. One type of enzyme is a series of lipoxygenases that peroxidize arachidonic acid at different carbon atoms, e.g., 5 and 12. These enzymes form unstable hydroperoxides, which then break down to the stable hydroxyacids or are further transformed into other products such as the recently described leukotrienes (see p. 319). Different lipoxygenases have been described in different tissues; the lung has the 11 and 12; platelets have the 12, and white cells have the 5 lipoxygenase (148, 149, 151).

The other enzyme is a cyclo-oxygenase that forms the prostaglandin endoperoxide PGG_2. This is converted to PGH_2, which then breaks down enzymically or nonenzymically to the stable substances PGE_2, $PGF_{2\alpha}$, PGD_2, and a 17-carbon hydroxy acid, 12-hydroxy-5,8,10 heptadecatrienoic acid (HHT), as well as malondialdehyde (MDA). The enzyme cyclo-oxygenase (sometimes called prostaglandin synthetase) seems to be present in all cell types (except erythrocytes) whereas lipoxygenases have been identified only in platelets, lungs, and white cells (148, 149, 151, 276).

The prostaglandin endoperoxides are also transformed enzymically into two other unstable products, prostacyclin and TXA_2. Unlike PGE_2, D_2, or $F_{2\alpha}$, these products cannot result from chemical breakdown.

Although MDA and HHT can be formed nonenzymically by spontaneous degradation of PGH_2 (154), the biosynthetic pathways of TXA_2 and HHT are linked since a purified enzyme that synthesizes TXA_2 also catalyses HHT formation (77, 398).

III Inhibition of Enzymes in the Arachidonic Acid Biosynthetic Pathway

The cascade of arachidonic acid metabolism that leads to the formation of the prostaglandins begins with the oxygenation and cyclization of the fatty acid to form the endoperoxide PGG_2 (fig. 1). These steps are catalysed by the enzyme known as cyclo-oxygenase. This enzyme complex has recently been solubilized and purified (236, 237).

In 1971, Vane and others discovered (95, 342, 366) that nonsteroid antiinflammatory drugs, notably aspirin and indomethacin, inhibited prostaglandin biosynthesis (point 1 in fig. 1). As a result of this, the general theory was proposed that this enzyme inbition accounts for the antiinflammatory effects (and perhaps the side effects) of aspirin-like drugs (366). Since then much evidence to support this theory has emerged and it is now generally accepted that the antipyretic, analgesic, and antiinflammatory actions of these drugs are mainly mediated via inhibition of prostaglandin biosynthesis at the cyclo-oxygenase step (96, 99, 253, 368). Moreover, arising from these results, new uses for aspirin-like drugs have been suggested. These include prevention of premature labour, stimulation of the closure of a patent ductus arteriosus after birth, and treatment of Bartter's syndrome (110, 118, 214). Besides their recognized therapeutic effects, aspirin-like drugs produce in man and other species side effects that include gastrointestinal bleeding, renal damage, delayed and prolonged parturition, and inhibition of the second phase of platelet aggregation [for review see Moncado and Vane (253)]. These activities have also been related to inhibition of cyclo-oxygenase. Because aspirin and indomethacin prevent biosynthesis of all known prostaglandins, they have been used extensively as experimental

tools to clarify whether prostaglandins are involved in physiological or pathophysiological events.

Since this review will not deal with the products of the lipoxygenase enzymes in depth (and several newly found ones are being described, apart from 12-HPETE and 12-HETE), there are only two other enzymes whose behaviour and selective inhibition will be described: thromboxane synthetase (point 2, fig. 1) and prostacyclin synthetase (point 3, fig. 1).

A. Thromboxane Synthetase

The enzyme that synthesizes TXA_2 from PG endoperoxides was first localized in the high speed particulate fraction of human and horse blood platelets (248, 267). The enzyme has recently been solubilized and separated from the cyclo-oxygenase (77, 154, 398), and detailed studies of human and bovine platelet thromboxane synthetase have been published (178, 344, 384). Several inhibitors of thromboxane synthetase have been developed but still await in vivo evaluation. Benzydamine (248) and a phenyl phosphonate derivative of phloretinphosphate (N-0164) (204) do inhibit the enzyme in microsomal preparations, but neither of these two compounds is highly selective since at slightly higher concentrations they also inhibit cyclo-oxygenase. Imidazole and several of its derivatives are much more selective inhibitors of thromboxane synthetase (26, 239, 355). Other inhibitors include a compound, l-(isopropyl 2-indole)-3-pyridyl-3-ketone, known as L-8027 (135, 144) and the more recently described 9,11-azoprosta-5,13-dienoic acid, a prostaglandin endoperoxide analogue (127). The evidence for L-8027 being a selective inhibitor of TXA_2 synthetase is scanty and has been challenged recently (300). Tissues in which TXA_2 formation has been described are listed in table 1.

B. Prostacyclin Synthetase

The enzymic formation of prostacyclin by vessel microsomes or fresh vascular tissues in vitro is strongly and selectively inhibited by 15-hydroperoxyarachidonic acid (15-HPAA) (136, 243, 318). Other peroxides of fatty acids and their methyl esters are also strong inhibitors of the enzyme in vitro (318). Tranylcypromine is a much weaker inhibitor of prostacyclin synthetase (136), although it is also a well known inhibitor of other enzyme systems. Attempts to use the lipid hydroperoxides in vivo as experimental tools to inhibit prostacyclin synthetase have so far been disappointing (84), presumably because of rapid reduction of the hydroperoxide moiety by enzymes such as glutathione peroxidase (63). However, the fact that these inhibitors can be formed in vivo (333) has important implications, for inhibition of prostacyclin production would favour thrombus formation (see below). Tissues that form prostacyclin are listed in table 2.

IV. Unstable Intermediates or Active Metabolites?

Rabbit Aorta Contracting Substance and Prostaglandin Endoperoxides

Piper and Vane, in 1969 (296), detected the release of an additional substance during anaphylaxis in isolated lungs from sensitized guinea pigs. Because of its activity they called it rabbit aorta contracting substance or RCS. RCS was unstable with a half-life of 1 to 2 min in aqueous solution at room temperature, and its release was inhibited by aspirin-like drugs.

Release of RCS from lungs in vitro has also been demonstrated after challenge with bradykinin, RCS-releasing factor (RCS-RF) (297), mechanical agitation, slow reacting substance of anaphylaxis (SRS-A), and, more importantly, the prostaglandin precursor, arachidonic acid (292, 297, 370). In addition, RCS is generated by the isolated dog spleen when stimulated with bradykinin or adrenaline (240), or by rabbit spleen slices or microsomes after mechanical agitation or incubation with arachidonic acid (142, 143). Platelet aggregation in vitro is also accompanied by the generation of an RCS (372), and addition in vivo of arachidonic acid or slow reacting substance of

TABLE 1

Tissues in which thromboxane A_2 synthetase has been described

Tissue	Means of Detection	Reference
Human platelets	Gas-liquid chromatography-mass spectrometry (GLC-MS)	151
Human platelet microsomes	Bioassay Radiochromatography	177, 267
Bovine platelets	Bioassay Radiochromatography GLC-MS	398
Rat platelets	Bioassay Radiochromatography	374
Rabbit polymorphonuclear leukocytes	Bioassay	166
Human polymorphonuclear leukocytes	Radioimmunoassay (RIA)	124
Mouse macrophages	RIA Thin-layer chromatography (TLC)	40
Rat and guinea-pig macrophages	Radiochromatography	258
Human lung fibroblasts	Bioassay Radiochromatography GLC-MS	181
Brain: guinea-pig cerebral cortex—homogenates and slices	Radiochromatography	394
Rabbit iris and conjunctiva	Radiochromatography	24
Human iris microsomes	Bioassay Inhibitors	204
Guinea-pig lungs	Radiochromatography GLC-MS	148
Guinea-pig lung microsomes	Radiochromatography	176
Challenged sensitized guinea-pig lungs	GLC-MS*	74
Anaphylactic guinea-pig heart	RIA	11
Rat kidney	GLC-MS	399
Rabbit kidney (ureter obstructed) Perfused kidney Cortical and medullary microsomes	Bioassay Radiochromatography	255
Cat spleen	Bioassay Inhibitors	271
Bovine gastric mucosal microsomes	Radiochromatography GLC-MS	6
Decidual tissue of the pregnant rat	GLC-MS	389
Human umbilical artery	GLC-MS	362
Human amnion, chorion, decidua, placenta	RIA	234
Bovine semen	GLC-MS	334
Rat inflammatory granuloma	Radiochromatography GLC-MS	53

* Detected as 15-oxo-13,14-dihydro TXB_2.

cobra venom (SRS-C) to whole blood in dogs releases RCS, which may originate in platelets (100).

Since RCS was released by the prostaglandin precursor, arachidonic acid, and its release was prevented by drugs that inhibit prostaglandin biosynthesis (366), it was suggested that RCS was an unstable inter-

TABLE 2

Tissues in which prostacyclin synthetase has been described

Tissue	Means of Detection	Reference
Pig aortic microsomes	Bioassay	136, 242
Rabbit aortic microsomes	Inhibitors	
Pig mesenteric artery microsomes	Radiochromatography	
Rabbit coeliac and mesenteric arteries	Bioassay Inhibitors	44
Human mesenteric arteries and veins	Bioassay Inhibitors	245
Bovine coronary artery	Bioassay Inhibitors	82
Hamster aorta	Bioassay	163
Cultured endothelial cells, human umbilical and bovine aortic	Bioassay Inhibitors Radiochromatography PGI_1-antibody	62, 381
Pig aortic endothelial cells	Bioassay	216
Pig post caval vein endothelium	Inhibitors	
Pig smooth muscle*		
Pig adventitial fibroblasts*		
Human arterial smooth muscle	Bioassay	17
Human skin fibroblasts	Inhibition of labelled 5HT release from stimulated platelets	
Mouse macrophages	Radiochromatography	183
Rabbit eye, iris and conjunctiva	Radiochromatography	24
Bovine iris and ciliary body	Bioassay Inhibitors	203
Guinea-pig perfused lung	GLC-MS	74
Cat lung	Bioassay Inhibitors	138
Rabbit lung	Bioassay Inhibitors PGI_1-antibody	247
Rabbit pleura, pericardium and peritoneum	Bioassay PGI_1-antibody Radiochromatography	161
Guinea-pig heart	Bioassay Inhibitors Radiochromatography	327
Rabbit heart	Bioassay Radiochromatography	75, 263
Ductus arteriosus (foetal lamb)	GLC-MS	289
Ductus arteriosus (foetal calf) Also, foetal calf aorta	Radiochromatography	299
Rat kidney	GLC-MS	399
Rabbit kidney	Bioassay Radiochromatography	263
Human renal cortex	Bioassay	307
Pig kidney	Radiochromatography	359
Rabbit renal collecting tubule cells	Radiochromatography	133
Cat spleen	Bioassay RIA	7

* Produced very little activity.

TABLE 2, *continued*

Tissue	Means of Detection	Reference
Rat stomach-fundus microsomes	Bioassay Inhibitors Radiochromatography	136
Rat stomach	GLC-MS	285
Rat gastric mucosa	Bioassay RIA GLC-MS Radiochromatography	249
Rat small intestine	Bioassay Inhibitors	385
Uterus; decidual tissue of pregnant rat	Radiochromatography GLC-MS	389
Myometrium of pregnant rat	Bioassay	388
Homogenates of pseudopregnant rat uterus	GLC-MS	94
Human placenta	Bioassay Inhibitors	261
Human chorion, amnion, and decidua	RIA	235
Ram seminal vesicles	Radiochromatography Inhibitors	69
Rat inflammatory exudate	Radiochromatography	52

mediate in the biosynthesis of prostaglandins (143, 371). The existence of such an unstable intermediate had been postulated by Samuelsson (325) who, while studying the conversion of 8,11,14-eicosatrienoic acid into PGE_1 by homogenates of sheep vesicular gland, found that the two oxygen substituents on the five-membered ring derived from the same oxygen molecule, which suggested the existence of an unstable endoperoxide intermediate.

The formation of a 15-hydroxy cyclic endoperoxide of arachidonic acid was later confirmed and called PGH_2 by Hamberg and Samuelsson (147) and PGR_2 by Nugteren and Hazelhof (277). 15-Hydroxy and 15-hydroperoxy endoperoxides have now been identified from arachidonic, dihomo-γ-linolenic, and eicosapentaenoic acids (153, 266, 277). Figure 1 shows the structures and nomenclatures used here.

PGG_2 and PGH_2 endoperoxides are unstable in aqueous solution (half-life approximately 5 min at 37°C), decomposing to the stable prostaglandins, E_2, D_2, and $F_{2\alpha}$. They also contract the rabbit aortic strip. These two characteristics led Nugteren and Hazelhof (277) to equate prostaglandin endoperoxides with the RCS of Piper and Vane.

In 1974, Willis and Kuhn (392) showed that short-term incubation of arachidonic acid with sheep vesicular gland generated an unstable principle that induced platelet aggregation and contracted the aortic strip. They called this principle, whose generation was inhibited by aspirin, "labile aggregation stimulating substance" or LASS. Vargaftig and Zirinis (372) also reported the release of arachidonic acid derivatives that were not PGE_2 or $PGF_{2\alpha}$ during platelet aggregation induced by arachidonic acid. Later, Willis et al. (393) described the isolation, purification, and some biological properties of LASS, which made it indistinguishable from PGH_2. At approximately the same time Hamberg et al. (153) reported that purified preparations of PGG_2 strongly induce platelet aggregation, and that during aggregation induced by other agents such as thrombin, prostaglandin endoperoxides were generated. A similar report was made by Smith et al. (336).

The involvement of endogenous cyclic endoperoxides in platelet aggregation pro-

vided 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 not been possible to explain why inhibitors of prostaglandin biosynthesis also inhibited platelet aggregation, for PGE_2 and $PGF_{2\alpha}$ have little or no proaggregatory activity (see below).

Prostaglandin endoperoxides contract vascular, gastrointestinal, and bronchial smooth muscle (146, 192, 241, 267) in vitro. On gastrointestinal smooth muscle (rat stomach strip and gerbil colon) their action is not qualitatively different from that of the stable PGE_2 and $PGF_{2\alpha}$ (146, 241) but in the guinea-pig tracheal muscle the endoperoxides are several times more active than $PGF_{2\alpha}$ (146). In vascular smooth muscle strips such as the rabbit aortic strip (267), the porcine coronary arteries (81, 349), bovine cerebral arteries (88), human umbilical artery (361), and dogs' intrapulmonary veins (192), prostaglandin endoperoxides induce contraction as a direct effect. In some tissues, the contraction could be partly due to their conversion into TXA_2; for example, thromboxane synthetase has been demonstrated in the umbilical artery (361).

In other vascular strips like the bovine coronary artery strip (82, 205) and the coeliac and mesenteric (46) artery of the rabbit, the endoperoxides induce relaxation or a short-lasting contraction followed by relaxation. The relaxation is caused by conversion of the endoperoxides into prostacyclin, for selective inhibitors of prostacyclin synthetase prevent the relaxation and unmask a pure contraction (82). Both PGG_2 and PGH_2 relax the isolated strip of the lamb ductus arteriosus but are less active than PGE_2 (65).

In the isolated Langendorff perfused heart preparation of the rabbit, PGH_2 induces coronary vasoconstriction (263), which is sometimes followed by a vasodilatation (327). In vivo, PGH_2 induces mainly vasodilatation comparable to that produced by PGE_2 or vasodilatation preceded by a short lasting vasoconstriction. These effects occur in mesenteric (84, 93, 293, 348), hind limb (84, 348), coronary (79, 185), and renal vascular beds (93) of the dog and cat and the microcirculation of the hamster cheek pouch (168, 213). In the pulmonary vascular beds of the dog and cat PGH_2 is a vasoconstrictor (184, 192).

As endoperoxides are rapidly converted enzymically or nonenzymically into other substances, their actions are sometimes difficult to interpret. For instance, their cardiovascular effects in the guinea pig consist of a fall in blood pressure followed by a short-lasting rise and then once more a prolonged fall (146). This type of complex response was described earlier in the rat by use of a crude sample of PG endoperoxides (393). PGG_2 and PGH_2 reduce the blood pressure both in normotensive and hypertensive rats and are more active when given intravenously than when given intraarterially, which suggests pulmonary transformation into a more active compound (12).

The intrinsic activity of endoperoxides, however, is constrictor for, at least in vitro, the relaxing activity on smooth muscle is blocked by 15-HPAA. In addition, there are several synthetic structural analogues of endoperoxides available (42) that are not substrates for conversion by the enzymes that convert endogenous endoperoxides. These all contract vascular strips in vitro (221), causing vasoconstriction in isolated vascular beds (84, 92, 193) and in vivo (84, 193, 312).

Not much work has been carried out with endogenous endoperoxides on bronchial muscle. PGG_2 and PGH_2 both increase the tracheal inflation pressure when given intravenously to the guinea pig (146). They were five to ten times more active than $PGF_{2\alpha}$. However, on aerosol administration, they caused a smaller increase than $PGF_{2\alpha}$ and had some protective effects against bronchoconstriction induced by 15(S) 15-methyl $PGF_{2\alpha}$ (146).

The stable analogues increase bronchomotor tone and pulmonary vascular resistance in the intact dog (192, 193). These substances increase airway resistance and

decrease dynamic compliance and lung volume (343, 376). The effects of these substances on the airways are probably direct, since mechanically induced increase in pulmonary vascular pressure had little effect on lung compliance (343).

V. Thromboxane A_2

The half-lives of RCS [<2 min; Piper and Vane (296)] and PG endoperoxides [<5 min; (296)] were different. Moreover, the amount of PG endoperoxides released from platelets during aggregation or from guinea-pig lungs by arachidonic acid was not sufficient to account for the observed rabbit aorta contracting activity. These observations led Samuelsson's group to look for the presence of an additional substance with RCS activity. Later they identified and named as thromboxane A_2 a highly unstable intermediate (half-life of 30 sec at 37°C) in the conversion of PGG_2 to "PHD" (a very polar compound, the hemiacetal derivative of 8-(l-hydroxy-3-oxopropyl)-9,12L-dihydroxy-5-10-heptadecaenoic acid) now known as thromboxane B_2 (TXB_2). A non-prostaglandin structure was suggested with an oxane ring like PHD but lacking the hemiacetal hydroxy group (148, 149) (fig. 1). This unstable metabolite strongly contracted the aortic strip and induced platelet aggregation.

RCS from guinea-pig lung as described by Piper and Vane (296) is, then, a mixture of prostaglandin endoperoxides and TXA_2. In this organ, the activity is mostly due to TXA_2 (46, 350). However, the composition of RCS from other organs may vary; for example, the RCS activity obtained by incubating arachidonic acid with ram seminal vesicles is due entirely to prostaglandin endoperoxides (312). In platelets as in lung, there is high conversion of endoperoxides to TXA_2, but in polymorphonuclear cells we and others have observed TXA_2 production alongside that of other prostaglandins (73, 124, 166).

As originally described by Vane's group (292), RCS released from the guinea-pig lungs contracted all vascular tissues tested. In many of these preparations, both the endoperoxides and TXA_2 contract the tissues, but TXA_2 is much more potent. There are, however, two interesting exceptions. The rabbit mesenteric and coeliac arteries are relaxed (often after a brief contraction) by prostaglandin endoperoxides and contracted by TXA_2 (46). As mentioned earlier, the relaxation is due to conversion to prostacyclin (82), but this distinguishing property has allowed differential bioassay of endoperoxides and TXA_2. In consequence, it has been shown that the biological activity generated by infusing arachidonic acid (AA) into guinea-pig isolated lungs is TXA_2 (46).

TXA_2 is generally more potent in contracting vascular and airway smooth muscle in vitro than the parent endoperoxides; such tissues include rabbit aorta (248, 267), human umbilical artery (361), guinea-pig trachea (146), bovine and pig coronary artery (81, 82, 349), bovine cerebral arteries (88), and lamb ductus arteriosus (65). In vivo, TXA_2 is a constrictor of vascular beds in the dog (84) and cat (348) and, moreover, it increases the tracheal inflation pressure in the anaesthetized guinea pig (146).

There are several problems in interpreting the apparent activity of TXA_2. First, the intrinsic instability of the compound makes any biological study difficult since, from the time of biosynthesis to its use, substantial decomposition into the less active thromboxane B_2 (TXB_2) has occurred. Moreover, most studies have used semipurified (either by filtration or rapid ether extraction) (152, 248) samples of material obtained from aggregated platelets, and these contain other vasoactive materials that complicate the effect (41, 225) such as adenosine diphosphate (ADP) and serotonin (5HT). Because of this it has been suggested that semipurified TXA_2 for biological use should be obtained from dog platelets that synthesize TXA_2 from platelets without aggregating in vitro (59, 60). The fact that dog platelets do not aggregate to arachidonic acid or its products is not generally accepted as some workers have

shown aggregation in a variable percentage of animals tested (our published results and communications from P. Ramwell and K. E. Eakins). The absolute potency of TXA_2 and its pharmacological profile will not be finally clarified until a better way is devised of obtaining pure samples or of stabilizing TXA_2 after preparation.

Longer half-lives (up to 24 h) for TXA_2 were reported at lower temperatures after rapid ether extraction (248) or filtration (132). The presence of albumin might also stabilize the TXA_2 molecule (338). However, in these experiments, because of the continued presence of aggregating platelets in the samples, it was difficult to separate "real stabilization" from further generation.

With a newly developed method based on the conversion of TXA_2 into a stable mono-O-methyl derivative by excess of methanol and measurement of this by radioimmunoassay, Granström et al. (132) showed that the presence of albumin stabilizes the TXA_2 molecule probably as a result of binding (108). Several substances, including acetylsalicylic acid, phenylbutazone, bilirubin, and warfarin, shortened the half-life of TXA_2 in the presence of albumin by interfering with this binding process (108).

All these results are different from those obtained by studying by bioassay the stability of TXA_2 in circulating blood of the dog. In whole blood, TXA_2 has a biological half-life similar to that reported in aqueous solutions (256). The explanation for these differences is not clear; possibly albumin stabilizes the chemical structure of the molecule, but at the same time removes its biological activity. The interpretation of these findings needs further research since there is controversy as to whether PGH_2 or TXA_2 binds covalently to albumin (87, 102).

Prostaglandin Endoperoxides and Thromboxane A_2 in Platelet Aggregation

Addition of cyclic endoperoxide to platelet suspensions induces the release of platelet constituents and aggregation. This was first shown by Hamberg et al. in 1974 (153), who demonstrated that addition of either PGG_2 or PGH_2 at 10 to 300 ng/ml to a suspension of platelets caused rapid aggregation. Surprisingly, PGG_2 was three times more potent than PGH_2. Both the extent and reversibility of the aggregation were concentration dependent. Low concentrations (<10 ng/ml) gave small reversible aggregations, whereas higher concentrations (50 to 300 ng/ml) elicited greater aggregations that were virtually irreversible. The aggregation was not significantly affected by aspirin.

In a more detailed study in human platelet-rich plasma (PRP), Salzman (321) found that, like ADP, PGG_2 produced reversible platelet aggregation without secretion. At higher doses there was an irreversible platelet aggregation with secretion that was inhibited by indomethacin, and with still higher doses these same effects occurred in the presence of indomethacin. The fact that intermediate doses of PGG_2 induced an aggregation and release reaction sensitive to indomethacin was attributed by Salzman to an "autocatalytic action of exogenous PGG_2 on cyclo-oxygenation of endogenous arachidonic acid."

Most of the studies on endoperoxide activity in human platelets have used PGH_2. Although this endoperoxide is as active as ADP in inducing aggregation (340), it has been suggested that it is a poor inducer of the platelet release reaction. When low concentrations of ADP and PGH_2 are added together to PRP, the extent of aggregation is greater than that observed with either agent alone. However, when given before ADP, PGH_2 had an inhibitory activity, due to the formation of PGD_2 (340, 341), a potent inhibitor of human platelet aggregation. This compound was mainly formed nonenzymically by decomposition of the endoperoxides (340, 341), although some enzymic PGD_2 synthesis has been postulated during aggregation induced by thrombin, collagen, noradrenaline, or ADP (5, 278).

Certainly, the nonenzymic formation of products that either modulate endoperoxide-induced aggregation, such as PGE_2,

which enhances endoperoxide aggregation (393), or in themselves have an inhibitory action on platelet aggregation (like PGD_2) (232, 272) creates problems in the interpretation of the results.

Hamberg et al. (152) incubated arachidonic acid or PGG_2 with suspensions of washed human platelets and demonstrated the formation ot TXA_2 by trapping experiments and by showing that the releasing and aggregating effects of arachidonic acid and PGG_2 declined with a half-life similar to that of TXA_2. They proposed that TXA_2 is the arachidonate metabolite that mediates aggregation and release.

Certainly, TXA_2 is a more potent inducer of platelet aggregation than the prostaglandin endoperoxides (152, 250). Whether TXA_2 is an obligatory mediator of aggregation, however, is still unclear, for exogenous PGG_2 added to platelets in vitro induces a very rapid aggregation and release of ^{14}C serotonin within 2 sec (64). Moreover, the degradation of PGH_2 in PRP to PGD_2 and PGE_2 with little conversion (<1%) into TXB_2 suggests a direct action (337). Indeed, several synthetic PGH_2 analogues (42, 68) are more active proaggregating and release-inducing agents (57, 68, 340) than PGH_2 without being converted into TXA_2, again suggesting that the endoperoxides can be active in their own right. However, another interpretation is that the endoperoxide analogues act more as TXA_2 analogues when they induce aggregation (340).

The question of whether the endoperoxides have proaggregatory activity in their own right or only after conversion to TXA_2 has been extensively studied with the use of thromboxane synthetase inhibitors, mostly imidazole (239) and the 9,11-azoprosta-5,13-dienoic acid (azo analogue I) (127) (see fig. 2). Imidazole inhibited endoperoxide-induced aggregation in indomethacin-treated human PRP (250). It also inhibited TXA_2 formation in washed human platelets (268). However, despite considerable inhibition of TXA_2 formation, platelet

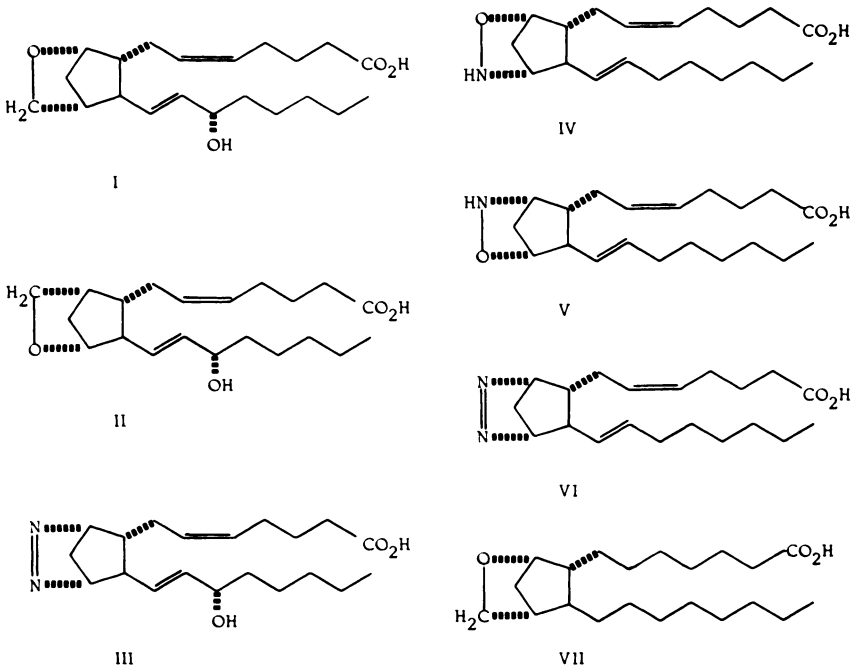


FIG. 2. Structure of some endoperoxide analogues: I, 9α,11α-epoxymethano-15-hydroxy-prosta-5,13-dienoic acid (42); II, 9α,11α-methanoepoxy-15-hydroxy-prosta-5,13-dienoic acid (42); III, 9α,11α-azo-15-hydroxy-prosta-5,13-dienoic acid (68); IV, 9α,11α-epoxyiminoprosta-5,13-dienoic acid (9,11 EIP) (43); V, 9α,11α-imino-epoxy prosta-5,13-dienoic acid (43); VI, 9α,11α-azoprosta-5,13-dienoic acid (AZO analogue I) (127); and VII, 9α,11α-epoxymethano prostanoic acid (344).

aggregation still occurred. Needleman et al. (268) concluded that TXA_2 synthesis was not obligatory for aggregation and that PGH_2 caused aggregation in its own right. It appears that imidazole can enhance PGH_2-induced aggregation in washed platelets but blocks PGH_2-induced aggregation in PRP (103, 264). Since imidazole has other activities (including stimulation of phosphodiesterase) that could affect platelet behaviour independently of the arachidonic acid metabolic products (301), the results obtained with imidazole are difficult to interpret.

The azo analogue I (see fig. 2) is a more potent inhibitor of TXA_2 synthesis, and this compound inhibited both TXA_2 synthesis and aggregation due to PGH_2 in PRP or washed platelets (103). In contrast to the studies of Needleman et al. (268), these results suggested that the conversion of PGH_2 into TXA_2 was obligatory for platelet aggregation and that PGH_2 is either inactive or much less active. However, results with the azo analogue I are also difficult to interpret for it can be argued that, due to structural similarity to the endoperoxides, azo analogue I is a receptor antagonist to PGH_2, as well as a potent thromboxane synthesis inhibitor. Experiments are needed to test this possibility.

Blackwell et al. (26) showed that l-*n*-butylimidazole, a more potent and selective inhibitor of TXA_2 synthesis, blocks PGH_2-induced platelet aggregation. Overall, then, it appears, but without unequivocal proof, that conversion of the PG endoperoxides into TXA_2 is necessary for platelet aggregation. The development of selective receptor antagonists or antibodies that will antagonize the biological effects of the endoperoxides (105) or of TXA_2 will help to clarify this point. Indeed, Fitzpatrick et al. (101) have recently shown that the compound (9α,11α-epoxyiminoprosta-5,13-dienoic acid (9,11 EIP) (fig. 2) inhibits aggregation induced by TXA_2, PGH_2, or arachidonic acid in human platelet suspensions through selective TXA_2 receptor antagonism. Possibly, when further metabolism is blocked, PGG_2 and PGH_2 exert a direct activity on platelets as pharmacological agents, perhaps at TXA_2 receptors, but normally when platelets are activated and the endogenous arachidonic acid cascade is triggered, the prostaglandin endoperoxides thus generated will exert their "physiological role" through conversion to the more potent TXA_2.

Another intriguing question is whether arachidonate products induce aggregation via other platelet constituents. Malmsten et al. (223) concluded that PGG_2 caused platelet aggregation via the release of ADP. This propositon was strengthened by the finding that furosemide, a competitive inhibitor of ADP-induced aggregation, also inhibited the aggregation response to PGG_2 (223), and by the later finding that in the presence of a rapid ADP-phosphorylating system the aggregating response of the endoperoxide was greatly inhibited (64). When PGG_2 is added to PRP there is a rapid release of ADP that occurs within a few seconds and is not inhibited by indomethacin (64). In this respect, PGG_2 is different from arachidonic acid or collagen, which induce a much slower release of ADP that can be blocked by indomethacin. Since ADP also causes aggregation in the presence of indomethacin (but no release reaction) it has been concluded that at least some of the aggregating activity of PGG_2 is due to released ADP (64).

More supporting evidence comes from experiments carried out in Marcus's laboratory with the use of the "oxygen burst" measurement secondary to platelet activation (38). It was shown that when washed platelets are stimulated by arachidonic acid there is an immediate burst of oxygen consumption and simultaneous aggregation. When the platelets were preincubated with apyrase, an enzyme that inactivates ADP, arachidonic acid induced the same burst of oxygen consumption but no aggregation (38). Salzman, on the other hand, has shown that "primary" aggregation is not affected by an ADP antagonist (2-n-amylthiro-5AMP). However, the second phase of aggregation is inhibited by this compound (321). Willis et al. (393) have sug-

gested that PGH_2 aggregates platelets by direct activation of the "aggregating machinery" of the platelets, and only in high concentrations can part of its effect be attributed to ADP release.

There are several investigations that seem to support the view that arachidonic acid metabolites can induce aggregation independently of ADP. Kinlough-Rathbone et al. (198, 199) showed that arachidonate produced aggregation in washed platelets that did not contain releasable ADP, for they had been previously degranulated by repeated thrombin treatment. Moreover, Charo et al. (56, 57) have demonstrated that endoperoxides can cause platelet aggregation in the absence of secretion. Marcus (225) has pointed out, however, that in all this work the presence of fibrinogen was not excluded. Since fibrinogen synergizes with endoperoxides in a washed platelet system (339), it is difficult to conclude that arachidonic acid metabolites have a direct action.

Whether prostaglandin endoperoxides or TXA_2 aggregate directly without the intervention of ADP needs further investigation. The interaction of products of arachidonic acid metabolism with the so-called third pathway of platelet aggregation (believed to be arachidonic acid- and ADP-independent) (198, 291) is unknown. Recent work suggests, however, that phospholipase A_2 activation is involved (295, 329) with the probable formation of a 1-lysophosphatidylcholine (58); because of this it is not unlikely that there will be interactions between the two pathways as happens with the ADP system.

It seems likely that normal "physiological" platelet aggregation is a multifactorial phenomenon in which there is participation by several proaggregating substances. The relative importance of each could depend upon the situation in which platelet aggregation occurs, e.g., haemostatic plug, thrombus on an ulcerated atherosclerotic plaque, platelet aggregation during disseminated intravascular coagulation, etc.

It is possible that the further study of platelets with specific defects will be of importance in the clarification of the relative importance of different intraplatelet components. So far, three patients with an absence of platelet cyclo-oxygenase have been reported (206, 223). The platelets from these patients did not respond to collagen or arachidonate but aggregated and released normally when exposed to PGG_2. Moreover, the platelets produced little TXA_2 or HHT after incubation with ^{14}C-arachidonic acid but did produce normal lipoxygense products.

In storage pool disease (a platelet defect in which there is a reduced dense granule content of Ca^{++}, ATP, ADP, and 5HT, and as a consequence less releasable ADP), platelets in PRP or after gel filtration respond less to the proaggregating activity of PGG_2 (224) or PGH_2 (379). Moreover, unlike its activity in normal platelets, PGE_2 is not able to potentiate PGH_2-induced aggregation (379). These findings suggest that aggregation induced by either PGH_2 or PGG_2 is ADP-dependent.

Many stimuli that aggregate platelets, including ADP, thrombin, collagen, epinephrine, arachidonic acid, and physical stimuli such as adsorption on kaolin particles or centrifugation, induce a decrease in cyclic AMP (cAMP) (319, 323). Since this effect can be inhibited by aspirin or indomethacin (323) and the above stimuli also lead to prostaglandin biosynthesis (320), it has been suggested that prostaglandin endoperoxide (PGG_2) formation might explain the reduction of the basal cAMP induced by the above stimuli (320). This proposal was strengthened by the finding that crude LASS can induce a decrease in cAMP (320), and later by the demonstration that PGG_2 decreases cAMP in human PRP (321). Although the concept of an ultimate effect on platelet adenylate cyclase is shared by Miller et al. (231) as a mechanism for the action of endoperoxides and TXA_2 in platelets, they do suggest that PG endoperoxides (probably after transformation into TXA_2) act indirectly on platelet adenylate cyclase by internal mobilization of calcium from intracellular stores (231). Miller et al. based this proposal on their

finding that TXA_2 or PGH_2 do not alter basal cAMP levels in the platelets when they induce platelet aggregation. In order to show an effect of PGH_2 or TXA_2, the cAMP levels must be stimulated with PGE_1 or PGI_2 (see below). Under these conditions, both PGH_2 and TXA_2 antagonized the increase in cAMP induced by PGE_1 or PGI_2. TXA_2 was the more active of the two.

Since they have shown that the cAMP-lowering effect of PGH_2 in PRP can be inhibited by imidazole and the azo analogue I (see fig. 2), Gorman et al. (129) have concluded that TXA_2 is the compound responsible for the abolition of the increase in cAMP induced by PGI_2. These results are in agreement with the suggestion by Gerrard et al. (115) that TXA_2 acts as a calcium ionophore and give support to the idea (116, 117) that calcium mobilization is the step necessary for the platelet to "contract" and start the release reaction. Later, Gorman (126) showed that the compound TMB-8 (8-N,N-diethylamino-octyl-3,4,5-trimethyoxylbenzoate), which acts as an intracellular calcium antagonist, dose-dependently prevents the inhibition by PGH_2 of the PGI_2-stimulated cAMP accumulation. Since it was found that increasing concentrations of TMB-8 were able to inhibit secretion dose-dependently without inhibiting the cAMP-lowering capacity of PGH_2, they concluded that the ability of PGH_2 or TXA_2 to lower cAMP is independent of the release of 5-HT or ADP, which have been suggested by Claesson and Malmsten (64) to be the mediators of this effect. Certainly, the ADP effect on cAMP is inhibited by aspirin, which in itself suggests that the ADP effect is mediated through arachidonic acid metabolites; moreover, attempts to demonstrate a direct effect of ADP on platelet adenylate cyclase have been unsuccessful (322).

The differences between the results obtained by Miller et al. (229, 231) and Salzman (321) on the effect of endoperoxides on basal cAMP levels are difficult to interpret. Salzman suggested that because Miller and Gorman (229) used only one dose of PGG_2 (2.8 μM), which is probably too high and breaks down substantially nonenzymically to PGD_2 (which stimulates cAMP), they missed the opportunity to detect an effect.

Alternatively, as we suggested (254), platelets in vitro are not subjected to the normal physiological stimulation of their adenylate cyclase by prostacyclin and have very low "basal levels" of cyclic AMP; any further effect in vitro is difficult to detect. Indeed, the discovery of prostacyclin as an endogenous substance that potently stimulates adenylate cyclase in the platelets has provided the missing link in a homeostatic mechanism of control of platelet aggregation in vivo and has underlined the insufficiencies inherent in the studies in vitro of platelet aggregation (see below).

VI. Prostacyclin

In 1976, in collaboration with S. Bunting and R. Gryglewski, we found that prostaglandin endoperoxides were transformed by a microsomal enzyme from blood vessels into an unstable substance that is a potent vasodilator and an inhibitor of platelet aggregation (242). This compound, originally called PGX (44, 136, 243), was later chemically identified by Johnson et al. (190) as an intermediate in the formation of 6-oxo-$PGF_{1\alpha}$, a compound already known (74, 285). PGX was then renamed prostacyclin and given the abbreviation of PGI_2 (see fig. 1).

Prostacyclin is formed by vascular tissues from all species so far tested including rabbit, ox, and human (44, 82, 245), and is the main metabolic product of arachidonic acid in isolated vascular tissue (190, 318). Prostacyclin is the most potent endogenous inhibitor of platelet aggregation yet discovered. It is 30 to 40 times more potent than PGE_1 (250) and more than 1000 times more active than adenosine (35). In vivo, prostacyclin applied locally in low concentrations inhibits thrombus formation due to ADP in the microcirculation of the hamster cheek pouch (167), and given systemically to the rabbit it prevents electrically-induced thrombus formation in the carotid artery

and increases bleeding time (363). The duration of these effects in vivo is short; they disappear within 30 min of administration. Prostacyclin disaggregates platelets in vitro (243, 363), in extracorporeal circuits where platelet clumps have formed on collagen strips (138, 139), and in the circulation of man (353). Moreover, it has also been shown that it inhibits thrombus formation in a coronary artery model in the dog when given locally or systemically (4) and protects against sudden death induced by intravenous arachidonic acid in rabbits (19).

Prostacyclin is unstable and its activity disappears within 15 sec on boiling or within 10 min at 22°C at neutral pH. In blood at 37°C, prostacyclin has a half-life of 2 to 3 min (80, 82). Alkaline pH increases the stability of prostacyclin (190) so that at pH 10.5 at 25°C, it has a half-life of 100 h.

In the anaesthetised dog, prostacyclin is hypotensive in doses ranged from 50 to 1000 ng/kg/min (13). Intravenously in the anaesthetized rabbit or rat, prostacyclin causes a fall in blood pressure and is four to eight times more potent than PGE_2. Prostacyclin is at least 100 times more active than its degradation product, 6-oxo-$PGF_{1\alpha}$ (13). Since it is not inactivated by the pulmonary circulation, prostacyclin is equipotent as a vasodilator when given either intraarterially or intravenously in the rat, rabbit, or dog (14, 80, 83). This is an important difference from PGE_1 or PGE_2, which, because of strong pulmonary metabolism, are much less active when given intravenously (98). Many authors have suggested a vasodilator role for locally generated PGE_2 in the vascular wall and others have suggested that PGE_1 is released. There is little evidence that PGE_1 is a naturally occurring prostaglandin in the cardiovascular system of mammals.

In the heart, local injections of arachidonic acid into the coronary circulation of the dog cause vasodilatation, and because this effect was abolished by indomethacin (174) it was assumed that PGE_2 was the likely mediator. However, there were some major difficulties with this proposal. In isolated Langendorff-perfused hearts of the rabbit, arachidonic acid dilated the coronary vasculature, but PGE_2 was inactive (28, 262). Isolated strips of bovine, canine, and human coronary artery were relaxed by arachidonic acid but PGE_2 contracted them (205). Arachidonate-induced relaxation of these strips was abolished by indomethacin and it was suggested, therefore, that the metabolite responsible must be the endoperoxide intermediate PGH_2 (205).

Later, Dusting et al. (82) showed that bovine coronary arteries were relaxed by prostacyclin and PGH_2 (which sometimes induced an initial transient contraction), and after treatment with 15-HPAA (an inhibitor of prostacyclin synthetase) the relaxation induced by arachidonic acid was abolished, whilst that induced by PGH_2 was reversed to a contraction. Thus, arachidonic acid-induced relaxation of coronary arteries is due to intramural metabolism to prostacyclin. This study further confirmed that the intrinsic activity of PGH_2 on isolated blood vessels is contractile (82). Needleman and associates (205), who originally suggested that the relaxation was probably due to the direct action of the endoperoxide, are now in agreement that the effect is induced by prostacyclin formation (263, 304).

In isolated Langendorff-perfused hearts of the guinea pig and rabbit, not only is prostacyclin a potent vasodilator but it is also the predominant metabolite of arachidonic acid (327). Similarly, by using chromatographic procedures, others have identified 6-oxo-$PGF_{1\alpha}$, the degradation product of prostacyclin, as the major product from rat and rabbit hearts perfused with arachidonic acid (75). We and others have investigated the coronary actions of prostacyclin in the intact heart of open chest dogs (13, 79, 185). Local injection of prostacyclin (50 to 500 ng) into the coronary circulation increased coronary blood flow without systemic effects and it was a more potent coronary dilator than PGE_2. Furthermore, profound and prolonged coronary vasodilatation was rapidly elicited by prostacyclin

(20 to 100 μg) absorbed through the myocardium after dripping a solution onto the surface of the left ventricle (79). Interestingly, the coronary circulation is sensitized to the vasodilator effects of exogenous prostacyclin, but not to those of PGE_2, when endogenous synthesis is inhibited by indomethacin or meclofenamate (79, 174). These inhibitors of cyclo-oxygenase decrease resting coronary blood flow in anaesthetised, open-chest dogs. Although this is not seen in conscious dogs without acute surgery (284), it does indicate that the generation of a vasodilator metabolite of arachidonic acid increases or maintains coronary blood flow during mildly traumatic conditions. It is clear that this metabolite is prostacyclin.

Bradycardia accompanying the hypotension induced by prostacyclin has been observed in anaesthetised dogs (13, 79, 175) and only transient weak tachycardia accompanied prostacyclin infusion in anaesthetized cats (210). In contrast, the hypotension induced by PGE_2 always causes tachycardia, which, presumably, is mediated by baroreceptors (220). Although there is no clear difference in the overall systemic vasodilator effects of these two prostaglandins as assessed by total peripheral resistance, PGE_2 has a more pronounced effect on cardiac output and myocardial contractility (as indicated by maximum acceleration of aortic blood flow). These observations indicate that in equihypotensive doses, prostacyclin reduces cardiac work more than PGE_2.

Recent experiments suggest (54, 55) that bradycardia induced by prostacyclin is a reflex response mediated at least partially by vagal pathways since atropine reduces or abolishes the bradycardia. However, the afferent arc is also subserved by vagal fibres, for vagotomy (but not atropine treatment) reduces the hypotensive effects of prostacyclin. Therefore, the hypotension induced by prostacyclin has at least two components: direct arteriolar vasodilatation and reflex, noncholinergic vasodilatation. Similar results have been obtained by Hintze et al. (175).

In the renal circulation of the dog, prostacyclin infused intravenously reduces renal vascular resistance and increases blood flow and urinary excretion of sodium, potassium, and chloride ions at doses below those needed for a systemic effect (29, 172). There is increasing evidence that prostacyclin mediates the release of renin from the renal cortex. Arachidonic acid, prostaglandin endoperoxides, or prostacyclin all stimulate renin release from slices of rabbit renal cortex, but PGE_2 has no such effect (377, 382). Furthermore, indomethacin reduces renin release in animals and man (72, 111, 209). Prostacyclin-like activity and 6-oxo-$PGF_{1\alpha}$ have been identified in incubates of PGG_2 or PGH_2 with renal cortical microsomes (307, 383, 399). Thus, prostacyclin may be the obligatory endogenous mediator of renin secretion by the kidney. Indeed, Gerber et al. (114) have demonstrated that prostacyclin induces renin release when infused intrarenally into dogs, and Hill et al. (173) have demonstrated increased concentrations of angiotensin II in arterial blood during intrarenal infusions of prostacyclin. 6-oxo-$PGF_{1\alpha}$ is also formed by collecting tubule cells isolated from rabbit papillae (133). It is interesting that angiotensin II releases prostacyclin from the rat kidney in vitro (330) and the dog kidney in vivo (257).

Prostacyclin is also a strong vasodilator in the mesenteric and hind limb circulations of the dog (where TXA_2 is a vasoconstrictor) (84) and on the precapillary side of the microcirculation of the hamster cheek pouch (170), where it also reverses epinephrine-induced vasoconstriction. In this preparation 6-oxo-$PGF_{1\alpha}$ had 1/20 the vasodilator activity of prostacyclin and was more potent than PGE_2. In the pulmonary circulation of the dog, prostacyclin is the only product of arachidonic acid that produces strong vasodilatation (191, 256). It also dilates the pulmonary vascular bed of the foetal lamb, where its potency is greater

than PGE_1 but less than PGE_2 (211). Prostacyclin also induces vasodilatation and hypotension in man when given either intravenously or by inhalation (141, 281, 352). This is accompanied by tachycardia.

Prostacyclin relaxes in vitro most vascular strips including rabbit coeliac and mesenteric arteries (44), bovine coronary arteries (82, 263), human and baboon cerebral arteries (36), and lamb ductus arteriosus (65). Exceptions to this include the porcine coronary arteries (81), some strips of rat venous tissue, and isolated human saphenous vein (212), which are weakly contracted by prostacyclin. Whether these same effects are induced in the corresponding circulations in the intact animal or man has not been studied. In the human umbilical arterial strip, prostacyclin induces a dose-dependent relaxation at low concentrations ($< 10^{-6}$ M) and a dose-dependent contraction at higher concentrations ($> 10^{-5}$ M) (298). As mentioned earlier, prostacyclin, and not PGE_2, is the main metabolite of arachidonic acid in isolated vascular tissue, and this has led to intense study for reassessment of the effects and role of arachidonic acid and its metabolites on vascular tissue and the cardiovascular system.

Indeed, until recently, there seemed substantial evidence to suggest that vascular tissue mainly synthesized PGE_2. Homogenates of rabbit aorta converted only 1% of exogenous radioactive precursor into PGE_1, which represented a low conversion when compared with 41% converted by the rabbit renal medulla (61). Low rates of conversion in aortae were also reported by Hollander et al. (179). Moreover, incubations of human umbilical artery produce an immunoreactive $PGF_{2\alpha}$-like material at a rate of 15 ng/g tissue wet weight in 2 h, and cultured human endothelial cells harvested from umbilical veins produce PGE as detected by radioimmunoassay (119). Terragno et al. (358) reported that slices of bovine mesenteric arteries and veins released into the incubating medium a material that was biologically and chromatographically characterized as PGE_2 and $PGF_{2\alpha}$. They found a high rate of conversion (20%) of arachidonic acid in bovine vessels. As mentioned earlier, prostacyclin is the main metabolite of arachidonic acid in the vessel walls and therefore plays the regulatory role previously ascribed to PGE_2. Indeed, in the ductus arteriosus, where it has previously been suggested that PGE_2 (66) maintained its open state, prostacyclin has been shown to be the main metabolite (289, 299). In vivo, in the anaesthetized dog, recent studies suggest that arachidonic acid infused intravenously is mainly converted into a substance with prostacyclin-like activity as measured by bioassay (256).

Several factors helped to mislead investigators studying arachidonic acid metabolism in vascular walls. First of all, prostacyclin is unstable and cannot be detected by chromatography. As pointed out by Fried and Barton (109), the claimed isolation of an isomer of prostacyclin in 1970 by Pace-Asciak and Wolfe (290) is incompatible with the known chemical properties. Secondly, the chromatographic mobility of the stable end product 6-oxo-$PGF_{1\alpha}$ is very similar to PGE_2 or $PGF_{2\alpha}$ in most of the solvent systems (69). Thirdly, the commonly used bioassay tissues (rat stomach strip, chick rectum, and rat colon) do not easily differentiate between PGE_2 and 6-oxo-$PGF_{1\alpha}$, even though 6-oxo-$PGF_{1\alpha}$ is much less active. This has led to the development of new bioassay tissues such as the bovine coronary artery (265), which contracts to PGE_2 and relaxes to prostacyclin (241). Fourthly, the cross reactivity of PGE antibodies with 6-oxo-$PGF_{1\alpha}$, although low (317), might have led to misinterpretations.

Finally, much of the previous work on arachidonic acid metabolism has been carried out in the presence of exogenous cofactors such as glutathione. Such additions may seriously disrupt the natural pathway of endoperoxide metabolism. For instance, in ram seminal vesicles where the formation of 6-oxo-$PGF_{2\alpha}$ had previously been described only in low concentrations (51),

Cottee et al. (69) have demonstrated that with low substrate concentrations and no cofactors, 6-oxo-$PGF_{1\alpha}$ is the main metabolite of arachidonic acid. Moreover, by using a microsomal enzyme from vessel microsomes, Salmon et al. (318) have demonstrated that the presence of glutathione diverts the pathway from 6-oxo-$PGF_{1\alpha}$ to PGE_2.

A. Prostacyclin Release and Role in Vascular Homeostasis

Vessel microsomes in the absence of cofactors can utilize prostaglandin endoperoxides but not arachidonic acid to synthesize prostacyclin (242). Fresh vascular tissue can utilize both precursors although it was far more effective in utilizing prostaglandin endoperoxides (44). Moreover, vessel microsomes, fresh vascular rings, or endothelial cells treated with indomethacin can, when incubated with platelets, generate a prostacyclin-like antiaggregating activity (44, 47, 136). The release of this substance is inhibited by 15-hydroperoxyarachidonic acid (15-HPAA), a selective inhibitor of prostacyclin formation (136, 243). From all these results it was concluded that the vessel wall can synthesize prostacyclin from its own endogenous precursors, but that it can also utilize prostaglandin endoperoxides released by the platelets, thus suggesting a biochemical cooperation between platelets and vessel wall (251, 252).

This latter hypothesis has proved to be controversial. Needleman and associates demonstrated that while arachidonic acid was rapidly converted to prostacyclin by perfused rabbit hearts and kidneys, PGH_2 was not readily used. The authors concluded that some degree of vascular damage is necessary for the endoperoxide to be utilized by the prostacyclin synthetase (263). On the other hand, incubation of PRP with fresh indomethacin-treated arterial tissue leads to an increase in platelet cAMP that parallels the inhibition of the aggregation (23) and that can be abolished by previous treatment of the vascular tissue with tranylcypromine, a less active inhibitor of prostacyclin formation (136). Additionally, Tansik et al. (356) showed that lysed aortic smooth muscle cells could be fed prostaglandin endoperoxides by lysed human platelets, and Nordoy et al. (273) have demonstrated that endothelial cells can be fed with endoperoxides released from platelets during collagen-induced aggregation. Further, undisturbed endothelial cell monolayers readily utilize PGH_2 to transform it into prostacyclin (226).

In contrast, recent work by Needleman et al. (270) and Hornstra et al. (182), who used vessel microsomes and fresh vascular tissue, suggests that the feeding of endoperoxides from platelets does not take place under their experimental circumstances. However, Needleman et al. (270) made the observation that when platelets were treated with a TXA_2 synthetase inhibitor then endoperoxides were available for utilization by the vessel wall. It is interesting that, in the presence of a thromboxane synthetase inhibitor, arachidonic acid or collagen added to blood in vitro leads to the formation of 6-oxo-$PGF_{1\alpha}$ rather than TXB_2, showing that some cell other than platelets has synthesized prostacyclin (26). These results support our suggestion that thromboxane synthetase inhibitors might have a superior antithrombotic effect to simple cyclo-oxygenase inhibitors (250, 251). It is important to realize at this stage, however, that all these observations have been made in in vitro systems and that in vivo experiments will be necessary to clarify further the nature of the interaction between platelets and normal or damaged vessel wall.

In the vasculature, the enzyme that metabolizes prostaglandin endoperoxides to prostacyclin (prostacyclin synthetase) is most highly concentrated in the intimal surface and progressively decreases in activity towards the adventitial surface (244). Production of prostacyclin by cultured cells from vessel walls also shows that endothelial cells are the most active producers of

prostacyclin (155, 216, 381); moreover, this production persists after numerous subcultures in vitro (62).

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

Vascular damage leads to platelet adhesion but not necessarily to thrombus formation. When the injury is minor, platelet thrombi are formed that break away from the vessel wall and are washed away by the circulation. The degree of injury is an important determinant, and there is general agreement that for the development of thrombosis, severe damage or physical detachment of the endothelium must occur. All these observations are in accord with the distribution of prostacyclin synthetase, for it is abundant in the intima and progressively decreases in concentration from the intima to the adventitia. Moreover, the proaggregating elements increase from the subendothelium to the adventitia. These two opposing tendencies render the endothelial lining antiaggregatory and the outer layers of the vessel wall thrombogenic (244).

The ability of the vascular wall actively to prevent aggregation has been postulated before (316). For instance, the presence of an ADPase in the vessel wall has led to the suggestion that this enzyme, by breaking down ADP, limits platelet aggregation (162, 215). We have confirmed the presence of an ADPase in the vascular wall. However, the antiaggregating activity of the vascular wall is mainly related to the release of prostacyclin, for 15-HPAA or 13-hydroperoxy linoleic acid (13-HPLA), two inhibitors of prostacyclin formation that have no activity on the ADPase system, abolish most if not all of the antiaggregatory activity of vascular endothelial cells (47). Similar results have been obtained with an antiserum that crossreacts with and neutralizes prostacyclin in vitro (45). Endothelial cells pretreated with this antiserum lose the ability to inhibit ADP-induced aggregation (45, 62). It is not yet clear whether prostacyclin is responsible for all the thromboresistant properties of the vascular endothelium. However, recent work by Czervionke et al. (71) with endothelial cell cultures has demonstrated that 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. This work suggests that prostacyclin, although probably not responsible for all the thromboresistant properties of vascular endothelium, plays a very important role in the control of platelet aggregability.

The fact that prostacyclin inhibits platelet aggregation (platelet-platelet interaction) at much lower concentrations than those needed to inhibit adhesion (platelet-collagen interaction) (165), suggests that, indeed, prostacyclin allows platelets to stick to vascular tissue and to interact with it, while at the same time it prevents or limits thrombus formation. Certainly, platelets adhering to a site where prostacyclin synthetase is present could well feed the enzyme with endoperoxide, thereby producing prostacyclin and preventing other platelets from clumping onto the adhering platelets, limiting the cells to a monolayer. Recently, Weiss and Turitto (378) 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.

It is also possible that formed elements of blood such as the white cells, which produce endoperoxides and TXA_2 (73, 124, 166), interact with the vessel wall to allow

formation of prostacyclin, as do the platelets. This suggestion, coupled with the fact that prostacyclin may modulate white cell behaviour (169, 380), could well mean that prostacyclin plays a role in the control of white cell migration during the inflammatory response (see below).

Unlike other prostaglandins, such as PGE_1 and $PGF_{2\alpha}$, prostacyclin is not inactivated on a passage through the pulmonary circulation (83), and this is probably because prostacyclin, although a good substrate for lung PGDH, is not a substrate for the uptake mechanism responsible for transport from the circulation to the intracellular enzyme (160). Indeed, the lung can constantly release small amounts of prostacyclin into the circulation (138, 247). The concentrations of prostacyclin are higher in arterial than in venous blood because of the overall inactivation of about 50% in one circulation through peripheral tissues (83). Thus, platelet aggregability in vivo is modulated by circulating prostacyclin, which will reinforce the actions of locally-produced prostacyclin throughout the vasculature. The possibility that other organs also release PGI_2 into the circulation as a result of a specific stimulus, such as bradykinin, has been suggested recently (257).

B. Mechanism of Action

Prostacyclin inhibits platelet aggregation by stimulating adenylate cyclase, leading to an increase in cAMP levels in the platelets (128, 357). In this respect prostacyclin is much more potent than either PGE_1 or PGD_2 (357). 6-oxo-$PGF_{1\alpha}$ has very weak antiaggregating activity and is almost devoid of activity on platelet cAMP (357).

Prostacyclin is not only more potent than PGE_1 in elevating cAMP but the elevation persists longer. The elevation induced by PGE_1 starts falling after 30 sec, while prostacyclin stimulation is not maximal until after 30 sec and is maintained for 2 min after which it gradually wanes over 30 min (128). Prostacyclin is also a strong direct stimulator of adenylate cyclase in isolated membrane preparations (128).

Prostacyclin, as well as the less active PGE_1 and PGD_2, seems to increase adenylate cyclase activity by acting on two separate receptors on the platelet membrane (230, 387). PGE_1 and prostacyclin act on one, whereas PGD_2 acts on another. This is shown both by differences in activity in different species (389) and by the use of a prostaglandin antagonist (86) that selectively prevents the inhibition of platelet aggregation induced by PGD_2 but not that induced by prostacyclin or PGE_1 (387). Moreover, studies of agonist-specific sensitization of cAMP accumulation in platelets show that PGE_1 or PGE_2 can desensitize for subsequent PGE_1 or prostacyclin activation and that subthreshold concentrations of prostacyclin desensitize PGE_1 stimulation. PGD_2, however, desensitizes to a further dose of PGD_2 but not to PGE_1 or prostacyclin (230). These results suggest (230, 387) that the previously recognized PGE_1 receptor in platelets (233) might be in fact a prostacyclin receptor.

There have not been many detailed studies on the mechanism of action of prostacyclin. In contrast to TXA_2 it enhances Ca^{++} sequestration (194). Moreover, an inhibitory effect on platelet phospholipase (208, 233) and platelet cyclo-oxygenase have been described (222). All these effects are related to its ability to increase cAMP in platelets. Moreover, prostacyclin inhibits endoperoxide-induced aggregation, which suggests additional sites of action still undefined but dependent on the cAMP effect (233). These observations have extended and given important biological significance to the original observation of Vargaftig and Chignard (369), who demonstrated that substances such as PGE_1 that increase cAMP in platelets inhibit the release of TXA_2 (measured as RCS) in platelets. Prostacyclin, by inhibiting several steps in the activation of the arachidonic acid metabolic cascade, exerts an overall control of platelet aggregability in vivo.

The fact that prostacyclin increases cAMP levels in cells other than platelets (130, 181) and the possibility that in those

cells an interaction with the thromboxane system could lead to a similar control of cell behaviour to that observed in platelets suggests that the PGI_2/TXA_2 system has wider biological significance in cell regulation and the definition of cell receptors for prostaglandins.

C. Prostacyclin, Thromboxane A_2- Thrombosis, and Haemostasis

We have explained above the role of prostaglandin endoperoxides in platelet aggregation in vitro. However, in vivo, it is now clear that prostaglandin endoperoxides are at the crossroads of arachidonic acid metabolism, for they are precursors of substances with opposing biological properties (see fig. 1). On the one hand, TXA_2 produced by the platelets is a strong contractor of large blood vessels and induces platelet aggregation. On the other hand, prostacyclin produced by the vessel wall is a strong vasodilator and the most potent inhibitor of platelet aggregation known. Each substance has opposing effects on cAMP concentrations, thereby giving a balanced control mechanism that will, therefore, affect thrombus and haemostatic plug formation. Selective inhibition of the formation of TXA_2 should lead to an increased bleeding time and inhibition of thrombus formation, whereas inhibition of prostacyclin formation should be propitious for a "prothrombotic state." The amount of control exerted by this system can be tested, for selective inhibitors of each pathway have been described (251, 271).

The utilization of aspirin as a pharmacological tool to investigate the interaction between these two substances has been fruitful. Aspirin is highly active against platelet cyclo-oxygenase in vivo and in vitro. Whereas the analgesic and antiinflammatory dose in people is about 1.5 g a day, a single tablet of aspirin (325 mg) inhibits the cyclo-oxygenase of platelets by about 90% (49). Moreover, this effect is long-lasting because aspirin acetylates the active site of the enzyme leading to irreversible inhibition (314, 315). Platelets are unable to synthesize new protein (225) and cannot replace the cyclo-oxygenase. Therefore, the inhibition will only be overcome by new platelets coming into the circulation after the block of cyclo-oxygenase in megakaryocytes has worn off (49). It is interesting that the cyclo-oxygenase of vessel walls is much less sensitive to aspirin than that of platelets (17). It has also been suggested that endothelial cells in vitro and in vivo recover from aspirin inhibition by regeneration of the cyclo-oxygenase (70, 195). This has been reinforced by the observation that the recovery of the endothelial cell synthetase in cell cultures can be prevented by treatment with the protein synthesis inhibitor, cycloheximide (71).

Studies in rabbits (9, 201) suggest that low doses of aspirin reduce TXA_2 formation to a greater extent than prostacyclin formation. These experiments also showed that inhibition of TXA_2 formation is longer lasting than that of prostacyclin. Indeed, infusions of arachidonic acid into rabbits and cats lead to an antithrombotic effect and to an increase in bleeding time that can be potentiated by low doses of aspirin and blocked by larger doses (which would inhibit prostacyclin and TXA_2 formation) (9, 201).

Any antithrombotic activity of dipyridamole can also be linked with the prostacyclin system, for this substance is an inhibitor of phosphodiesterase and thus amplifies the effects of the increase in cAMP induced by circulating prostacyclin (246). Dipyridamole is most effective when there is a favourable PGI_2/TXA_2 ratio, after a small dose of aspirin or more than 24 h after a high dose. These experiments have provided the explanation for the well recognized synergism of small doses of aspirin and dipyridamole in experimental models or in clinical experience (156, 180). A selective inhibitor of thromboxane formation and a phosphodiesterase inhibitor should now be tested for antithrombotic efficacy, since theoretically this provides an advantage over aspirin in leaving endoperoxides

from platelets available for the vessel walls or other cells to synthesize prostacyclin.

These results also suggest that, when aspirin is used, a small daily dose or large doses at weekly intervals, alone or in combination with a phosphodiesterase inhibitor such as dipyridamole, would be a useful therapeutic combination. Clearly, it is important not to use too high a dose of aspirin, for that will neutralize the whole system including prostacyclin formation and might lead to deleterious effects.

Until the discovery of prostacyclin, the use of aspirin as an antithrombotic, based on its effects on platelets, looked very clear (219). Now, however, the situation needs further clarification, especially with respect to the optimal dose of aspirin. Aspirin in high doses (200 mg/kg) increases thrombus formation in a model of venous thrombosis in the rabbit (195), and in vitro treatment of endothelial cells with aspirin enhances thrombin-induced platelet adherence to them (70). In addition, there is an inverse correlation between platelet adhesion and aggregation and the amount of prostacyclin produced by the tissue. Moreover, aspirin treatment of arterial tissue in vitro increases its thrombogenicity (Baumgartner, personal communication).

In people, O'Grady and Moncada showed that a low single dose of aspirin (0.3 g) increased bleeding time 2 h after ingestion, whereas a high dose (3.9 g) had no effect (280). Some workers have confirmed these results (302), but others have been unable to do so (121a). The variability might be linked to the differences in methodology or in the age range of the subjects. Moreover, after a single high dose of aspirin (3.9 g) platelet aggregation and TXA_2 formation are blocked 2 h after aspirin. The bleeding time is unchanged at that time but 24 and 72 h after aspirin it is increased and slowly recovers toward pretreatment levels over a period of 168 hr in a manner that is a mirror image of the recovery of TXA_2 formation and platelet aggregability (8). An extension of the concept comes from the demonstration that tranylcypromine, an inhibitor of prostacyclin formation, enhances platelet aggregation in an experimental model of thrombosis in the microcirculation of the brain of the mouse (313). All these results clearly demonstrate that the balance between TXA_2/PGI_2 is an important factor in the control of platelet aggregability in vivo. Clearly, manipulation of this control mechanism might lead to pro- or antithrombotic states of clinical relevance. In this context it is interesting that Mustard's group has shown that hydrocortisone treatment of normal or thrombocytopenic rats blocks prostacyclin formation in the vessel wall and decreases the bleeding time (27), a result which would be expected from the interference with arachidonic acid release induced by steroids (106). Blajchman et al. (27) mention that for years it has been the clinical impression that steroids decrease the bleeding time in thrombocytopenic patients without increasing the platelet count.

Whether other drugs exert their antithrombotic effect by acting on the prostacyclin/thromboxane system mechanism is not yet known but studies with sulphinpyrazone in cultured endothelial cells (125) and ticlopidine given orally to rats (15) suggest that these compounds have little or no effect on prostacyclin formation at concentrations at which they affect platelet behaviour. A compound that might stimulate prostacyclin formation in people after oral ingestion has also been described (373).

Selective inhibition of prostacyclin formation by lipid peroxides could also lead to a condition in which platelet aggregation is increased and this could play a role in the development of atherosclerosis. Indeed, lipid peroxidation takes place in plasma as a nonenzymic reaction (158) and it is known to occur in certain pathological conditions (333). Hence, lipid peroxides present in these conditions could be shifting the balance of the system in favour of TXA_2 and predispose to thrombus formation. In this context it is interesting that Gryglewski's group (76) has found that there is a strong reduction in prostacyclin formation by hearts or vessel walls of rabbits made ath-

erosclerotic. Similarly, it has been reported that human atherosclerotic tissue does not produce prostacyclin, whereas tissue obtained from a nearby normal vessel does (10).

The role of lipid peroxides in the development of atherosclerosis has been debated for the last 25 years since Glavind et al. (121) described the presence of lipid peroxides in human atherosclerotic aortae. They found the peroxide content in diseased arteries to be directly proportional to the severity of the atherosclerosis. Subsequent investigations by Woodford et al. (396) suggested that Glavind's findings were artifactual, ascribing the presence of lipid peroxides to their formation during the preparative procedure (396). Despite this, the presence of conjugated diene hydroperoxides in lipids of human atheroma has again been reported (112, 113) and lipid peroxides have been found in atherosclerotic rabbit aortae (186) subjected to an extraction procedure that avoids lipid peroxidation in vitro. Some authors (39, 157) favour the suggestion that lipid peroxides are present in atherosclerotic plaques, whether or not these peroxides act by inhibiting prostacyclin formation and as a consequence reduce the wall's defence mechanism. This theory is of interest, especially since other substances related to atherosclerosis such as the cholesterol carriers, low density lipoproteins (LDL), have also been shown to inhibit prostacyclin formation in endothelial cell cultures (275).

D. Therapeutic Potential of Prostacyclin

Prostacyclin or chemical analogues may find a use as "hormone replacement" therapy in conditions such as acute myocardial infarction or "crescendo angina" and other states in which excessive platelet aggregation takes place in the circulation. Moreover, we have suggested its use in extracorporeal circulation systems such as cardiopulmonary bypass and renal dialysis (252). In these systems the main problems are platelet loss with the formation of microaggregates that, when returning to the patient, are responsible for the cerebral and renal impairment observed after bypass (1, 37). In addition, there are side effects associated with the chronic use of heparin, especially the development of osteoporosis (134).

Several antiplatelet drugs have been suggested to deal with these two problems and some have been used with moderate success. PGE_1 has been reported to be beneficial during cardiopulmonary bypass (18). However, prostaglandins of the E type induce diarrhoea (218), an effect not shared by prostacyclin (309, 363). Therefore prostacyclin is not only more potent but more specific in achieving platelet protection. Prostacyclin has now been beneficially used in several systems of extracorporeal circulation in experimental animals, including renal dialysis, cardiopulmonary bypass, and charcoal haemoperfusion (48, 67, 215a, 397). In renal dialysis, prostacyclin can replace heparin altogether (397). In charcoal haemoperfusion, heparin is also necessary since charcoal particles seem to activate directly the clotting cascade (48). Following reports that PGE_1 has been used successfully in the treatment of peripheral vascular disease (50), prostacyclin has been shown to have a similar effect, producing a long-lasting increase in muscle blood flow, disappearance of ischemic pain, and healing of throphic ulcers after an intraarterial infusion to the affected limb for 3 days (354).

VII. Unstable Derivatives of Other Fatty Acids

Endoperoxides derived from dihomo-γ-linolenic acid and eicosapentaenoic acid have been described and some of their biological activities have been studied. Needleman et al. (266) showed the formation of PGG_1, PGH_1, PGG_3, and PGH_3 when sheep seminal vesicles were incubated with the appropriate precursor. Moreover, they showed that when PGH_3 was incubated with indomethacin-treated platelet microsomes (thromboxane synthetase) TXA_3 was formed. However, they could not detect the formation of TXA_1 when PGG_1 or H_1

were incubated with the same enzyme preparation. The existence of TXA_1 has been demonstrated by Falardeau et al. (91), although the conversion rate of the endoperoxide precursor is low.

PGH_1 contracts the rabbit aortic strip (about one-fifth as active as PGH_2), the pig coronary artery strip (equiactive to PGH_2), and the bovine coronary artery, a preparation on which PGH_2 is relaxant or has a biphasic effect (265). PGH_3 also relaxes the bovine coronary artery, but is less potent than PGH_2 (265). It contracts the rabbit aortic strip where it is one-fifth as potent as PGH_2. TXA_3 also contracts the three bioassay tissues and is approximately one-fifth as active as TXA_2.

In Platelets

In contrast to arachidonic acid, dihomo-γ-linolenic acid and eicosapentaenoic acid do not induce aggregation but prevent the second phase of ADP-induced aggregation (331). Originally, Willis et al. (393) showed that PGG_1 and PGH_1 do not aggregate human platelets, nor do they inhibit the aggregation induced by LASS (PGH_2). However, Gorman and Miller (131) later showed that PGH_1 blocks PHG_2 aggregation and elevates cyclic AMP. This has been confirmed by Needleman et al. (269). On the other hand, PGH_3 or TXB_3 have little or no proaggregating effect (305).

The study of the unstable intermediates of dihomo-γ-linolenic and eicosapentaenoic acid has gained momentum as a result of the theories on the use of these fatty acids as dietary components with "antithrombotic" properties. Before the discovery of prostacyclin, it was suggested that the use of dietary dihomo-γ-linolenic acid, the precursor of the E_1 series of prostaglandins, could be an approach to the prevention of thrombosis, for PGG_1 was not proaggregating (391). Furthermore, if the platelet made PGE_1 it might inhibit aggregation. Some reports tended to agree with this proposal (332) but there is some controversy, for, in the rabbit, feeding with dihomo-γ-linolenic acid leads to an increase in the tissue content of this acid without change in platelet responsiveness, at least to ADP (279). Most of the positive studies are performed in vitro in situations in which platelets have no contact with vessel walls (196).

The use of dihomo-γ-linolenic acid in an attempt to direct the synthetic machinery of the platelets is not the most rational approach for prevention of thrombosis, because the endoperoxides PGG_1 and PGH_1 are not substrates for prostacyclin synthesis and an accumulation of these substances or their precursor might adversely affect the prostacyclin protective mechanism. Indeed, it has recently been shown (274) that in endothelial cell cultures the addition of dihomo-γ-linolenic acid to the culture medium reduces the release of prostacyclin-like material.

Eicosapentaenoic acid, the precursor of the PG_3 series, can, however, act as a precursor for an antiaggregating agent, probably Δ^{17}-prostacyclin (140, 269). This compound has been synthesized chemically and has similar properties and potency to prostacyclin (189). Thromboxane A_3 is synthesized by platelet microsomes and is less proaggregatory than thromboxane A_2 (140, 269, 305, 335). Originally, Needleman and colleagues (269) reported that TXA_3 and PGH_3 directly stimulate platelet adenylate cyclase and inhibit aggregation. However, it is now clear that in this work the formation of PGD_3 and PGE_3 from PGH_3 and their possible interaction were underestimated. Since these compounds strongly counteract the proaggregatory effect of PGH_3 and TXA_3 (140), the net effect of the transformation of eicosapentaenoic acid in the platelets, unlike that of arachidonic acid, is not a proaggregatory effect. Thus the use of this fatty acid could afford a dietary protection against thrombosis (85). Indeed, we and others (85) have suggested that the low incidence of myocardial infarction in Eskimos and their increased tendency to bleed could be due to the high eicosapentaenoic acid and low arachidonate content of their diet and consequently of their tissue lipids. Most of the results de-

scribed in this section are preliminary and more work is needed particularly in whole animals before a less tentative conclusion can be made.

VIII. Gastrointestinal Tract

6-oxo-$PGF_{1\alpha}$ was first isolated as the major product of endoperoxide metabolism in homogenates of rat stomach (285). We have shown that prostacyclin is the major prostaglandin product of the gastric mucosa of several species (249) and, furthermore, that it is a potent vasodilator of rat stomach mucosa in vivo (386), where it also reduces acid secretion induced by pentagastrin. Thus, prostacyclin release may be involved both in functional hyperemia of the mucosa during acid secretion and in acting as a natural brake on the secretion, a role previously ascribed to PGE_2 (217). Inhibition of prostacyclin production by prostaglandin synthetase inhibitors, such as aspirin and indomethacin, could explain why this general group of substances tends to cause gastrointestinal irritation. In addition, prostacyclin, in contrast to PGE_2, does not cause diarrhoea, but inhibits diarrhoea and enteropooling induced by several agents (309). This fact, together with the demonstration of prostacyclin formation in intestinal homogenates (385), suggests a role in the regulation of intestinal motility and probably fluid transport.

There is until now only one report that TXA_2 is formed by bovine stomach mucosa microsomes (6). The significance of this finding remains to be assessed.

IX. Reproductive System

6-oxo-$PGF_{1\alpha}$ has been identified as a major prostaglandin product of the pseudopregnant and pregnant rat uterus (78, 94). Moreover, a prostacyclin-like substance increases in the myometrium and decidual tissue during pregnancy in the rat (388). Prostacyclin-like activity has also been identified in human placenta (261).

Prostacyclin or 6-oxo-$PGF_{1\alpha}$ contract rat uterine strips in vitro but are much less active than PGE_2 or $PGF_{2\alpha}$ (282). In the nonpregnant human myometrium in vitro, prostacyclin induces a biphasic response consisting of a short-lasting contraction followed by a lasting relaxation and disappearance of spontaneous tone (283). Prostacyclin also inhibits the contractions induced by $PGF_{2\alpha}$ but not those induced by $BaCl_2$ or vasopressin. 6-oxo-$PGF_{1\alpha}$ weakly contracts uterine muscle (283).

Prostacyclin relaxed the human fallopian tube and partially reversed $PGF_{2\alpha}$ contractions. 6-oxo-$PGF_{1\alpha}$ was a weak contractor (283). The role played by prostacyclin in uterine physiology is not at present defined, but it is possible that it regulates the increasing blood flow as the uterus expands. It could also play a modulatory role in the contractions induced by other prostaglandins. Formation of prostacyclin or 6-oxo-$PGF_{1\alpha}$ has also been demonstrated in amnion, chorion, and decidua (235). TXB_2 has also been isolated from rat decidual tissue (389).

X. Inflammation and Anaphylaxis

In the last four years there have been increasing reports involving TXA_2, prostacyclin, and prostaglandin endoperoxides in the inflammatory process.

Willis et al. (393) reported that LASS (later characterized chemically as PGH_2) did not show any pain-producing activity in mice and very little proinflammatory activity when injected into the paws of rats. More recent reports have indicated that prostaglandin endoperoxides or their stable analogues have similar effects to PGE_2, potentiating the proinflammatory effects of other mediators (145, 367). As in other systems, it is not clear whether the effects of prostaglandin endoperoxides are direct or due to their conversion into other substances such as PGE_2, prostacyclin, or TXA_2.

Kuehl et al. (202) have pointed out that the conversion from PGG_2 to PGH_2 gives origin to an oxygen moiety that might be responsible for some of the signs and symptoms of inflammation. Although this hy-

pothesis is attractive, recent work by Williams and Peck (390) and our own observations (238) show that PGG_2 is not as potent as PGH_2 in inducing exudation or in potentiating bradykinin-induced exudation in a rabbit skin model or in carrageenin-induced oedema in the rat paw.

So far there are no reports on the effects of TXA_2 in inflammation, and its role will be difficult to assess in view of its very short half-life. However, phagocytosing white cells (73, 124, 166) and aggregating platelets (152) produce TXA_2. Thromboxane B_2, the stable end product of TXA_2, has been reported to be chemotactic for mouse polymorphonuclear leukocytes (PMNs) (31), although a purified sample of TXB_2 free of HHT was not chemotactic for human PMNs (122). Moreover, fluid obtained from carrageenin-induced granuloma converts arachidonic acid into TXB_2 in vitro (53). TXB_2 levels are increased in the synovial fluid of rheumatoid arthritis patients (360) and in the fluid of carrageenin-induced inflammation in the rat (171). In view of this, selective inhibition of TXA_2 formation could modify the inflammatory process. Recent work with imidazole in a knee-joint inflammatory model in the pigeon suggests that in this model imidazole is acting more as a cyclo-oxygenase than a thromboxane synthetase inhibitor, since it lowers the levels both of prostaglandins and of TXB_2 (294).

6-oxo-$PGF_{1\alpha}$ has been identified in the inflammatory exudate of chronic granulomas and, moreover, prostacyclin and 6-oxo-$PGF_{1\alpha}$ induce erythema when injected into the rabbit skin although they are less active than PGE_2. Prostacyclin also potentiates carrageenin-induced oedema in the rat paw (164, 200), increases vascular permeability, and enhances vascular permeability induced by other inflammatory mediators (259). It is also more potent than PGE_1 or PGE_2 in enhancing carrageenin-induced hyperalgesia in rats, although its activity is short-lived (97, 164). Because of these effects, prostacyclin could be involved, along with other prostaglandins and TXA_2, in the genesis and maintenance of some of the signs of the acute inflammatory reaction. On the other hand, 6-oxo-$PGF_{1\alpha}$ is produced by macrophages (183) and it may be produced by other formed elements of blood such as white cells. The facts that prostacyclin inhibits chemotaxis of human PMNs without inhibiting phagocytosis (380) and inhibits white cell margination in the hamster cheek pouch model (169) suggest that prostacyclin might also play a role in modulating white cell movement during inflammation. At this stage, however, the precise role of prostacyclin vis a vis other products of arachidonic acid in inflammation has not been determined. The use of lipid peroxides, which are selective inhibitors of prostacyclin formation (135), or of the recently developed rabbit antiserum that inactivates prostacyclin (45, 104) will help to clarify this problem.

Although prostaglandins and RCS were identified several years ago in the effluent of normal or sensitized lungs in vitro after different stimuli, it has only recently been recognized that the main metabolites of arachidonic acid via the cyclo-oxygenase pathway in the lung are prostacyclin and TXA_2 (74, 150). In consequence, the existing hypotheses about the opposing roles played by PGE_2 and $PGF_{2\alpha}$ on bronchial muscle (227) have been losing support. Sensitization followed by immunological challenge increases the amount of TXA_2 (measured as 15-oxo-13-14 dihydro TXB_2) and decreases the amount of prostacyclin (measured as 6-oxo-$PGF_{1\alpha}$ or 6-15 dioxo 13, 14 dihydro $PGF_{1\alpha}$) released from endogenous substrate or from exogenously added arachidonic acid (30). TXA_2 is bronchoconstrictor and vasoconstrictor whereas prostacyclin, although not a very potent bronchodilator (352), is effective in antagonizing bronchoconstriction induced by other agents (25) and is a vasodilator. Thus, it is tempting to speculate that the balance between these compounds plays a role in lung physiology. The release of these compounds appears to be compartmentalized,

the parenchyma being the tissue source of TXA_2 (137), and tracheal and vascular tissue being the source of prostaglandin-like material (137) and probably prostacyclin. It is possible that pathological conditions shift the balance away from prostacyclin towards TXA_2 or lead to an increased concentration of prostaglandin endoperoxides that are bronchoconstrictors (393) (see above) and probably strong stimulants of vagal, lung "irritant" receptors like their stable analogues (120). It has been postulated recently that lipid peroxides synthesized by the lungs via the lipoxygenase pathway increase the formation of TXA_2 and other mediators in the guinea-pig lungs "in vitro" (2, 3). In addition, there have been several indications in the last few years that slow reacting substance in anaphylaxis (SRS-A) could be a product of arachidonic acid (16). Recently, the structure of a slow reacting substance (SRS) from mouse mastocytoma cells has been reported as a novel product of the lipoxygenase pathway. It is formed by addition of cysteine to an unstable epoxide (at C5) intermediate in the formation of dihydroxylated arachidonic acid metabolites in leukocytes. The name leukotriene has been introduced as a generic name for eicosanoids like SRS, which are noncyclized C20 carboyxlic acids with one or two oxygen substituents and three conjugated double bonds (260, 326).

Leukotrienes share certain structural features with some oxygenated products of arachidonic acid in human and rabbit PMNs (33, 34). It is not know whether these compounds are identical to SRS-A released from the lungs, but these findings highlight the importance of the products of the different lipoxygenases (5, 12, 15) in cell migration, inflammation, and in other conditions such as increased mediator release and airway reactivity during asthma or anaphylaxis. The study of interactions between lipid peroxides, these newly described leukotrienes, and the prostacyclin/thromboxane A_2 system will undoubtedly lead to further research and to new and exciting developments.

XI. Metabolism of Endoperoxides

While all tissues can synthesize prostaglandin endoperoxides, the further metabolism of these endoperoxides differs according to the tissue and it is crucial for the understanding of the biological significance of arachidonic acid metabolism to recognize the controlling factors. Unfortunately, the metabolism of endoperoxides depends on several factors that are not clearly elucidated. Certainly the presence of different isomerases varies from tissue to tissue (136, 277, 288, 345) and sometimes great specialization is found. Whilst lung and spleen can yield the whole range of cyclo-oxygenase products (PGE_2, D_2, $F_{2\alpha}$, HHT, TXA_2, and prostacyclin), other cells like platelets make mostly TXA_2 (152) and the vessel wall makes mainly prostacyclin (190, 226, 242).

Even though PGE_2, PGD_2, and $PGF_{2\alpha}$ can be formed nonenzymically, isomerases for PGE_2 and PGD_2 have been clearly established (277). However, the existence of a reductase that synthesises $PGF_{2\alpha}$ is in doubt (277).

The biochemical conditions in which the enzymes are studied influence the substances produced. For example, the presence of reducing agents such as ferriheme increases the production of $PGF_{2\alpha}$, and the presence of proteins such as albumin increases the isomerization of the endoperoxide towards PGD_2. On the other hand, the presence of glutathione favours the generation of PGE_2 (277, 325, 364).

It is not clear whether conversion of *exogenous* arachidonic acid or endoperoxides (used in many biochemical studies of substrates) follows the same pattern of metabolism as arachidonic acid released within the cell environment from *endogenous* sources. Finally, it is possible that hormonal or other influences like immunological sensitization or mechanical damage (74, 255) change the direction of the metabolism of the endoperoxides. This possibility, although only superficially explored at the moment, might open fascinating areas of research. Indeed, we have suggested that a

diversion of pathway from some products of metabolism to others might explain physiopathological changes (250). Studies in the next few years along these lines will clarify a great deal about the fate and final biological effect of prostaglandin endoperoxides.

XII. Metabolism of Thromboxane A_2

The conversion of TXA_2 into TXB_2 seems to be nonenzymic and occurs very rapidly (152). It is not known whether TXA_2 or TXB_2 are circulating substances. Most of the evidence suggests that TXA_2 is a local hormone released during pathological (74, 255) or near-pathological conditions, such as during the formation of a haemostatic plug (9).

In monkeys some TXB_2 injected intravenously is excreted unconverted (13%), and 32% of the urinary radioactivity is in the form of dinorTXB_2 (a single-step oxidation product) (197). In another study (310), in which TXB_2 was infused into monkeys, the major product in urine was also found to be dinorTXB_2. In man, after intravenous infusion of tritium labelled TXB_2, 74% of the total radioactivity was recovered in the urine within 13 h. The major metabolite recovered (16.8%) was 2,3-dinorTXB_2, a product of a single-step oxidation. Other ill-defined products were also detected in considerable amounts (311). Boot et al. (32) have found that sensitized lungs of guinea pigs when challenged produce among other things a metabolite of TXB_2, namely 15-oxo-13, 14-dihydroTXB_2 (42% of total label).

XIII. Metabolism of Prostacyclin

Prostacyclin or 6-oxo-$PGF_{1\alpha}$ metabolism has been studied in whole animals and in vitro in tissue slices or homogenates (395). In vivo in the rat, 6-oxo-$PGF_{1\alpha}$ is partially excreted intact and partially as dinor-6-oxo-$PGF_{1\alpha}$ and dinor-ω-1-hydroxy-6-keto $PGF_{1\alpha}$ (286). Sun and Taylor (347) have identified seven metabolites of prostacyclin in urine after intravenous administration. Of the administered dose, 77% is excreted within 3 days (33% in urine and 44% in faeces). Most of the metabolism was by 15-hydroxy-prostaglandin dehydrogenase giving 15-oxo-PGI_2 as the first degradation step. However, some compounds formed suggested that some of the compound had first been converted nonenzymically to 6-oxo-$PGF_{1\alpha}$ and then metabolized. This study demonstrates that in vivo 6-oxo-$PGF_{1\alpha}$ is not a major metabolite of prostacyclin and that 6-oxo-$PGF_{1\alpha}$ should be considered mainly as a chemical degradation product.

Studies in vitro have demonstrated that in lung (228) and blood vessel (346) prostacyclin is rapidly oxidized by the 15-hydroxy prostaglandin dehydrogenase to the corresponding 15-oxo compound. However, under the same conditions, 6-oxo-$PGF_{1\alpha}$ was a poor substrate for this enzyme (228, 346).

XIV. Thromboxane A_2 and Prostacyclin Imbalance in Other Pathological States

Increased production of prostaglandin endoperoxides or TXA_2 in vitro by platelets has been found in patients with arterial thrombosis, deep venous thrombosis, or recurrent venous thrombosis (207). These conditions are associated with a shortened platelet survival time (207). In addition, increased sensitivity to aggregating agents and increased release of RCS have been described in rabbits made atherosclerotic by diet (328) and in patients who have survived myocardial infarction (351). Moreover, platelets from rats made diabetic release more TXA_2 (159, 188). Diseases associated with changes in prostacyclin production have been described. An increased production has been suggested in uraemic patients to explain their haemostatic defect (306). On the other hand, a lack of prostacyclin production has been suggested in patients with idiopathic thrombocytopaenic purpura (308). Both diseases are linked by the accumulation during uraemia or the lack of production during idiopathic thrombocytopaenia purpura of an ill-de-

fined "plasma factor" that stimulates prostacyclin synthesis (216).

More recently, a decreased production of prostacyclin by the blood vessels of rats made diabetic has also been described (159, 188); this decreased production can be corrected by chronic treatment with insulin (159). Finally, increased prostacyclin production has been described in blood vessels of spontaneously hypertensive rats (287).

As yet, a clear relationship between different diseases and the PGI_2/TXA_2 balance is not established. However, it seems that conditions that favour the development of thrombosis are associated with an increase in TXA_2 and a decrease in prostacyclin formation, whereas increased prostacyclin formation plus decreased TXA_2 is present in some conditions associated with an increased bleeding tendency. These are, however, wide generalizations that need much more experimental and clinical evidence.

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