PROSTAGLANDINS, PLATELETS, AND ATHEROSCLEROSIS

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I. INTRODUCTION

The aggressive behavior of blood platelets[310,311,318] and the defensive role of vascular endothelium[317,321] in the development of arterial thrombosis and atherosclerosis are unquestionable. On the other hand an interaction between platelets and vascular endothelium is a morphological phenomenon,[1] the pathophysiological significance of which is explained as the "support" offered by platelets to the endothelium.[2-4]

I believe that a common biochemical link for these two diverse platelet functions is closely associated with the metabolism of arachidonic acid in platelets and in arteries. Recent discoveries of a vasoconstrictive, proaggregatory factor (thromboxane A_2) in blood platelets[5,6] and a vasodilative and antiaggregatory factor (prostacyclin) in arteries[7-10] focused the attention of many cardiologists on the metabolism of arachidonic acid in the circulation.

Thromboxane A_2 and prostacyclin are formed from prostaglandin endoperoxides, during the progression of the so-called "arachidonic acid cascade."[10a] A part of the "cascade" is constituted by "classical" prostaglandins (PGE_2, PGD_2 and $PGF_{2\alpha}$). Prostaglandins are of no interest to us since they are not major metabolites of arachidonic acid either in platelets[323] or in blood vessels.[325] The word "prostaglandins" in the title has been used as an old-fashioned designation for prostacyclin and thromboxane A_2.

An attempt will be made to predict the possible impact of the discovery of thromboxane A_2 and prostacyclin on our understanding of the pathogenesis, prevention, and treatment of arterial thrombosis and atherosclerosis.

II. THE ARACHIDONIC ACID CASCADE

Arachidonic acid (eicosa-5,8,11,14-tetraenoic acid) (Figure 1) is a polyunsaturated fatty acid (PUFA). In order to stay healthy we need several PUFAs of plant origin to supplement our diet. These PUFAs are jointly named "vitamin F" or more correctly, "essential fatty acids" (EFA). In mammalian organisms, EFA can be desaturated elongated. Arachidonic acid and its trienoic analog (dihomo-γ-linolenic acid) seem to be the most important products of the desaturation of linoleic acid (18:2ω6).

In 1964 Bergstrom et al.[11] and Van Dorp et al.[12] demonstrated that in mammalian seminal vesicles arachidonic acid is the substrate for the generation of prostaglandin E_2 (PGE_2). As far as the circulatory system is concerned, arachidonic acid is transformed in platelets to proaggregatory thromboxane A_2 (TXA_2)[5,6] and in blood vessels to antiaggregatory prostacyclin (PGI_2).[7-10] We are not so sure about the site and extent of the in vivo generation of two other antiaggregatory prostaglandins, i.e., PGD_2 from arachidonic acid[13] and PGE_1 from dihomo-γ-linolenic acid.[14]

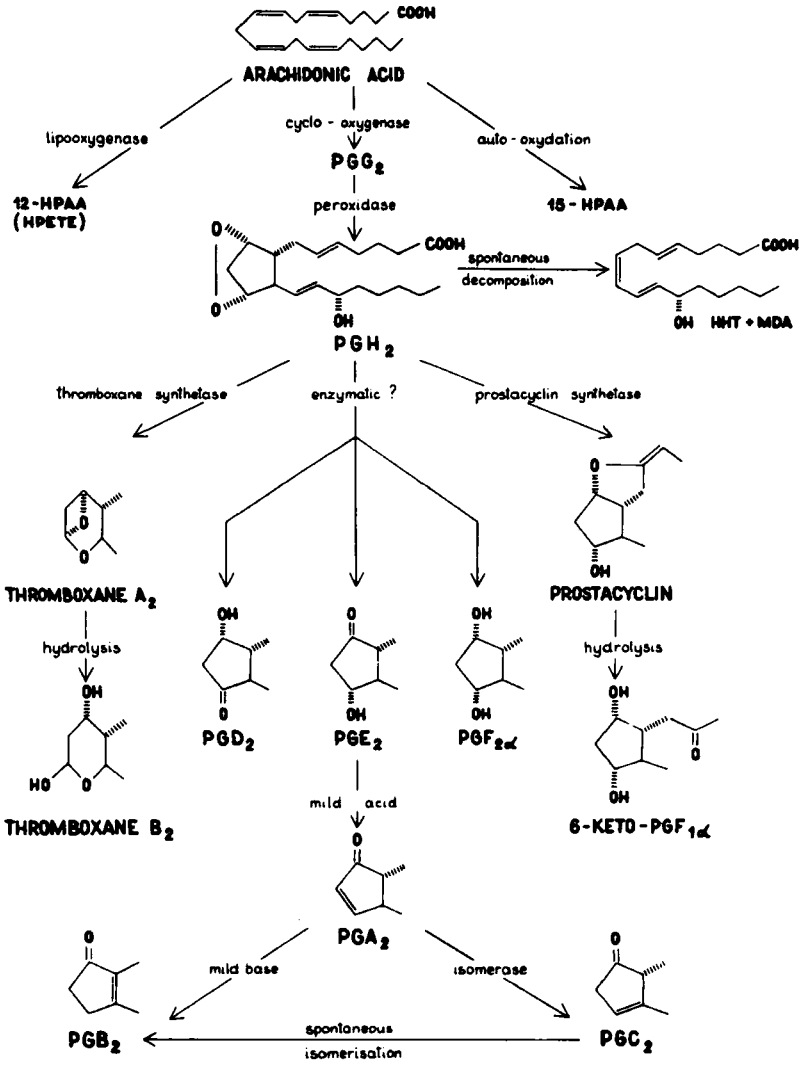


FIGURE 1. The scheme of the arachidonic acid cascade. PGG_2 and PGH_2 (cyclic prostaglandin endoperoxides), 12-HPAA (HPETE) (12-L-hydroperoxy-5,8,10,14-eicosatetraenoic acid), 15-HPAA (15-hydroperoxyarachidonic acid), HHT (12-L-hydroxy-5,8,10-heptadecatrienoic acid), MDA (malondialdehyde), PGs (prostaglandins).

The pathways by which arachidonic acid is oxygenated in the body are named "the arachidonic acid cascade." Both in platelets[15,16] and in endothelial cells,[17] arachidonic acid is esterified and fixed mainly in membrane phospholipids. In order to be metabolized by oxidative tissue enzymes, arachidonic acid has to be liberated from the membrane phospholipids by phospholipase A_2.[18-20] Phospholipase A_2 in platelets requires Ca^{++} for its biochemical activity[20,21] and it is likely that the availability of this cation regulates platelet phospholipase A_2 activity.[22] The activation of this enzyme causes the release of arachidonic acid mainly from diacyl phosphatidyl ethanolamine[15,16] which compromises only 15% of platelet membrane phospholipids.[15]

We have proposed[23,24] that the activation of phospholipase A_2 in intact tissues is hindered by glucocorticosteroids as well as by steroid anti-inflammatory drugs. Other authors have since been able to confirm our hypothesis.[25-27] Mepacrine is a direct inhibitor of phospholipase A_2 activity.[28]

In platelets[5,29] and in lung homogenates[30,31] free arachidonic acid may be the substrate for the animal lipoxygenase catalyzed synthesis of 12-hydroperoxyarachidonic acid (HPETE) and its corresponding hydroxy acid (HETE). The physiological significance of HPETE and HETE remains unknown, although HPETE has been claimed to inhibit thromboxane A_2 synthetase.[32]

During spontaneous autooxidation in animal tissues[33] or in vitro arachidonic acid is peroxidized to 15-hydroperoxy-arachidonic acid (15-HPAA), which is also the product of the enzymic peroxidation of arachidonic acid by soybean lipoxygenase.[34] Vitamin E and butylated hydroxytoluene (BHT) are selective inhibitors of spontaneous lipid peroxidation and lipoxygenase-catalyzed peroxidation.[35]

The most important pathway of the enzymatic transformation of arachidonic acid involves its cyclooxygenation to intermediate prostaglandin endoperoxides (PGG_2 and PGH_2)[36,37] and then to prostaglandins (PGE_2, PGD_2, and $PGF_{2\alpha}$), thromboxanes (TXA_2 and TXB_2), and prostacyclin (PGI_2).

Previously, when only prostaglandins were known, this multienzymic, membrane-bound, microsomic complex was called "prostaglandin synthetase". Owing to the separation and partial purification of its components[38-40] the complex was separated into a number of distinct enzymatic units. For our purposes, it is sufficient to know that *cyclo-oxygenase* transforms arachidonic acid to PGG_2, while the corresponding synthetases convert prostaglandin endoperoxides to thromboxane A_2[5,6] and to prostacyclin.[7-10] The conversion of prostaglandin endoperoxides to the "classical" prostaglandins is conceived to be an enzymatic process,[37,40] however, there are no known specific inhibitors of this conversion, and an isomerization of PGH_2 to PGD_2 takes place in the presence of serum albumin[41] alone.

Prostaglandin endoperoxides are unstable in aqueous solution and are spontaneously broken down to malondialdehyde (MDA) and to 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT).[30]

The activity of cyclooxygenase is selectively and irreversibly inhibited by aspirin, indomethacin, and by other nonsteroidal, anti-inflammatory drugs.[42-46] Any type of enzymic peroxidation of arachidonic acid is inhibited by its acetylenic analog — eicosa-5,8,11,14-tetraynoic acid (TYA).[45,47] The only known prostacyclin synthetase inhibitors are lipid peroxides (e.g., 15-HPAA) and tranylcypromine.[7] Thromboxane synthetase inhibitors are reviewed in Section X.

Prostaglandin endoperoxides play a pivotal role in the metabolism of arachidonic acid, however, as far as blood platelets and arteries are concerned, I believe that prostaglandin endoperoxides are no more than unstable intermediates in the synthetic pathway of thromboxane A_2 or prostacyclin. The inherent biological activity of prostaglandin endoperoxides reported so far[48-51] may be attributed to the generation of either of their two hormonal products. Indeed, PGH_2-induced relaxation of a mesenteric artery strips is abolished by pretreatment with a prostacyclin synthetase inhibitor (15-HPAA)[9] and PGH_2-induced platelet aggregation is inhibited by a thromboxane synthetase inhibitor (azo analogue of PGH_2).[52]

It may well be that in the circulation there are only two enzymatic routes opened to prostaglandin endoperoxides, namely, conversion to thromboxane A_2 in platelets and conversion to prostacyclin in vascular endothelium. The transformation of prostaglandin endoperoxides to "classical" prostaglandins perhaps acts as an "outlet" which is used by the organism when the regular pathways are blocked or when a surplus of endoperoxides is formed. I believe that the biological effects previously assigned to endogenous "classical" prostaglandins (e.g., vasodepression by PGE_2, vasoconstriction by $PGF_{2\alpha}$, or suppression of platelet aggregability by PGD_2) are actually manifestations of prostacyclin and thromboxane A_2 actions. Prostaglandins are only imitators of these two hormones.

III. ARACHIDONIC ACID

In mammalian organisms most of arachidonic acid stays "frozen" in triglycerides, cholesterol esters, and in phospholipids. There is little chance for free arachidonic acid to reach a blood level which would induce systemic biological effects. That is why prostaglandins are "local hormones". Nonetheless, it is interesting to follow the pharmacological effects of exogenous arachidonic acid in the circulation as the preference of enzymatic pathways of arachidonic acid is then disclosed.

Smith and Silver[324] reviewed the action of arachidonic acid on platelets. Arachidonic acid is avidly oxidized, both by cyclooxygenase and lipoxygenase in washed platelets which are resuspended in saline. A high concentration of arachidonic acid (300 to 600μM) has to be used in order to stimulate cyclooxygenase when platelets are suspended in human plasma. This suggests that the affinity of plasma albumin for arachidonic acid is greater than the affinity of platelet cyclooxygenase for the same substrate. The high affinity of albumin for arachidonic acid has been utilized in order to trap this fatty acid and thus to shift its metabolism in the perfused organs.[53]

On the other hand [^{14}C]-arachidonic acid, when added to platelet rich plasma, is easily incorporated into platelet phospholipids,[16] although the distribution of the incorporated arachidonic acid does not match its relative abundance in various types of natural platelet phospholipids.[15]

The binding of arachidonic acid by plasma albumin and its incorporation into tissue phospholipids[19,53] may be considered as a defensive mechanism against dangerous elevations of free arachidonic acid in the blood. An intravenous injection of arachidonic acid (1.4 mg/kg) in rabbits causes sudden death due to the obturation of their pulmonary circulation with platelet aggregates.[54] Rabbits can be protected from this arachidonate-induced death by the pretreatment with aspirin but not with heparin. Obviously, arachidonate-induced fatal thrombosis in rabbits is caused by a burst of conversion of arachidonic acid in their platelets to proaggregatory thromboxane A_2.

An intravenous infusion of arachidonic acid (0.3 mg/kg) into dogs results in thrombocytopenia, increased platelet aggregability, and vasodepression.[55] This last effect is not dependent on the presence of platelets in the circulation.[56] Unfortunately, dogs are not the best animals for this kind of study, since the involvement of the cyclooxygenase pathway in their reactions to arachidonate has been questioned.[57] On the other hand an intravenous infusion into dogs of dihomo-γ-linolenic acid (2.0 mg/kg) (the precursor of antiaggregatory PGE_1) causes decrease of platelet aggregability.[58]

Four healthy, brave volunteers ingested ethyl arachidonate at a dose of 6 g daily for a period of 2 to 3 weeks. In all these volunteers, platelet aggregability to ADP was increased.[59]

The above experiments show that an elevation in the blood level of arachidonic acid stimulates platelet cyclooxygenase. If vascular cyclooxygenase could utilize sufficient amounts of arachidonic acid, then antiaggregatory prostacyclin would be formed and, consequently, the rabbits would not die because of intravascular platelet aggregation. Also, platelet aggregability in humans would not be enhanced after administration of arachidonic acid.

Unlike arachidonic acid, prostaglandin endoperoxides, when injected at a low dose of 1 to 2 μg into rats,[51] cause a vascular reaction typical to the prostacyclin response. Vascular responses both to prostaglandin endoperoxides and to prostacyclin are "activated" across the pulmonary circulation.[51,60,61] It seems that intravenously injected PGG_2 and PGH_2 are better substrates for the endothelial prostacyclin synthetase than for the platelet thromboxane synthetase.

Summing up, the following assumptions are proposed. Firstly, in the circulatory system, a potent cyclooxygenase activity is located in platelets but not in vascular en-

dothelium. Secondly, when competing with platelet thromboxantase for prostaglandin endoperoxides, vascular prostacyclin synthetase prevails. Indeed, these two assumptions have gained additional support from the in vitro data.[7,9,62] Furthermore, in vitro a biochemical association between platelets and vascular walls has been shown to exist. Platelets are assumed to supply endothelial prostacyclin synthetase with their own prostaglandin endoperoxides.[7,62] This interaction will be the subject of Section VI.

IV. THROMBOXANE A_2

Arachidonic acid induces platelet aggregation,[63,64,324] and this effect has been assigned to vigorous conversion of arachidonic acid in platelets to prostaglandin endoperoxides. This assumption is experimentally well-founded since PGG_2 and PGH_2 are strongly proaggregatory[65,66] and cyclooxygenase inhibitors (e.g., aspirin) abolish arachidonate-induced aggregation.[63] Prostaglandin endoperoxides are labile intermediates in the arachidonic acid cascade (t ½ = 4 to 5 min, at 37°C) and teleologically thinking (as we are always inclined to do) further products of their biotransformation rather than prostaglandin endoperoxides themselves are expected to induce platelet aggregation. Neither "classical" prostaglandins nor HHT, however, are proaggregatory.

In 1974/75 Swedish scientists discovered that platelets[5] and lung homogenates[30,31] convert arachidonic acid via endoperoxides to a new, stable, nonprostaglandin compound that was later named thromboxane B_2 (TXB_2). TXB_2 is devoid of any biological activity aside from being chemotactic for leukocytes.[68]

The breakthrough came when Samuelsson and colleagues[6] were able to demonstrate that between PGH_2 and TXB_2 there exists for a short while (t ½ = 32.5 ± 2.5 (S.D.) sec. at 37°C)[67] a substance with powerful proaggregatory[67,69] and vasoconstrictor[70-72] properties. This ephemeride has been named thromboxane A_2 (TXA_2). TXA_2 is thought to be responsible for arachidonate-induced platelet aggregation and its life span in human plasma seems to be somewhat longer than in a buffer.[323]

The discovery of TXA_2 was facilitated by its earlier biological characterization as a "rabbit aorta contracting substance" (RCS). RCS is a labile factor released from guinea pig lungs during anaphylaxis,[73] by infusion of arachidonic acid[74] and histamine,[75] as well as by the activation of phospholipase A_2.[27] Rabbit spleen slices release RCS.[76] Platelets aggregated with arachidonic acid,[64] collagen,[64] thrombin,[74] and ADP[72] also release RCS. At present we know that the major component of RCS in all the above situations is TXA_2.

The only direct way to detect and quantify TXA_2 is based on its contractile action on vascular strips and on its instability. Confusion may arise from the fact that rabbit aortic strips are contracted not only by TXA_2 but also by prostaglandin endoperoxides, and, therefore, a factor characterized biologically as RCS may in fact be either TXA_2, PGG_2, PGH_2, or a mixture of these three substances.[67,74,77] In comparison to TXA_2, prostaglandin endoperoxides are more stable and 7 to 20 times less active as contractile agents on a rabbit aortic strip.[323] This, however, is a poor basis for differentiation between PGH_2 and TXA_2 by bioassay.

Bunting et al.[78] have introduced a new bioassay technique that differentiates between prostaglandin endoperoxides and TXA_2. The assay organ (a rabbit mesenteric or celiac artery strip) is relaxed by prostaglandin endoperoxides and contracted by TXA_2 (Figure 2). Amazingly enough, some of the stable synthetic endoperoxide analogues[79,80] behave like TXA_2, i.e., they contract a strip of rabbit mesenteric artery. We have successfully used an 11,9-epoxymethano analogue on PGH_2 (U 46619) as the reference substance for quantification of the TXA_2 that is generated in stimulated platelet-rich plasma (Figure 2).[72,81]

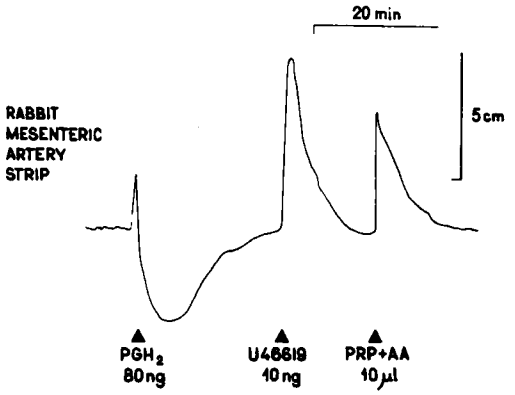


FIGURE 2. Bioassay of TXA_2 that is generated in human platelet-rich plasma (PRP) during aggregation induced by arachidonic acid (AA, 300 μM). The assay organ is a superfused strip of the rabbit mesenteric artery. This organ differentiates between TXA_2 (contraction) and prostaglandin endoperoxides (PGH_2) (relaxation). Stable prostaglandin endoperoxide analog, (U 46619 (11,9-epoxymethano analog of PGH_2, EMA), behaves like TXA_2, i.e., it contracts the assay organ.

Many authors[31,32,69,82] estimate TXB_2 by mass spectrometric or by radioimmunological techniques. Bioassay, however, allows us to get results which would not otherwise be obtainable.

For example, using bioassay we have recently found that 45 sec after instillation of ADP to platelet-rich plasma a small peak of TXA_2-like activity appears, which wanes within the next minute.[72] When TXB_2 is measured in the stimulated platelet-rich plasma, a sigmoid curve is obtained and the concentrations of TXA_2 at different time intervals may only indirectly be calculated.[69] Freshly generated TXA_2 can be trapped in methanol and then its derivative mono-*O*-methyl TXB_2 is formed and assayed using radioimmunological technique.[83] This method is the next best alternative to the direct bioassay of TXA_2.

Assay of TXA_2, mono-*O*-methyl TXB_2, or TXB_2 released from the stimulated platelets is the best way to determine the activity of platelet cyclooxygenase. Depending on the local laboratory facilities, other methods may be used to reach the same goal. Measured "markers" of the decomposition of prostaglandin endoperoxides, in platelets are available e.g., $PGF_{2\alpha}$ by radioimmunoassay,[84] a C17 hydroxyacid (HHT) by mass spectrometry,[85] or malondialdehyde (MDA) by its reaction with thiobarbituric acid.[86-88] The HHT and MDA assays may become as good as the TXB_2 assay under the condition that Anderson et al.[89] are right that TXA_2 along with HHT are produced simultaneously from two molecules of PGH_2.

A burst in oxygen consumption which accompanies platelet stimulation by thrombin and collagen in the presence of antimycin A is due to the oxidation of endogenous arachidonic acid.[22] In these conditions oxygen consumption by platelets is measured by a Clark electrode. This method does not differentiate between cyclooxygenase and lipoxygenase activities.

Thromboxane synthetase has been isolated from platelet microsomes, characterized, resolved, and solubilized.[32,71,82] This enzymatic system converts prostaglandin endoperoxides to a less stable substance with an enhanced contracting potency on a rabbit

aortic strip. This substance is rapidly and spontaneously decomposed to TXB_2, which can be identified by mass spectrometry.

TXA_2-like activity has been detected in aggregating platelet-rich plasma[64,72,74] in phagocytizing leukocytes,[90] in stimulated lungs,[27,73-75] in vibrated spleen slices,[76] and in incubated human iris microsomes.[91] Our interest is focused on platelets. What is the biological significance of TXA_2 in platelets? Is TXA_2 required for platelet aggregation? Does TXA_2 influence arterial blood flow?

When platelets transform arachidonic acid into prostaglandin endoperoxides[63-65] and subsequently into TXA_2[6,67] aggregation occurs. Proaggregatory concentrations of arachidonic acid in human platelet-rich plasma are 300 to 600 μM,[81] while 0.03 to 0.015 μM of PGH_2 will do the same job.[65] It is understandable that arachidonate-induced platelet aggregation is inhibited by cyclooxygenase inhibitors (e.g., by aspirin), while PGH_2-induced aggregation is not. Will it be inhibited by TXA_2 synthetase inhibitors? In other words, is conversion of PGH_2 to TXA_2 necessary for platelet aggregation? Needleman et al.[92,93] say no. They consider PGH_2, alone, as the intrinsic proaggregatory factor in the arachidonic acid cascade, whereas, according to these authors, the biological significance of TXA_2 is restricted to its vasoconstrictor action.

The above point of view is not shared by the others.[7,52,69,94,95] For example, we have found[95,97] that a selective inhibition of TXA_2 biosynthesis in platelet-rich plasma by low concentrations of nictinodole makes platelets resistant to the proaggregatory action of arachidonic acid. Direct evidence has been obtained by Fitzpatrick and Gorman,[69,94] who have demonstrated that platelet-rich plasma transforms exogenous PGH_2 to TXA_2 and when this transformation is abolished by imidazole[98] or by 9,11-azaprosta-5,13-dienoic acid[94] then PGH_2-induced platelet aggregation is inhibited at all concentrations of PGH_2. The experiments of Fitzpatrick and Gorman[69,94] clearly indicate that PGH_2 has no intrinsic proaggregatory potency and it must be converted to TXA_2 in order to induce platelet aggregation. TXA_2 is the intrinsic substance in the arachidonic acid cascade that results in irreversible platelet aggregation.

There is little doubt that endogenous arachidonic acid is released and metabolized in platelets aggregated by thrombin,[74] collagen,[64] ADP,[72] and epinephrine.[85] It seems that the formation of endogenous TXA_2 plays an essential role in the second (irreversible) phase of ADP-induced and epinephrine-induced platelet aggregation[72,85] and most of all in collagen-induced, but not in thrombin-induced, aggregation.[72,99,100]

The mechanisms by which TXA_2 contributes to platelet aggregation are not known. The original concept was that PGH_2 and hence TXA_2 cause platelet aggregation by provoking dense granule secretion.[85] The secreted ADP was considered to be the final proaggregatory messenger. Recently, the first direct evidence has been provided showing that TXA_2 causes primary aggregation of human platelets without the stimulation of dense granules to secrete ADP.[101]

Miller et al.[102] have demonstrated that TXA_2 inhibits the PGE_1-stimulated accumulation of cyclic AMP in human platelets. These authors consider that platelet aggregability in the circulation is controlled by a balance between prostacyclin and TXA_2. Prostacyclin stimulates platelet adenylate cyclase,[103-105] inhibits Ca^{++} mobilization, and thus prevents platelets from aggregation. TXA_2 lowers the cyclic AMP level in platelets, stimulates Ca^{++} mobilization, and therefore leads to platelet aggregation.[102]

Holmsen[313] postulates the existence of a "basic platelet reaction" defined as a response of platelets to a great variety of stimuli. According to his hypothesis the "basic platelet reaction" consists of the following sequential events: shape change, aggregation, PGH_2/TXA_2 biosynthesis, dense granule secretion, and α-granule secretion. These events are supposed to be independent of each other and result from an increase in the cytoplasmic concentration of Ca^{++}, which rises due to the interaction of an exogenous stimulus within the platelet cell membrane. The extent to which this process

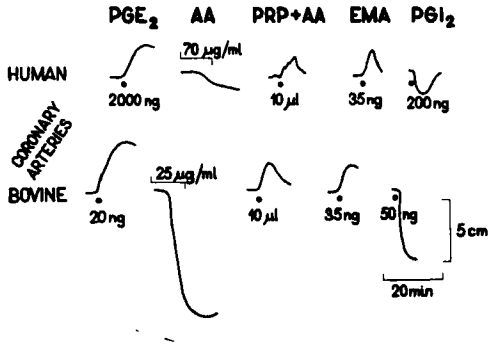


FIGURE 3. The effects of PGE_2, arachidonic acid (AA), TXA_2 generated by platelet-rich plasma (PRP) from AA, 11,9-epoxymethano analog of PGH_2 (EMA) and prostacyclin (PGI_2) on the tone of superfused strips of the human and bovine coronary arteries. The bovine coronary artery was excised from the bull's heart immediately after the animal was slaughtered, whereas the specimen from the corpse of a 36 year old man was obtained 8 hr after a fatal street accident.

occurs in a platelet depends on the strength of exogenous stimulus and on the release of endogenous, platelet-produced amplifiers, e.g., TXA_2. TXA_2 sets the positive feedback loop into motion. Let us give an example. A weak, exogenous stimulus would probably cause only a shape of the platelets, if not for the accompanying release of TXA_2. TXA_2 potentiates the weak action of an exogenous stimulus and thus aggregation occurs. Cyclooxygenase and TXA_2 synthetase inhibitors block this positive feedback loop and therefore are considered to be antiaggregatory drugs.[318] A similar role for TXA_2 in the positive feedback mechanism has been proposed in the histamine-induced contraction of lung parenchyma.[75]

TXA_2 is a powerful vasoconstrictor. It is at least as active as angiotensin II in causing rabbit aortic strip to contract[71] and it is 26 to 308 times more potent than $PGF_{2\alpha}$ in causing contractions of a strip of swine coronary artery.[323] Needleman et al.[92,93] consider that the vascular action of TXA_2 is sufficient to explain its pathological significance in the organism. TXA_2 contracts strips of coronary arteries from man,[70] swine,[106] and bull (Figure 3). It is tempting to speculate that continuous generation of labile TXA_2 by platelets sticking to the damaged walls of coronary arteries results in their localized vasoconstriction. At the same time the released TXA_2 induces the circulating platelets to aggregate and to form a mural thrombus. At the site of thrombus formation the coronary artery will be more and more constricted by increasing amounts of labile TXA_2. The central role of platelets in the development of coronary heart disease is generally accepted.[312,315] Might it be that the intravascular generation of TXA_2 by platelets constitutes a part of the natural history of ischemic heart disease? Arachidonate-aggregated platelets of many of our patients suffering from myocardial infarction generated more TXA_2 than platelets of the healthy subjects.[81]

A few words should be said about TXB_2, the only product of spontaneous decomposition of TXA_2 in vitro. When administered to humans, TXB_2 is metabolized to a couple of derivatives. Less than 3% is excreted unchanged into the urine.[107] This fact complicates the quantitative studies on thromboxane biosynthesis in vivo.

V. PROSTACYCLIN

In 1976 we were looking for thromboxane synthetase activity in various microsomal

preparations. When incubating aortic microsomes with prostaglandin endoperoxides we observed[7-10] that PGH_2 is not converted to expected "classical" prostaglandins. Neither TXA_2 nor HHT are formed, and yet biological activity of PGH_2 instantly disappears from the incubation mixture. After several unsuccessful attempts to find out what aortic microsomes were generating from PGH_2 we were able to demonstrate that an unknown, labile vasodilator, and antiaggregatory substance is produced. This substance had many nicknames, but finally we named it PGX.[7-10]

PGX, when left standing, is broken down within a few minutes to a stable, biologically inactive compound with chromatographic mobility close to that of PGE_2.[7] The biological significance of the discovery of PGX was fully realized by us when we demonstrated that prostaglandin endoperoxides generated by platelets can feed arterial "PGX synthetase"[9] and that this enzyme is selectively inhibited by low concentrations of lipid peroxides, e.g., by 15-hydroperoxyarachidonic acid (15-HPAA).[7]

When speculating as to the possible chemical structure for PGX we came across the paper of Pace-Asciak and Wolfe[108] in which the authors describe a novel transformation of arachidonic acid (and PGG_2)[109] to 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid by rat stomach homogenates. The existence of an unstable recursor of this new metabolite of arachidonic acid had been hypothesized.[108,109] Could this hypothetical labile precursor be similar to our PGX? Indeed, rat stomach microsomes when incubated either with PGH_2 or PGG_2 produce a principle which we previously characterized as PGX.[7]

At this stage the chemists[110] isolated, characterized, and determined the chemical structure of PGX, which was renamed to prostacyclin. Some anonymous people[333] gave another name to the same substance (PGI_2) although the basic chemical structure and the conformation of prostacyclin differ from those of "classical" prostaglandins. As compared to prostaglandins, the prostacyclin molecule has an additional furane ring which is formed due to the presence of an oxygen bridge connecting carbon atoms 6 and 9 (Figure 1). The other characteristic feature of the prostacyclin molecule is the presence of a 5,6 double bond which "stiffens" the rectangular shape of the prostacyclin molecule, so different from the hair pin-like shape of the prostaglandin molecules. Prostacyclin is hydrolyzed rapidly to biologically inactive 6-keto prostaglandin ($F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), a compound first described by Pace-Asciak[111,112] and Dawson et al.[113] The methods of chemical synthesis of prostacyclin are relatively simple.[114-117]

In aqueous solution at pH 7.4 and at 37°C prostacyclin is not stable. Its half-life in extravasated dog blood at 37°C is 3 min.[118] On the other hand at pH 9.5 to 10.5 and at a low temperature aqueous solutions of prostacyclin are stable for at least several weeks.

Bioassay is unbeatable in handling the unstable members of the arachidonic acid cascade. The quantitative bioassay of prostacyclin is based on its ability to relax a bovine coronary artery strip[119] (Figure 3) or to inhibit platelet aggregation.[7-10] Using this last methodological principle, prostacyclin can be monitored continuously in the circulating blood of anesthetized animals (Figure 4).[120,121] Recently, antisera against 9-deoxy-6,9-epoxy-$PGF_{1\alpha}$[123] have been developed. These antisera also cross-react with prostacyclin. What an elegant way to assay prostacyclin! The above discoveries not only offer a new assay method for prostacyclin but they will also encourage investigation on extracellular phenomena regulated by prostacyclin, since these antisera can remove prostacyclin from body fluids. 6-Keto-$PGF_{1\alpha}$ is quantified by radioimmunoassay[124] and by mass spectrometry.[111,112,125]

The product of prostacyclin hydrolysis in vitro, 6-keto-$PGF_{1\alpha}$ is not the major product of prostacyclin biotransformation in vivo. In rats injected with prostacyclin several different metabolites are formed.[126] Biotransformation of prostacyclin seems to be initiated by its enzymatic to 15-keto-prostacyclin, not by its hydrolysis to 6-keto-

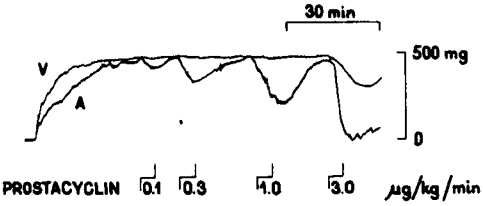


FIGURE 4. Assay of prostacyclin blood levels in vivo. Assay is based on the deaggregatory properties of prostacyclin. Collagen strips are superfused with mixed venous (V) and aortic (A) blood or an anesthetized and heparinized cat.[120,121] Platelet clumps are formed and cause a gain in weight of the blood superfused strips (0 to 500 mg). Intravenous 3 min lasting infusions of prostacyclin (0.1 to 3.0 μg/kg/min) cause a dose-dependent deaggregation of platelet clumps in aortic blood, which is seen as a decrease in weight of the blood-superfused collagenstrip. The deaggregatory response to prostacyclin at doses 0.1 to 1.0 μg/kg/min occurs only in aortic blood, since there is found endogenous prostacyclin which is generated by the breathing lungs.[60]

$PGF_{1\alpha}$.[126] Blood vessels transform prostacyclin to 6,15-diketo-$PGF_{1\alpha}$.[127] Relatively large amounts of prostacyclin metabolites may constitute the immunoreactive PG-like material in the human urine.[128]

In vitro the following cardiovascular tissues generate prostacyclin (sometimes detected as 6-keto-$PGF_{1\alpha}$): aortic microsomes from pig and rabbit,[7,8,129] arterial and venous ring slices obtained from man,[130,131] rabbit,[10,132] and rat;[133] homogenates of ductus arteriosus and fetal arteries from the calf[134,135] and lamb;[125] perfused hearts from rabbit and rat,[119,132,136,137] and cultured arterial cells.[62,138,139] Prostacyclin synthetase is localized mainly in the endothelial layer of the vascular wall. Other layers have little capacity to generate prostacyclin.[140]

Prostacyclin is the main metabolite of prostaglandin endoperoxides in the vascular wall.[7] The early findings that vascular tissue converts arachidonic acid to various types of prostaglandins[141,142] and generates prostaglandin-E like material in response to hormonal stimulation[23,143-145] need to be reassessed in the light of the discovery of prostacyclin.[7-10] Chromatographic separation, bioassay, and radioimmunoassay techniques which have been used in the above experiments are not sufficiently specific to differentiate between prostaglandins on one side and prostacyclin and 6-keto-$PGF_{1\alpha}$ as well as their metabolites, on the other.

Prostacyclin (or 6-keto-$PGF_{1\alpha}$) is also produced in vitro by nonvascular tissues such as ram[129,146] and bovine[147] seminal vesicle microsomes, rat stomach homogenates and microsomes,[7,111,112,148] rat stomach mucosa,[149] rat, guinea pig, and sheep uteri,[150,151] bovine corpora lutea,[129] and placenta.[152] Rat lung homogenates[148] and immunologically-triggered, perfused guinea pig lungs[113] also generate considerable amounts of 6-keto-$PGF_{1\alpha}$ and so do 23 other microsomal preparations of various mammalian tissues.[153]

This flood of information reminds me of smething we used to call creative bliss in our country. Before any conclusions can be drawn, we have to assure ourselves that prostacyclin is produced by all three organs in vivo. Prostacyclin is only one of the numerous members of the arachidonic acid cascade[319] and the isolated enzymes of this cascade are not, as yet, readily available in laboratory practice. The

crosomes and homogenates are far removed from the properties of pure enzymatic preparations on one side, and from the in vivo situation on the other. Most of the homogenates and microsomal preparations will convert arachidonic acid to any of its metabolites, provided that the incubation mixture will be supplied with adequate cofactors and set at pH, temperature, and substrate concentration optimum for a given metabolic route. Sometimes these crucial experimental conditions are simply accidental. A convincing example of my thesis is arachidonate metabolism in the lungs. There is no single known metabolite of arachidonic acid which would not be proposed as a major product generated by the lungs.[23,27,30,31,75,113,148]

Does this mean that at the moment I am typing these words my lungs produce this mess? I hope not. I am sure they do not, since we have shown that quietly perfused guinea pig and rat lungs, as well as the lungs of anesthetized cats, spontaneously and continuously generate only small amounts of prostacyclin.[60,61] Thromboxane A_2 and prostaglandins can be produced by the lungs in response to anaphylactic shock, during intoxication with histamine or with arachidonic acid,[23,60,75] as well as when the lungs are squeezed, chopped, minced, homogenized, anything but quietly breathing. There exists a tremendous discrepancy between the potential routes of metabolism of arachidonic acid in a tissue in vitro and the actual performance in vivo. The former cannot be mistaken for the latter.

The antiplatelet and vasodilator actions of prostacyclin determine its biological significance.[7-10] When prostacyclin (0.3 to 3.0 n*M*) is preincubated for a couple of seconds with platelet-rich plasma, human, rabbit, guinea pig, or feline subsequent addition of ADP, collagen, or arachidonic acid fail to induce platelet aggregation. On the average, prostacyclin is a 20 to 30 times more potent antiaggregatory agent than PGE_1.[8]

The antiaggregatory properties of PGE_1[154] and PGD_2[155] were early associated with their potencies to stimulate platelet adenylate cyclase.[156-159] Cyclic AMP phosphodiesterase inhibitors enhance the antiaggregatory action of PGE_1.[157] Prostacyclin is 30 times more potent than PGE_1 and 10 times more potent than PGD_2 as a stimulator of human platelet adenyl cyclase.[103-105] Have all of them a common receptor on the platelet membrane or are there three separate regulatory subunits of adenylate cyclase specific for PGE_1, PGD_2, and PGI_2? We do not know, however, PGE_1 and PGD_2 can hardly be considered as physiological mediators in platelets. Dihomo-δ-linolenic acid, the precursor of PGE_1, constitutes only a minor portion of the fatty acid pool available to cyclooxygenase in platelets.[324] PGD_2 is not detected in healthy arteries,[7] although minute amounts are formed during platelet aggregation.[160]

The discovery of prostacyclin has had a direct impact on research dealing with the mode of antiaggregatory cyclic AMP activity.[161] The concept of Holmsen[313] is that cyclic AMP inhibits platelet aggregation by affecting the distribution of intracellular Ca^{++}. Does cyclic AMP inhibit the generation of TXA_2 in platelets? Some authors say yes[162,163] and the others say no.[164] Malmsten et al.[163] consider cyclic AMP to be an inhibitor of platelet cyclooxygenase and therefore an increase of platelet cyclic AMP levels is accompanied by a decrease in the generation of PGG_2, PGH_2, TXA_2, and, consequently, platelet aggregation is inhibited. Recently Minkes et al.[165] and Lapetina et al.[161] put forward another concept stating that cyclic AMP is the inhibitor of Ca^{++}-dependent phospholipase A_2[19-22] and thus it restricts the availability of free arachidonic acid which is necessary for the generation of cyclic endoperoxides and TXA_2. According to Gerrard et al.[164] this last concept may constitute the basis for the unifying theory on the mode of the antiaggregatory action of cyclic AMP. Cyclic AMP, by the sequestration of Ca^{++} inside cellular stores, has two parallel inhibitory actions: (1) it hinders phospholipase A_2 activity and hence the release of TXA_2 precursors, and (2) it inhibits Ca^{++}-dependent activation of the platelet contractile proteins.

In vivo prostacyclin not only prevents platelets from aggregation (antiaggregatory

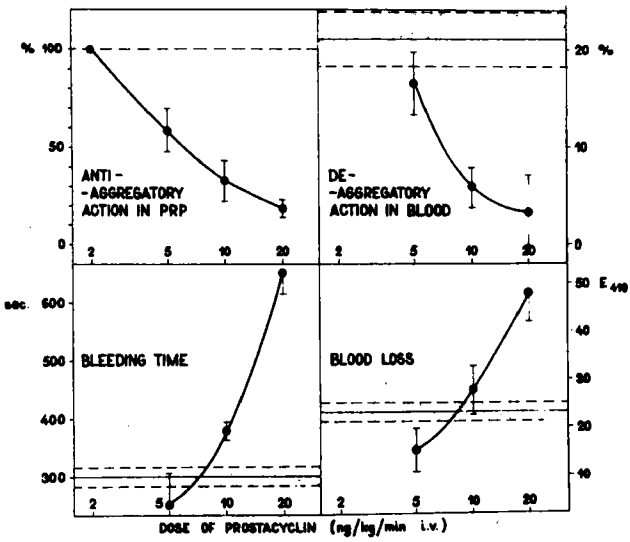


FIGURE 5. The effects of intravenous infusions of prostacyclin (2 to 20 ng/kg/min) into six healthy men on ADP-induced platelet aggregation (antiaggregatory action), dissipation of circulating platelet aggregates (deaggregatory action), template bleeding time, and blood loss after the skin incision. Each point represents mean of three to six observations ± S. E.

action) but it also reverses platelet aggregation (Figure 4) by dissipation of the preformed platelet clumps (deaggregatory action).[120] Prostacyclin (10 to 100 ng/kg/min, i.v.), infused into anesthetized rabbits, inhibits electrically induced thrombus formation in the carotid artery, prolongs bleeding time, and inhibits ex vivo ADP-induced platelet aggregation. All these effects of prostacyclin are potentiated by theophylline, an inhibitor of phosphodiesterase.[166]

We have shown in anesthetized, heparinized cats[121] the deaggregatory action of prostacyclin.[120] Prostacyclin in a single bolus injection of 7.5 μg/kg, i.v., "dissolves" 50% of the preformed white thrombi weighing 300 to 500 mg. We have also seen a potentiation of the deaggregatory action of prostacyclin by theophylline (3 mg/kg, i.v.). At this dose theophylline has little effect on the hypotensive action of prostacyclin.[120]

My colleagues and I were the first men to experience the effects of intravenously infused prostacyclin.[167,168] When infused intravenously for a period of 30 min, prostacyclin (2 to 20 ng/kg/min) inhibits ex vivo ADP-induced platelet aggregation, dissipates circulating platelet aggregates,[169] and prolongs bleeding time (Figure 5). Prostacyclin does not influence the platelet count or the functional integrity of coagulation and fibrinolytic systems. The most spectacular feature of prostacyclin action is vasodilatation appearing in the regions of the face, neck, and palms. Due to the profound erythema the temperature of the forehead skin rises by 0.3 to 0.8°C. Prostacyclin, at a dose of 20 ng/kg/min, evokes feelings of restlessness and lightheadedness. In some of us it has also elicited a slight diuretic effect. A moderate tachycardia precedes a discrete lowering in diastolic blood pressure (Figure 6). There is little effect on the mean arterial blood pressure, unless a dose of 50 ng/kg/min is infused. This dose of prostacyclin may result in collapse in which case the hypotensive action of prostacyclin produces a distinct bradycardia. Any action of prostacyclin disappears briefly after termination of the infusion.

In general, the vascular action of prostacyclin is weaker than its antiplatelet action. Isolated strips of blood vessels are unevenly affected by prostacyclin. The strongest relaxation is evoked by prostacyclin in strips of bovine coronary arter

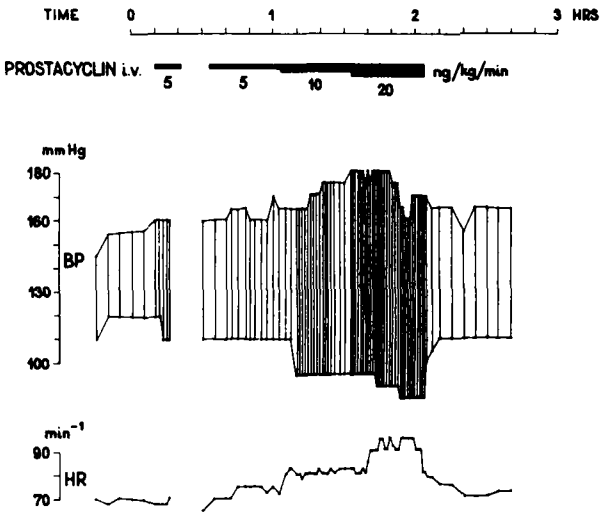


FIGURE 6. The circulatory effects of prostacyclin infused intravenously at doses of 5 to 20 ng/kg/min into a healthy man. BP — blood pressure measured at the left thigh, HR — heart rate.

This relaxation is dose dependent and fairly specific for prostacyclin. Strips of bovine coronary arteries are used as the assay organs for prostacyclin. The reaction of the human coronary artery is weaker (Figure 3), while swine coronary artery is alleged to be contracted by prostacyclin.[171] In our laboratory we have seen that the coronary artery strips from all three species are relaxed to varying extents by prostacyclin, whereas PGE_2 contracts all of them. Perfused rabbit hearts produce prostacyclin[119,132,136,137] and the hormone seems to derive mainly from the coronary arteries.[132,172]

Strips of the rabbit celiac and mesenteric arteries[7,10,170] and of the lamb ductus arteriosus[173] are moderately relaxed by prostacyclin, whereas PGE_2 is a much more powerful relaxant in these vascular preparations. We have shown that the PGH_2-induced relaxation of rabbit mesenteric artery occurs because of the intramural conversion of PGH_2 to prostacyclin. When the strip is pretreated with 15-HPAA (a prostacyclin synthetase inhibitor) then prostaglandin endoperoxides contract the rabbit mesenteric artery as they contract the rabbit aorta.[10] On the other hand, strips of rabbit aorta and vena cava are neither contracted nor relaxed by prostacyclin.

The above in vitro data seem to favor the concept that prostacyclin is an endogenous vasodilator generated by the coronary vascular bed, at least in some species.[119,172] Not only is the coronary vascular bed sensitive to the vasodilator action of prostacyclin in vivo, the hypotensive action of prostacyclin in laboratory animals is greater than those of PGE_1, PGE_2, and PGD_2,[174-177] and in humans a low dose of prostacyclin (5 ng/kg/min, i.v.) results in a distinct erythematous reaction. Therefore, the statement of Coceani et al.[173] that " . . . the prime function of PGI_2 concerns platelet aggregability and not the regulation of vascular tone . . . " might be premature.

As I am writing this chapter, the available reports on cardiovascular responses to prostacyclin in vivo are few and conflicting.[174-177] Dose-dependent vasodepression has been reported in rats, rabbits, and dogs, however, in dogs prostacyclin is claimed to be cardiodepressive,[174] while in rats it is cardiostimulant.[175] The positive chronotropic effect of prostacyclin in rats is supposed to be either reflectory[176] or direct.[175] We have to wait for further information on the cardiovascular action of prostacyclin, especially in man.

Prostacyclin is synthesized in the endothelium, but its action is directed to blood platelets and vascular myocytes.

VI. INTERACTION BETWEEN THE VASCULAR WALL AND BLOOD PLATELETS

In thrombocytopenic animals an artificial injury to the arterial wall is not followed by formation of a thrombus[178] while in normothrombocytemic animals myocardial infarction is followed by an enhancement of platelet aggregability.[179] These two facts indicate the importance of platelets in formation of intra-arterial thrombi.

The platelet life span is around 10 days,[86] daily production being 1.5×10^{11} platelets.[180] A suspension of washed platelets shows a high respiration rate. This is dramatically accelerated by exogenous and endogenous[22] arachidonic acid, owing to the stimulation of lipoxygenase[5,29] and cyclooxygenase[36,37] pathways.

In contrast to platelets, the mitotic and respiratory activities of the vascular endothelium are very low.[321] The life span of aortic endothelial cells is 100 to 180 days.[181] They have a very low basic respiratory activity which is hardly stimulated by exogenous arachidonic acid. An interaction between these stationary, "slow going", "antithrombotic" endothelial cells and the circulating, highly reactive, "prothrombotic" blood platelets is responsible for maintaining homeostasis in the arterial portion of the circulation.

The importance of vascular endothelium in maintaining blood fluidity has been assumed for many years,[317] however, too much attention was paid to the passive role of the endothelium in preventing thrombosis. Glycoproteins of the endothelial cell plasma membrane in forming a blood-compatible surface[182,183] were thought to be solely responsible for the repulsion of blood platelets from vascular walls. One of the active roles of the endothelium in prevention of thrombosis was recognized due to the discovery of Vane[184] that pulmonary endothelium removes a variety of vasoactive substances including kinins, catecholamines, 5-hydroxytryptamine, and adenine nucleotides from the circulation.[185,186] These biologically active substances potentiate or induce platelet aggregation. There are other mechanisms by which the endothelium can actively defend blood vessels against thrombosis, e.g., the endothelium contains a plasminogen activator,[187] binds thrombin,[188] and releases a low molecular weight labile factor that acts as a broad-spectrum inhibitor of platelet function.[189]

However, the main active antithrombotic function of the vascular[7-10] and pulmonary[60-61] endothelium is to generate prostacyclin. Indeed, we believe[60] that the pulmonary endothelium is a vast endocrine organ that secretes a hormone into the circulation, namely, prostacyclin. Hormonal prostacyclin potentiates the antithrombotic action of vascular prostacyclin, mainly in coronary and cerebral arteries which are in the closest vicinity of the "endocrine gland" (i.e., the lung). It might well be that this interaction between platelets and endothelial cells enhances the efficacy of the endothelium to generate prostacyclin, at least the existence of this interaction was shown for the vascular tissue.[10]

Ten years ago it was recognized that endothelial cell function appears to depend on the presence of blood platelets in the fluid perfusing the arteries.[2,3] Ultrastructural studies have revealed that platelets can be assimilated into vascular endothelial matrix or even incorporated into endothelial cells.[1,4] This phenomenon was described as the platelet "support" to endothelium. Very little is known concerning the biochemical character of this platelet-endothelium interaction. We have suggested that the morphological interaction enables the transfer of prostaglandin endoperoxides from platelets to endothelial cells in order to accelerate the generation of prostacyclin.[7]

When incubated in a buffer, the chopped rings of rabbit mesenteric arteries avidly

generate prostacyclin either from exogenous prostaglandin endoperoxides (80 to 90% conversion) or from the endogenous substrates; however, the conversion rate of exogenous arachidonic acid to prostacyclin does not exceed 0.5 to 1% conversion.[9] Indomethacin-treated arterial rings do not produce prostacyclin at all unless prostaglandin endoperoxides or blood platelets are added to the incubation mixture. In both instances the generation of prostacyclin by arterial rings is abolished by a prostacyclin synthetase inhibitor (w)15-HPAA).[7] The only conclusion that may be drawn from these experiments is that platelets supply arterial rings with their own prostaglandin endoperoxides. In vitro, using the arterial cell cultures, it has been confirmed[62,139] that endothelial cyclooxygenase is the rate-limiting enzyme in generation of prostacyclin and that arterial cells can utilize endoperoxides from blood platelets in order to generate prostacyclin.

We believe that the in vivo synthesis of prostacyclin by arterial walls could partially derive from prostaglandin endoperoxides supplied by blood platelets which try to adhere to the endothelium. In health, this might be the mechanism by which endothelium repels platelets and protects itself against the deposition of mural thrombi.

Let us imagine that, when subjected to mild mechanical deformations in rheologically "hazardous" regions of the vascular bed,[190] blood platelets are prone to generate minute amounts of prostaglandin endoperoxides from arachidonic acid which is liberated from their temporarily deformed cell membranes.[16,191] The resulting concentration of prostaglandin endoperoxides in platelets is too low to stimulate the enzymatic machinery of thromboxane synthetase, but it is sufficiently high to trigger endothelial prostacyclin synthetase.[7,62] The nascent endothelial prostacyclin repels these deformed "feeding" platelets and the deposition of mural platelet aggregates does not occur. This transfer of prostaglandin endoperoxides could be the key to the understanding of the mysterious platelet endothelial "support" mechanism,[1-4] especially since an endothelium-repairing function of prostacyclin has already been proposed.[7]

Our hypothesis offers a reasonable explanation for the fact that aortic microsomes reverse ADP-induced platelet aggregation in platelet-rich plasma.[192] The activation of platelet cyclooxygenase by ADP is so weak that only a trace of thromboxane A_2 is formed and this occurs exclusively during the second wave of aggregation.[72] However, these minute amounts of cyclic endoperoxides from ADP-aggregated platelets are sufficient to trigger prostacyclin synthetase in aortic microsomes. The resulting amounts of prostacyclin in platelet-rich plasma are high enough to reverse ADP-induced platelet aggregation,[192] although this fact has not been established by the authors of this observation.

Summing up, when thromboxane synthetase and prostacyclin synthetase are brought together and supplied with a small concentration of prostaglandin endoperoxides (derived from ADP-aggregated platelets)[72] only prostacyclin will be formed and a paradoxical antiaggregatory or even deaggregatory effect of ADP will be observed.[192]

The above phenomenon explains the fact that ADP, the most powerful proaggregatory agent in vitro also has a slight thrombolytic effect in vivo.[193,194,316] ADP-aggregated platelets, while trying to adhere to the intact intimal surface of arteries, automatically initiate the generation of prostacyclin and this hormone disperses platelet clumps.

I assume that *conditio sine qua non* for the formation of intra-arterial platelet thrombi is the inability of endothelial prostacyclin synthetase to generate enough prostacyclin. Platelets are then free to adhere to the defenseless arterial wall, produce TXA_2, and give way to the full progression of various phases of their aggregation and release reactions.[313]

Thus arterial thrombosis develops when endothelial prostacyclin synthetase is inactivated by lipid peroxides,[7] when endothelium becomes detached exposing the under-

lying connective tissue to platelets,[195,196] or when polymerizing fibrin prevents the interaction between platelets and endothelial cells.[197]

On the other hand, minor injury might even increase endothelial capability to produce prostacyclin.[10] Indeed, in vitro, platelets do not adhere to endothelium scraped from rabbit aorta[198] and there is little platelet adhesion to patches of endothelium in the mechanically injured aorta in vivo.[196] We have observed that spirally cut strips of rabbit aorta are not covered with platelet clumps when superfused with blood[121] unless the aortic strips are prepared from atherosclerotic animals.[132] All these phenomena may be ascribed to the accelerated rate of prostacyclin formation by endothelium as a consequence of cutting, squeezing, scraping, crushing, etc.

There are, however, certain limits beyond which trauma no longer stimulates the endothelium but rather begins to destroy the prostacyclin synthetase apparatus. Experimentally this effect is probably reached after heat injury from a laser beam directed onto the arterial wall.[199] On the basis of our experimental data[132,200] we hypothesize that the contamination of endothelial cells with lipid peroxides in experimental atherosclerosis has a similar injurious effect on the arterial wall. The details of our hypothesis will be presented in Section VIII.

VII. PLATELETS — EFA — ATHEROSCLEROSIS — MYOCARDIAL INFARCTION

Although many factors in the pathogenesis of atherosclerosis still remain unknown, some evidence that intra-arterial platelet aggregation is an important factor in the development of this disease has appeared in the literature. The thrombogenic hypothesis of atherosclerosis (the so called "incrustation theory") was originally proposed by von Rokitansky[201] in 1842, but Virchow's "infiltration theory" prevailed and dominated the academic community for many years, owing greatly to Anichkov's experimental demonstration[202] that atherosclerosis in rabbits could be induced by a diet high in lipid and cholesterol content. The yellow streaks of lipids which "infiltrated" the rabbit aorta could be seen with the naked eye and under a microscope.

More than a century after von Rokitansky had put forward his original hypothesis, pathologists could see that myointimal cells of arteries were growing over mural thrombi and thus forming an atherosclerotic plaque.[312,320] They noted that platelets and fibrin seemed to "incrustate" the vascular wall. In view of the fact that intra-arterial platelet adhesion and aggregation is the key event in the initiation of arterial thrombosis (Mustard and Packham, 1975 and Baumgartner and Muggli, 1976) and that the concept of von Rokitansky has gained a wide acceptance, one can deduct that atherosclerosis arises from some kind of imbalance between platelets and arterial walls. Indeed, aggregating platelets produce a factor that stimulates proliferation of arterial myocytes and promotes their migration to the atherosclerotic cap.[203,204] The experimental intimal thickening due to smooth muscle cell hyperplasia following injury to the arterial endothelium is mediated through substances released from a carpet of platelets that covers the site of the injury.[205] Homocysteinemia is an inborn error of metabolism which is associated with premature atherosclerosis. A baboon model of homocysteine-induced atherosclerosis is characterized by focal vascular loss of endothelium and an immense increase in platelet consumption. This is followed by arterial myocyte proliferation.[206]

Other data suggests that platelets play an important role in the development of atherosclerosis. The epidemiological risk factors of coronary heart disease are known to activate platelets.

High fat diet may be one of the risk factors for humans. The hardened coconut oil induces intra-arterial occlusive thrombosis in rats.[207,208]

High cholesterol blood levels are considered to be a predisposing factor in atherosclerosis. The aggregability of platelets is increased after cholesterol is incorporated into their plasma cell membrane.[209]

Tobacco smoking is a well-recognized risk factor. Cigarette smoking increases platelet aggregability.[210]

Atherosclerosis and resulting myocardial infarction are less common in women before menopause than in men. Testosterone enhances platelet aggregability in man[211] and in animals.[212,213] Estradiol and progesterone attenuate the platelet response to proaggregatory agents.[213] Platelet responsiveness to the proaggregatory action of ADP is ten times greater in male than in female rats. Castration reduces aggregability in males four times and increases it in females by three times.[214] These data of the Ramwell's group provide the significant experimental evidence for an association between sex, platelet reactivity, and atherosclerosis.

Long lasting diabetes mellitus may be a predisposing factor towards atherosclerosis. Platelets obtained from diabetic patients show increased activity of cyclooxygenase[215] and this is probably related to the enhanced platelet aggregability reported many times in this disease.[216]

A variety of platelet abnormalities have been described in patients with coronary heart disease[318] and in experimental arterial insufficiency. Increased platelet sensitivity to proaggregatory agents has been reported in acute myocardial infarction[217,218] and long after its occurrence.[81,219] Experimentally this same effect has been noted 20 to 80 days after coronary artery ligation in dogs[179] and 5 months after feeding rabbits an atherogenic diet.[200] Spontaneous platelet aggregation in various types of arterial insufficiency has also been shown.[81,218,220] In 40% of the survivors of myocardial infarction an increased platelet aggregability to arachidonic acid was associated with an augmented rate of its conversion to TXA_2 (Figure 7).[81] Platelet survival time in men with diagnosed arterial atherosclerosis is significantly shortened.[221-223]

In humans[208] and in animals[207] platelet aggregability is suppressed by a diet rich in linoleic acid.[314] Linoleic acid (18:2ω6), the principal EFA present in sunflowerseed oil and in corn oil, is a common precursor both for dihomo-γ linolenic acid (20:3ω6) and for arachidonic acid (20:4ω6). Dihomo-γ-linolenic acid constitutes only a tiny portion of the EFA pool available to cyclooxygenase in mammalian tissues.[224] Probably this precursor of monoenoic prostaglandins is rapidly desaturated to arachidonic acid and has little chance to be incorporated into the tissue phospholipids.

A 12-year long trial carried out in hospitalized Finnish patients has shown that a high linoleic acid diet reduces the incidence of death by coronary heart disease in men.[225] There is a number of similar reports claiming that the high linoleic acid diets have antithrombotic and antiatherosclerotic action .[314] An elevation of the linoleic acid content in the diet to 4 to 12 cal% has also been shown to have an antihypertensive effect in man[226,227] and in animals.[228]

The rationale for the antithrombotic, antiatherosclerotic, and antihypertensive action of dietary linoleic acid has been seen in the accumulation of dihomo-γ-linolenic acid in tissues, followed by an increased generation of PGE_1. Dihomo-γ-linolenic acid, injected into rats or dogs, inhibits platelet aggregation ex vivo.[58,230] Because of an instant desaturation of dihomo-γ-linolenic acid to arachidonic acid in the mammalian organism[224] and in light of our discovery of prostacyclin,[7-10] I am inclined to explain the beneficial effects of the high level linoleic acid diet as a result of an increase in the generation of prostacyclin by vascular and pulmonary endothelium. Would an increased generation of PGE_1 really be so desirable? PGE_1 has only 3 to 5% of the antiaggregatory activity of prostacyclin,[7] while both of them seem to compete for the

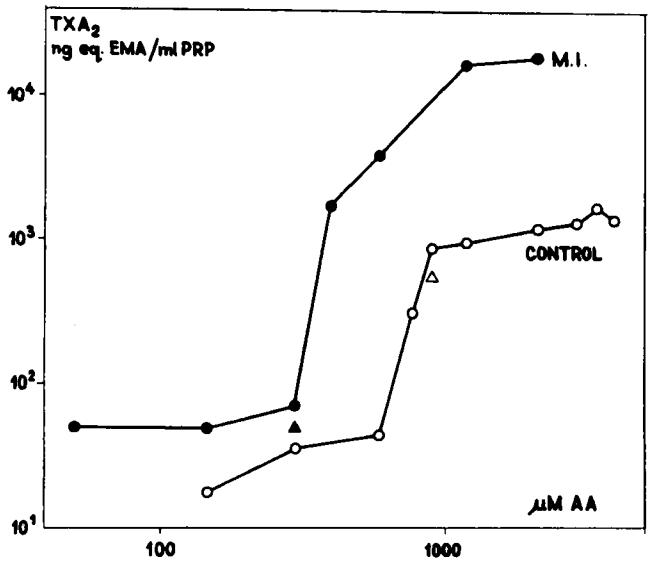


FIGURE 7. The generation of TSA_2 (see Figure 2) in platelet-rich plasma (PRP) of a myocardial infarction survival (M.I.) and in PRP of a control subject (control). PRP was aggregated by arachidonic acid (AA) at concentrations of 50 to 5000 μM. Triangles indicate the threshold proaggregatory concentrations of AA.

same active site on the platelet membrane. An increased generation of PGE_1 at the expense of prostacyclin might be a hazardous game, which actually seems to be played by the atherosclerosis organism.

Atherosclerosis in man is associated with a dramatic drop in the plasma ratio of arachidonic acid to dihomo-γ-linolenic acid.[231] The plasma level of arachidonic acid is lowered by more than 50%, while the level of dihomo-γ-linolenic acid is raised by 40%. The plasma levels of eight other free fatty acids remain within the range of the controls, except for an understandable fall in the 18:3ω6 plasma level. These data may suggest that in survivals of myocardial infarction (clinical evidence for coronary artery atherosclerosis) there exists a partial block in the last biosynthetic step of arachidonic acid, resulting in an accumulation of its immediate precursor, dihomo-γ-linolenic acid. When feeding an atherosclerotic person with sunflowerseed oil we will probably cause an accumulation of dihomo-γ-linolenic acid in his plasma and subsequently the tissue ratio of PGG_1/PGG_2 and PGE_1/PGI_2 will be increased. As has been previously mentioned this probably isn't a desirable shift in the efficacy of the antiaggregatory mechanism.

Another aspect of the high degree of unsaturation of the dietary fats is a danger arising from their autooxidation either in vitro or in the biophase. The lipid peroxides and the corresponding free radicals thus generated are potent prostacyclin synthetase inhibitors.[7] The same peroxides and free radicals are also thought to be responsible for the complex biological phenomena of aging.[232,233]

Harman[232] has shown that an increase in degree of unsaturation of dietary fat causes a significant decrease in the mean life span of female C3H mice. The same tendency has been observed in a group of Charles River male rats. One of the most hazardous diets for animals was one with a high content of sunflower seed oil (8.2% of saturated fatty acids and 76.4% of 18:2 acids). The safest was a mixed diet based principally upon menhaden oil (42.3% of saturated fatty acids and 1.1% of 18:2 acids).

The role of the degree of unsaturation of dietary fats in the prevention and treatment of atherosclerosis and aging is still disputable.

Myocardial infarction in humans is usually associated with a preexisting atherosclerosis of coronary arteries. Acute myocardial ischemia rarely results from the slowly developing atherosclerotic stenosis of a coronary artery without any complicating factors such as the formation of the platelet clump, hemorrhage into an atherosclerotic plaque, or discharge of atheromatous debris into the blood.[316] What is the role of platelets in precipitation of acute myocardial infarction? Even those scientists who are convinced about the key role of platelets in the etiology of atherosclerosis[320] still consider coronary thrombosis to be secondary to the myocardial infarction. There are two basic clinical observations which have led to the above suggestion: firstly the incorporation of ^{125}I-labeled fibrinogen into coronary arterial thrombi still occurs after myocardial infarction has been completed, and secondly one can rarely see arterial thrombi in patients dying suddenly because of a heart attack. Nevertheless these two facts do not exclude platelet-mediated intra-arterial thrombosis as the cause of myocardial infarction. The appearance of radioactivity from ^{125}I-fibrinogen upon the heart need not be explained by the *de novo* formation of thrombi. It may arise from the hardening of a preexisting platelet clump, which was originally responsible for the myocardial infarction by fibrin.

The formation of transient platelet clumps inside coronary arteries, and not the formation of a fibrinous "red thrombus," may constitute the principal cause of sudden cardiac death. These "white thrombi" are loose and after causing an immediate death they spontaneously deaggregate and hence, they are not detected by pathologists. These platelet clots need time in order to undergo the process of fibrinous transformation. Therefore the frequency of coronary thrombosis increases in direct proportion to the survival time which elapses after myocardial infarction.[312,316] It is also worthwhile to mention the fact that aggregating platelets would be expected to produce significant amounts of TXA_2 which would exacerbate the occlusive effect of the incipient thrombi by contracting the coronary artery around them.

In his elegant review, Jorgensen (1976) has presented a vast number of experimental and clinical evidence which clearly indicates that the occurrence of platelet aggregates in myocardial microcirculation is the causative factor leading to myocardial ischemia, cardiac arrhythmias, and sudden death. Whatever the mechanisms of the occurrence of these microthrombi, TXA_2 has to participate in the formation of the platelet aggregates and in trapping them in locally constricted fine coronary blood vessels (Section IV). This is a rationale for the administration of cyclooxygenase inhibitors (Section IX) and thromboxane synthetase inhibitors (Section X) in prevention of coronary thromboembolism.

While aspirin and thromboxane synthetase inhibitors may be used in the prevention of arterial thrombosis, prostacyclin and its analogues have a great chance to become the drugs that will cure acute myocardial infarction and other forms of arterial insufficiency. Indeed, we have shown that prostacyclin "dissolves" preformed platelet clumps in animals,[120,121] disintegrates circulating platelet aggregates[169] in men,[167,168] and that its deaggregatory action is enhanced by theophylline.[166]

Prostacyclin is also a circulating hormone generated by the lung.[60,61] The endocrine-like activity of this organ is increased during hyperventilation.[60] Could this be the reason for "deep breathing" exercises which one starts to perform involuntarily when chest pain occurs? Arterial blood would not only be better oxygenated but also enriched with hormonal prostacyclin which would help to disperse platelet aggregates which are trapped in the myocardial microcirculation. Upon reaching the coronary

circulation, hormonal prostacyclin induces bradycardia.[234] Thus in addition to its antiaggregatory,[7-10] deaggregatory,[121,167,168] coronary vasodilator[119,172] and peripherial resistance suppressing[174-177] properties, prostacyclin reduces cardiac energy expendi-ture by slowing the heart rate.[234] Since prostacyclin is not removed from the circulation across the lung, its intravenous infusion may serve to aid myocardial performance while recovering from injury.

Pharmacological stimulation of the endocrine-like function of the lung is another possible approach to the treatment of arterial thromboembolism. Dextran has been recently reported to release "prostaglandin-like activity" from perfused rabbit lungs.[235] On the other hand, air pollutants, such as tobacco smoke, which have been linked with coronary heart disease, should be carefully studied as potential inhibitors of prostaglandin production by the lung.

VIII. THROMBOXANE A_2 — PROSTACYCLIN — ATHEROSCLEROSIS (A HYPOTHESIS ON THE ETIOLOGY OF ATHEROSCLEROSIS)

We postulate that the beginning of atherosclerosis is causally associated with an increase in lipid peroxidation either in the human body (i.e., in vivo) or in the dietary fat (i.e., in vitro). The accumulation of lipid peroxides in certain regions of the vascular bed inhibits the formation of prostacyclin in these areas of arterial endothelium. The endothelium when deprived of its powerful antiaggregatory hormone becomes a surface prone to platelet adhesion and aggregation. As a consequence, adherent platelets release harmful substances and cause endothelial damage which is followed by a known sequence of events leading to the formation of an atherosclerotic plaque.[322]

The details of our concept are as follows. Potentially atherogenic lipid peroxides can be easily formed in dietary fats by autooxidation. It has been pointed out[236] that standards for some polyunsaturated dietary fats limit lipid peroxides to 20 μg/kg, which seems to be unduly high. One can imagine that high concentrations of lipid peroxides may be formed in an oil used repeatedly day and night for frying french fries. The same can happen to fat-containing products kept in a refrigerator for a period of several months. In vitro cholesterol esters of polyunsaturated fatty acids of human serum can be peroxidized in air.[237] It may well be that an overloading of the organism with dietary lipids induces a similar spontaneous peroxidation in vivo. It is more likely, however, that lipid peroxides are formed in the body due to a disturbance in fat metabolism which could result from exposure to previously mentioned risk factors of atherosclerosis. Pathological, i.e., controlled lipid peroxidation, is known to occur in mammalian tissues during vitamin E deficiency, intoxication with carbon tetrachloride, exposure to ionizing irradiation, carcinogenesis, and aging,[233,238] and we believe that it occurs during the hyperlipidemia which precedes the development of atherosclerosis.[239]

The rheologically hazardous regions of arteries represent the highest risk of lipoprotein absorption,[240] and, possibly, of lipid peroxides if their accumulation in body fluids occurs. Indeed, lipid peroxides have been detected in atherosclerotic aortas.[241-243] It may be predicted that when the local concentration of lipid peroxides in the arterial endothelium attains a level equivalent to 2.0 to 5.0 μEq/l of 15-hydroperoxyarachidonic acid (15-HPAA),[7] then the affected region of an artery will stop its production of prostacyclin.

At this stage of development of atherosclerosis there is no anatomical damage to arterial wall. The only defect is a lack of active prostacyclin synthetase. The patches of the prostacyclin-deprived endothelial cells are soon covered with carpets of adhering and aggregating platelets. A similar picture is seen when endothelial cells are damaged

by dessication[205] or by a biolaser beam.[199,244] The difference is that, in the above experimental models, endothelial cells are washed away after injury by the blood stream and the subendothelial layers are immediately exposed to platelets. In contrast, during the first stage of atherosclerosis, although their biochemical capabilities are severely compromised, endothelial cells still protect the arterial wall. Platelets form mural microthrombi, the boundaries of which are circumscribed by healthy endothelium which is capable of generating prostacyclin and thus of rejecting platelets from its surface. Aggregated platelets, adhering to the endothelium, release TXA_2, 5-hydroxytryptamine, nucleotides, lysosomal proteases, and phospholipases.[313,318] The released content of platelets accumulates between the "platelet carpet" and the "biochemically dead" endothelium. The artery is locally contracted by TXA_2 and 5-hydroxytryptamine, while the endothelium is digested by lysosomal enzymes. Morphological injury to endothelium occurs. The second stage of atherosclerosis is characterized by a massive platelet aggregation which is activated by the naked subendothelial layers. High concentrations of prostaglandin endoperoxides, TXA_2, and PGE_2 released from platelet aggregate help other platelet factors (Ross and Glomset, 1976) set myocytes out on their migration from media to intima. The proliferative inflammatory focus in the arterial wall is burning as a result of the action of chemical mediators released from platelets. Their inflammatory action is temporally limited by their short life span and by their diffusion into the blood. The inflammatory response of the arterial wall is also spacially restricted by the boundaries set by the surrounding, healthy endothelium which can eventually cover the area of injury. If, however, the process is repeated on several occasions over a long period of time, an irreversible damage to the vascular wall occurs which opens the way to secondary changes such as lipid and cholesterol infiltration. The full-scale proliferative and reparative responses develop. Myocytes and fibrous tissue grow over the mural thrombus and over the tissue debris and an atherosclerotic plaque is formed.[322]

Indirect evidence supporting our hypothesis is as follows.

1. Lipid peroxides are consistently found in human atherosclerotic aortas[241-243] but are absent in healthy arteries. Trace amounts of lipid peroxides are detectable in human plasma[233,237,245] but not in the plasma of laboratory animals (e.g., mice and rats)[237] in which spontaneous atherosclerosis is rare.
2. In premature infants inhaling pure oxygen a marked rise in plasma lipid peroxides occurs. At the same time in retinal blood vessels lesions develop which closely resemble those characteristic for atherosclerosis. Both the rise in plasma lipid peroxide levels and the vascular damage can be prevented by administration of a potent antioxidant (Hibon®).[245,246]
3. In vitro, prostaglandin endoperoxides from blood platelets are converted by arterial slices[9] and by cultured arterial cells[62] into prostacyclin. This last conversion is not inhibited by indomethacin, but it is abolished by 15-hydroperoxyarachidonic acid (15-HPAA).[9] This fact points to the possibility of an existence of a similar biochemical link between platelets and endothelial lining in vivo, a link that is broken by lipid peroxides.
4. Human veins obtained from surgical specimens generate about eight times more prostacyclin than human arteries.[130] This difference might account, at least in part, for the fact that atherosclerosis does not develop in human veins. In the rat, an animal which is notably resistant to spontaneous atherosclerosis, arteries possess as high a capacity to generate prostacyclin as reins.[247]
5. Many patients with atherosclerosis of coronary arteries have platelets which generate more TXA_2 than platelets of healthy subjects.[81] These subjects are also

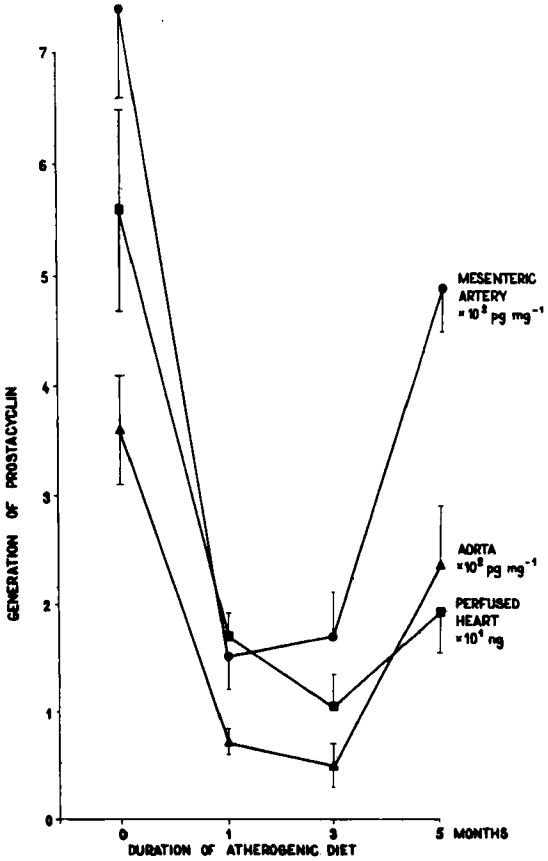


FIGURE 8. Suppression of prostacyclin generation by the incubated slices of mesenteric arteries, aortas, and by perfused hearts of the rabbits fed for 1, 3, and 5 months an atherogenic diet.[132]

hyperreactive to aggregatory agents.[217-223] Could this be due to the limited transfer of prostaglandin endoperoxides from platelets to atherosclerotic arteries? If so, then platelets would have to convert more of their PGH_2 to TXA_2.

6. The above concept is favored by the fact that the deep suppression of prostaglandin synthetase activity in arteries of atherosclerotic rabbits[132] (Figure 8) appears earlier than the "activation" of platelet aggregability in these animals. Platelet "activation" is detected only after 5 months of feeding an atherogenic diet[200] to a bull 4 months after the radical decrease of arterial prostacyclin synthetase activity.
7. The selective inhibition of thromboxane synthetase in platelets by imidazole[93,98] causes a diversion of prostaglandin endopejoxide metabolism to "classical prostaglandins".[248] A similar phenomenon occurs in atherosclerotic arterial walls which generate large amounts of PGE_2[249] and PGD_2[334] and small amounts of prostacyclin (Figure 8).[132]sTherefore it could be concluded that atherosclerosis causes damage to prostacyclin synthetase and not to cyclooxygenase in arterial walls. Lipid peroxides are thesonly known endogefous substance which selectively inhibit prostacyclin synthetase.[7] On the other hand, the generation of PGE_2 and PGD_2 by atherosclerotic arteries may represent an effect on the part of the organism to substitute the lacking prostacyclin with prothetic substances, a vasodilator PGE_2 and an antiaggregatory PGD_2.[155]

If further evidence were to be obtained in favor of our hypothesis more attention should be paid to the possible harmful effects of an excess of polyunsaturated, easily peroxidizable fats in the diet, especially in the developed countries, the inhabitants of which have become increasingly exposed to this kind of the diet. Prophylactic and therapeutic trials with chosen antioxidants (e.g., vitamin E) cyclooxygenase inhibitors, thromboxane synthetase inhibitors, prostacyclin, and its analogues would also be warranted.

Over the past 30 years several clinical studies have appeared concerning the preventive or therapeutic actions of vitamin E in arterial thrombosis, angina pectoris, myocardial infarction, and intermittent claudication. These were short-term studies, limited to small numbers of patients and platelet behavior was the main concern of the investigators. It is not surprising that their opinions on the role of vitamin E in the prevention of thromboembolism and arterial insufficiency are controversial and inconclusive.[250-256]

Vitamin E (α-tocopherol) is a physiological antioxidant whose mechanism of action is still uncertain. In most tissues, the ratio of vitamin E to unsaturated fatty acids is 1:200, while blood platelets take up vitamin E until a ratio of 1:20 is reached.[250] In man, an oral dose of 1,800 i.v. results in a complete saturation of platelets with vitamin E,[251] which, in turn, reduces platelet aggregability to collagen[251] but not to ADP and epinephrine.[252] In vitro a concentration of 1 m*M* of vitamin E inhibits platelet aggregation induced by ADP, arachidonate,[35] collagen, thrombin, and epinephrine.[253] In vitamin E deficient rats, platelet aggregability is enhanced[254] and prostaglandin biosynthesis stimulated[255] although, in vitro, vitamin E (1 to 4 m*M*) has no direct effect on cyclooxygenase activity.[35]

If vitamin E exerts any beneficial effect in prevention of atherosclerosis and in intraarterial thrombosis it does not necessarily do so via platelets which have been thought until now to be the main target for the antithrombotic action of vitamin E. Vitamin E may inhibit lipid peroxidation in body fluids and inside the arterial wall, thus protecting prostacyclin synthetase against its inactivation by lipid peroxides. The question as to whether vitamin E is effective in the prevention of atherosclerosis still remains open and the answer can be obtained in long-term, multicenter, prospective clinical studies and in basic research on the antioxidant action of vitamin E in the arterial endothelium.

Among synthetic antioxidants, special attention should be paid to butylated hydroxytoluene (BHT) which shares some important biochemical properties with vitamin E. BHT, at a concentration of 400 μM, does not inhibit the activity of mammalian cyclooxygenase, whereas it is a powerful inhibitor of soybean lipoxygenase (IC_{50} = 0.01 μM).[35] According to our concept of the development of atherosclerosis an antioxidant that will block all pathways of lipid peroxidation except for the cyclooxygenase pathway will be the ideal potential antiatherosclerotic drug (Figure 1).

IX. ASPIRIN — CYCLOOXYGENASE INHIBITORS — THROMBOEMBOLISM

The clinical aspects of the effectiveness of aspirin in arterial and venous thrombosis have been reviewed by Jobin.[315] Aspirin and a number of other cyclo-oxygenase inhibitors, when administered at theurapeutic doses in humans, abolish ex vivo platelet aggregation induced by collagen and arachidonic acid. They also reduce the second wave aggregation (TXA_2 mediated)[72] induced by ADP and epinephrine.[257] Platelet adhesion to subendothelium and collagen fibrils in flowing blood[258] is not reduced following

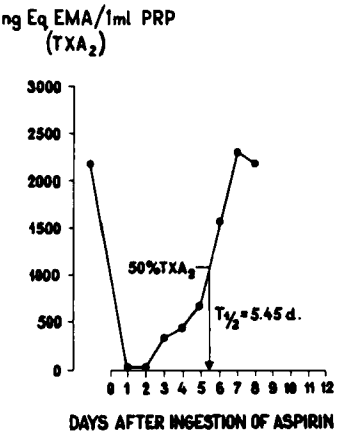


FIGURE 9. Irreversible inhibition of platelet cyclooxygenase by aspirin as the basic for assessment of platelet life span in humans. In arachidonate (1500 μM) — aggregated platelet rich plasma (PRP) of a 46 year old man there was bioassayed TXA_2 (see Figure 2) in nanogram equivalents of 11,9-epoxymethano analog of PGH_2 (EMA). The TXA_2 assay started at the "0" day before an oral ingestion of aspirin at a single dose of 600 mg and the assay of TXA_2 was repeated each day during the next eight days. Aspirin completely suppressed the generation of TXA_2 by PRP for a period of 2 days. A recovery up to the initial TXA_2 synthetizing capacity gradually appeared. In the subject studies TXA_2 synthetizing capacity in PRP recovered up to 50% of the initial value after 5.45 days from the moment of the aspirin ingestion.

ingestion of aspirin,[259,260] while the template bleeding time is doubled.[261] These properties of aspirin in man seem to be directly related to a blockade of the arachidonic acid cyclooxygenation to prostaglandin endoperoxides[262,263] and hence to TXA_2.[72,264] Aspirin does not change platelet morphology.[265]

Aspirin irreversibly acetylates an active site on the cyclooxygenase molecule (mol wt 85.000) in platelets.[266] Since platelets have no nuclei they cannot synthetize new enzyme proteins and, therefore, a single ingestion of two tablets of aspirin (600 mg) causes a long lasting suppression of platelet aggregation[267,268] and inhibition of the generation of prostaglandins,[269,269] TXA_2,[264] and malondialdehyde.[86] This suppression of platelet function lasts for several days until new platelets from bone marrow replace the "aspirin-blocked" ones. The persistence of the antiplatelet action of aspirin is reproducible in an individual who has undergone aspirin trials for the evaluation of the platelet life span (Figure 9).[86,264]

The impairment of platelet function by aspirin is persistent, but mild, and cannot be enhanced by an increase in the dosage of the drug.[315] As a matter of fact, the optimum dose of aspirin which will induce maximum platelet dysfunction and minimum inhibition of endothelial cyclooxygenase remains to be determined. We should realize that the ingestion of a tablet of aspirin leads not only to the inhibition of TXA_2 for-mation by platelets, but also creates the potential danger of silencing the endothelial production of prostacyclin, firstly, because of a direct inhibition of endothelial cyclooxygenase activity, and secondly, because of the disappearance from platelet of prostaglandin endoperoxides which could be the substrate for the endothelial prostacyclin synthetase.[9] Some hope remains that cyclooxygenase from platelets is more sensitive to the inhibitory action of aspirin than the isoenzymes in other cells and tissues. As compared to ram seminal vesicle cyclooxygenase, the platelet enzyme is 31 times more sensitive to the inhibitory action of aspirin.[270] Nonetheless, low concentrations of indomethacin[7-10] and aspirin[247] efficiently inhibit the generation of prostacyclin by vascular tissues in vitro. Villa and Gaetano[133] have shown that in rats a bolus intravenous injection of a lysine salt of aspirin at a dose of 10 mg/kg causes substantial reduction in the spontaneous generation of prostacyclin by arterial tissue ex vivo. This effect is still observed 24 hr after injection of the drug. In our laboratory we have seen that in rabbits an intravenous injection of the sodium salt of aspirin at a dose of 15 mg/kg does not inhibit the generation of prostacyclin by arterial slices while the platelet cyclooxygenase is inhibited significantly. Aspirin at a dose of 25 mg/kg results in a nearly equipotent suppression of TXA_2 generation in platelets and of prostacyclin in the mesenteric artery slices of rabbits (Figure 10). The aspirin-mediated removal of prostacyclin from arteries may explain the failure of this drug (20 to 200 mg/kg) to affect platelet-mediated myointimal cell hyperplasia in rat carotid arteries subjected to endothelial injury,[205] as well as the lack of protection aspirin (30 to 60 mg/kg) against myocardial ischemia in cats[271] and against coronary thrombosis in dogs.[272]

The above laboratory data should be a warning against using excessive doses of aspirin in antithrombotic therapy in humans. In humans, a single oral dose of 5 mg/kg (a tablet of 325 mg) of aspirin inhibits platelet cyclooxygenase activity by 89% for a period of 2 days.[270] In most clinical antithrombotic studies, aspirin is used within a range of doses from 900 to 1500 mg daily.[315]

In various models of experimental thrombosis, aspirin has been found effective[244,273,274] or ineffective[272,275] as a preventive countermeasure. Similarly, clinical studies on the prevention of thromboembolism by aspirin have yielded mixed results.[315] No firm conclusions can be drawn. Generally speaking, aspirin seems to be less effective in venous than in arterial thrombosis, although successful aspirin prophylaxis of venous thromboembolism after a total hip replacement has been reported.[276] Interestingly, only male patients benefit from the aspirin treatment. Also, only men, among patients who previously suffered from transient cerebral ischemia, benefited from the aspirin treatment.[315]

Most controversial and fascinating is the problem of protecting the myocardium against infarction by the regular intake of aspirin. Reports have repeatedly appeared stating that patients who undergo long-term, sustained aspirin treatment for rheumatoid arthritis have a considerably lower incidence of myocardial infarction than appropriate control.[277-280] Epidemiological studies seem to confirm the protective action of aspirin against fatal coronary thrombosis,[281] however, the results of double blind prospective studies on secondary prevention of incidence and mortality from myocardial infarction are not conclusive.[282-284] Two long-term, prospective, multicenter, randomized studies — (1) Aspirin Myocardial Infarction Study, 1975 (AMIS) and (2) Persantine-Aspirin Re-infarction Study, 1975 (PARIS) — have been undertaken by the Med-

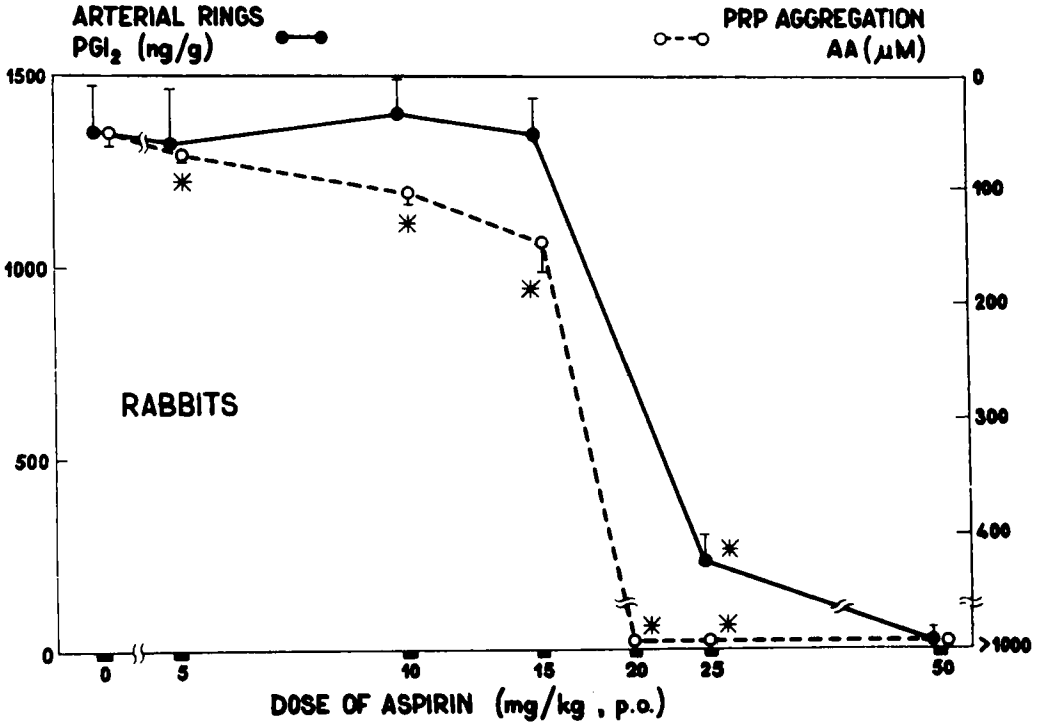


FIGURE 10. The effects of aspirin in rabbits on the generation of prostacyclin (PGI_2) by their mesenteric artery slices and on their platelet aggregability to archidonic acid (AA). Aspirin was administered orally at doses of 5, 10, 15, 20, 25, or 50 mg/kg into 43 rabbits. Blood was withdrawn 3 hr later by cardiac puncture in order to prepare platelet-rich plasma (PRP). The animals were killed and mesenteric artery was sliced into small rings. The generation of prostacyclin by arterial rings was bioassayed in parallel to the assessment of the threshold proaggregatory concentration of AA in PRP. It has been found that the platelet cyclooxygenase is more sensitive than the arterial cyclooxygenase to inhibitory action of aspirin, however, this difference in the sensitivity of the enzymes may be spotted only after administration of low doses of aspirin (5 to 15 mg/kg). The asterisks denote a statistically significant ($p < 0.01$) difference in comparison to the aspirin nontreated animals.[308] The unpublished data of R. Gryglewski et al.

ical Research Council of Great Britain and will hopefully answer the questions, "Does aspirin protect against myocardial infarction?"

Among the many cyclooxygenase inhibitors endowed with antiplatelet properties,[285] sulfin pyrazone (Anturan®) gained a wide popularity in the prevention of thromboembolism.[318] Sulfinpyrazone, a drug first introduced as an uricosuric agent in vitro inhibits its collagen-induced platelet aggregation and in vivo protects against thrombogenesis and prolongs the shortened platelet life span in patients suffering from various types of vascular insufficiency.[223] When compared with aspirin, dipyridamole, clofibrate, and hydroxychloroquine, sulfinpyrazone has the broadest spectrum of efficacy in the treatment of various types of arterial and venous thromboembolism.[286] Recently, sulfinpyrazone was reported to protect a high percent of MI survivors against reinfarction.[287]

Aspirin is used in the treatment of rheumatoid arthritis, influenza, and headache. My guess is that the best chance for aspirin-mediated antithrombotic therapy lies in the combined administration of low doses of aspirin along with either prostacyclin analogues or phosphodiesterase inhibitors. Fleming et al.[244] have shown the existence of a supraadditive interaction between aspirin and PGE_1 in preventing intra-arterial thrombosis in rabbits. PGE_1 just mimics the antiplatelet action of prostacyclin.[7,8] The antiplatelet action of PGE_1 is potentiated by phosphodiesterase inhibitors,[157] including

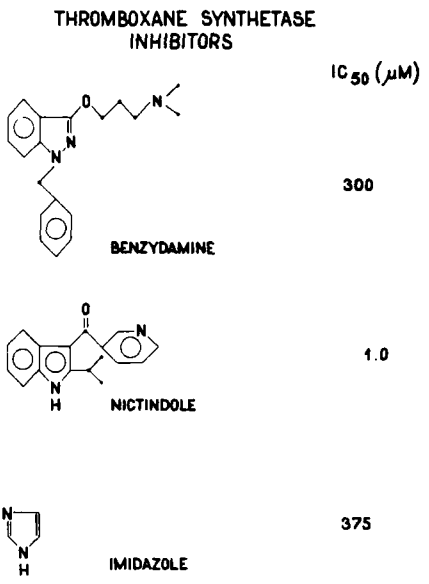


FIGURE 11. Nonacidic heterocyclic thromboxane synthetase inhibitors and their antienzymatic potency (IC_{50} μ M).

dipyridamole.[157,288] Benefit of combined therapy with aspirin plus dipyridamole has been claimed in arterial thrombosis in man.[220,318] On the other hand, the potentiation of the antithrombotic action of prostacyclin by another phosphodiesterase inhibitor (theophylline) has been demonstrated in cats[120] and rabbits.[166] Another therapeutic approach to thrombosis is the simultaneous administration of aspirin and heparin. In humans, these drugs synergistically depress the availability of platelet factor 3. The enhanced aggregability of platelets due to heparin is suppressed by a simultaneous administration of aspirin.[289] In my opinion, however, selective thromboxane synthetase inhibitors and prostacyclin analogues (Section X) will soon replace aspirin in antithrombotic therapy.

X. FUTURE TRENDS: THROMBOXANE SYNTHETASE INHIBITORS — PROSTACYCLIN ANALOGUES

By definition, thromboxane synthetase inhibitors invalidate the conversion of prostaglandin endoperoxides to TXA_2. A selective thromboxane synthetase inhibitor is expected not to influence the activities of cyclooxygenase and prostacyclin synthetase. The superiority of thromboxane synthetase inhibitors over cyclooxygenase inhibitors is based on the fact that the former do not inhibit generation of prostacyclin by arterial walls. A search for selective thromboxane synthetase inhibitors is a search for potential antithrombotic drugs, which may replace or supplement heparin, K antivitamins, aspirin, sulfinpyrazone, dipyridamole, and clofibrate in the prevention of myocardial reinfarction and arterial thromboembolism.

The known thromboxane synthetase inhibitors do not constitute a homogenous group, either chemically or pharmacologically.[290]

Two nonacidic anti-inflammatory drugs, first known as cyclooxygenase inhibitors, benzydamine[291] and nictindole (L 8027),[95-97] have recently been shown to inhibit thromboxane synthetase at a lower range of concentrations than that needed for inhibition of cyclooxygenase activity (Figure 11). Benzydamine is a weak thromboxane

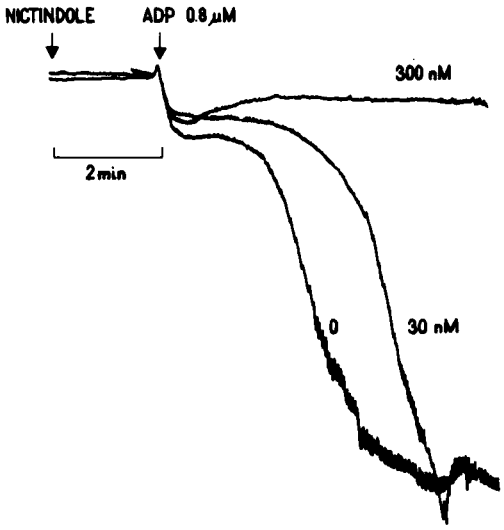
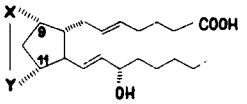


FIGURE 12. Influence of nictindole on the second (TXA_2 - mediated) wave of ADP-induced platelet aggregation in cat platelet rich plasma.

synthetase inhibitor with an IC_{50} = 300 μM, but it is even weaker as a cyclooxygenase inhibitor with an IC_{50} = 750 μM.[291] The corresponding IN_{50}s for nictindole are 1.0 μM and 6.0 μM.[95] Nictindole inhibits in vitro platelet aggregation in human and rabbit platelet rich plasma. At threshold, proaggregatory concentrations of arachidonic acid, the antiaggregatory activity of nictindole appears at concentrations as low as 2×10^{-15} M (2 fM!). There exists a distinct difference between the mode of antiplatelet action of any cyclooxygenase inhibitor (e.g., indomethacin) and the mode of action of nictindole. The latter is obviously a competitive inhibitor of arachidonate-induced platelet aggregation. Nictindole also inhibits platelet aggregation precipitated by collagen as well as the second phase of aggregation induced by epinephrine and by ADP (Figure 12), i.e., the phase that is mediated by TXA_2.[72] In vitro nictindole is a potent and fairly selective thromboxane synthetase inhibitor. In vivo nictindole is also a potent antiaggregatory agent,[121] however, one cannot be sure about the actual mechanism of its antiplatelet action. The maintenance of low "antithromboxane synthetase" nictindole levels in the blood is difficult since the drug seems to be avidly removed from the circulation.[121]

Imidazole, a compound first known as a stimulator of cyclic AMP phosphodiesterase,[292] has recently been found to inhibit thromboxane synthetase.[93,98,290] Imidazole is a weak but selective thromboxane synthetase inhibitor with an IC_{50} = 375 μM (Figure 10).[96] Therefore imidazole derivatives,[98] histamine and antagonists of histamine H_2 receptors, such as burimamide, metiamide, and cimetidine,[293] have been studied as potential thromboxane synthetase inhibitors. Only 1-methyl-imidazole (IC_{50} = 180 μM)[98] and burimamide (IC_{50} = 25 μM)[293] inhibit the enzyme activity. Unexpectedly, the influence of imidazole on platelet aggregation is rather erratic. Imidazole causes a delay in platelet aggregation in platelet-rich plasma but it is without any effect in washed platelet suspensions aggregated with arachidonic acid or with prostaglandin endoperoxides.[93] At high concentrations, imidazole may, in fact, enhance platelet aggregability through a mechanism independent of thromboxane synthesis.[52] Perhaps a direct stimulatory effect of imidazole on phosphodiesterase activity[292] or on Ca^{++} transport through biomembranes[294] may offer an explanation for the paradoxical proaggre-



SUBSTITUENTS	BIOLOGICAL EFFECTS	
	PRO-AGGREGATORY	VASOCONSTRICTOR
X – Y = O – O	1.0	1.0
X – Y = N = N	7.9	6.9
X – Y = O – CH_2	0.6	3.6
X – Y = CH_2 – O	3.7	6.2
X – Y = CH = CH	0.1	0.1
X – Y = S – S	~2.0	24.0

FIGURE 13. Relative proaggregatory and vasoconstrictor potencies of prostaglandin endoperoxide analogs as compared with the activity of PGH_2 (relative potency = 1).[79,80,297,298]

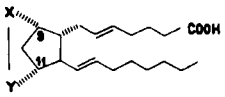
gatory action of this compound. Imidazole is also an irritant to mammalian tissues. Thus for different reasons nictindole and imidazole are both useless for the in vivo administration as thromboxane synthetase inhibitors.

The failure of imidazole to block the aggregation of washed platelet suspensions has been used by Needleman et al.[93] as evidence for the hypothesis that the generation of TXA_2 is not necessary for platelet aggregation and that prostaglandin endoperoxides can do the same job. The problem has been elucidated by the synthesis of antimetabolites of PGH_2 which are endowed with a selective and potent inhibitory action against thromboxane synthetase.[52,94,295,296] Using these pharmacological tools, it has been shown that TXA_2 plays a crucial role in the spontaneously irreversible wave of platelet aggregation,[69] as discussed in Section IV.

The chemical group of prostaglandin endoperoxide analogues and related prostanoic structures are of great biological interest. The first representative of this group was the 9,11-azo analog of PGH_2.[297] This compound and some other analogues of PGH_2[79,80,298] have the biological activity of the parent structure, i.e., they induce platelet aggregation and contract aortic strips (Figure 13). Interestingly enough, the biological activity of some PGH_2 analogues resembles the biological activity of TXA_2 rather than that of prostaglandin endoperoxides (Figures 2 and 3).

Replacement of the 15-hydroxy group in the PGH_2 molecule by a hydrogen atom and replacement of the –O–O– bridge by azo or epoxyimino bridges[295] yields a series of potent and markedly selectively thromboxane synthetase inhibitors (Figure 14).[52,69,94,296] The most potent is 9,11-azaprosta-5,13-dienoic acid (U-51605)[94] which simultaneously inhibits thromboxane synthesis and PGH_2-induced platelet aggregation, both in washed platelet suspensions[290] and in platelet-rich plasma.[69] U-51605 also inhibits the activity of cyclooxygenase in ram seminal vesicles and prostacyclin synthetase from sheep aorta and from rabbit lungs. It is in this respect 10 to 40 times weaker than a thromboxane synthetase inhibitor in human platelet microsomes.[94,296] A little bit weaker, but much more selective, is 9,11-iminoepoxyprosta-6, 13-dienoic acid.[295,296] Indeed, this last compound opens a new class of the selective thromboxane synthetase inhibitors which are antimetabolites of the substrate for TXA_2 biosynthesis.

Eakins et al.[299] have discovered that p-benzyl-4-[1-oxo-2-(4-chlorobenzyl)-3-phenyl propyl] phenyl phosphate (N-0164) at concentrations of 1 to 10 μM antagonizes the contractile action of prostaglandins at their "receptor sites" in gastrointestinal smooth muscle. In addition, high concentrations of N-0164 (20 to 100 μM) inhibit the formation of TXA_2 from prostaglandin endoperoxides by human platelet microsomes,[300,301]



SUBSTITUENTS	INHIBITORY EFFECT ON ENZYMES (μM)		
	TXA_2 SYNTHETASE	PGI_2 SYNTHETASE	AA-CYCLOOXYGENASE
X – Y = N=N	0.23	8.0	—
X – Y = HN–O	4.3	» 100	> 100
X – Y = O–NH	52.0	39.0	> 100

FIGURE 14. Prostanoic acid derivatives which inhibit thromboxane synthetase. Comparison of their inhibitory potency on TXA_2 synthetase in platelet microsomes, PGI_2 synthetase in arterial microsomes, and arachidonic acid (AA) cyclooxygenase in seminal vesicle microsomes.[52,69,94,295,196]

and block the cyclooxygenation of arachidonic acid in platelets.[290] N-0164 inhibits platelet aggregation by acting at the extracellular site as a TXA_2 receptor antagonist rather than as an inhibitor of thromboxane synthetase or cyclooxygenase. Therefore N-0164 is not a selective thromboxane synthetase inhibitor. The complex action of N-0164 on platelets will probably stimulate Eakins and colleagues to look to derivatives of N-0164 as the selective receptor antagonists of TXA_2. This, as yet, undiscovered group of TXA_2 antagonists might be as interesting as the group of thromboxane synthetase inhibitors.

Nicotinic acid inhibits TXA_2 synthesis in platelets and suppresses their aggregations,[302] but its mechanism of antiplatelet action is not clear.

Inhibition of the biosynthesis of TXA_2 in platelet or neutralization of its action on the platelet membrane may be beneficial in the prevention and treatment of atherosclerosis and arterial thrombosis when platelet reactivity is heightened and the platelet generation of TXA_2 is accelerated.[81,200]

Another approach to the therapy of arterial insufficiency and thromboembolism is based on the antiplatelet and vasodilator properties of prostacyclin. When infused intravenously into humans, the natural hormone inhibits ex vivo platelet aggregation and dissipates the circulating platelet aggregates,[167,168] however, at a high dose it can cause collapse because of its vasodepressor action. Because of its instability the route of administration of prostacyclin is restricted to intravenous infusion. When a spray of prostacyclin is inhaled, circulatory and antiplatelet effects also appear, however, the dosage of the hormone is not precise.[335]

The chemical synthesis of prostacyclin analogues is undertaken for the following reasons: (1) to improve its stability, and (2) to separate the antiplatelet and vasodepressor properties of the natural hormone. The third possible reason, i.e., an increase in the antiplatelet activity, is not an obligatory requirement. The natural hormone is powerful enough to allow for a 100-fold reduction of its biological activity in the form of a hypothetical analogue, and still be a highly active, antiplatelet drug. There is no doubt that at least two big industrial firms, deeply involved in prostacyclin research, synthesize and study hundreds of prostacyclin analogues, although only a few published reports have appeared regarding their synthesis, nomenclature, and biological properties.[303,304] In our laboratory we use the following outline for the screening of the biological activity of prostacyclin analogues.

A. Assessment of the General Pharmacological Profile

Synthetic prostacyclin analogues may be endowed not only with prostacyclin-like activity, but also with the pharmacological properties typical for prostaglandins or for TXA_2. The life span of the aqueous solution of an analogue is also an important fact

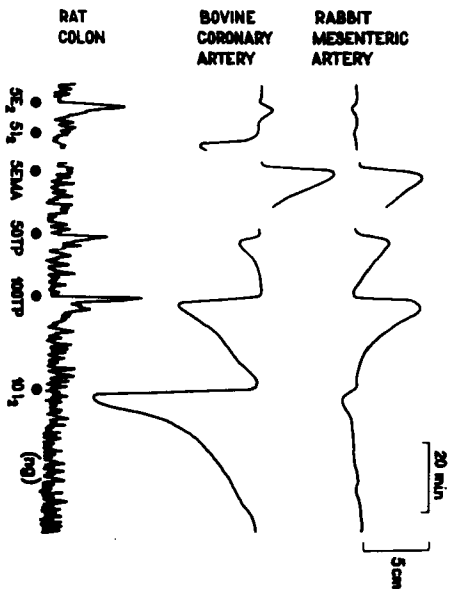


FIGURE 15. A comparison of the smooth muscle activity of a prostacyclin analog (6,9-thiaprostacyclin, TP)[303] with those of prostaglandin E_2 (E_2), prostacyclin (I_2), and 11,9-epoxymethano analog of PGH_2 (EMA). The doses are in nanograms (ng). It may be seen that in a molecule of TP there are combined prostacyclin-like properties (relaxation of bovine coronary artery), prostaglandin-like properties (contraction of rat colon), and thromboxane-like properties (contraction of rabbit mesenteric artery).

to be obtained. These goals are reached in a single experiment. A strip of bovine coronary artery,[119] a strip of rabbit mesenteric artery,[78] a rat colon, and a second strip of bovine coronary artery are superfused in cascade with Kreb's solution (at 37°C, pH 7, 6, 3 m*l*/min) which also contains a mixture of antagonists of biogenic amines.[170] The changes in length of the assay organs are recorded by auxotonic levers. In this assay system, prostacyclin causes a strong relaxation of the bovine coronary artery, a weak relaxation of the rabbit mesenteric artery, and sometimes an inhibition of spontaneous movements of the rat colon. PGE_2 contracts the bovine coronary artery and rat colon and relaxes the rabbit mesenteric artery. $PGE_{2\alpha}$ evokes a strong contraction of the rat colon and has no effect on the other two organs. TXA_2 contracts both arterial strips and has no effect on the rat colon (Figure 15). Thus prostacyclin can be easily distinguished from the other biologically active metabolite of arachidonic acid. The strip of bovine coronary artery at the bottom of the cascade is separated from the one at the top by a warmed (37°C) delay coil. The delay time can be regulated from 1 to 10 min. A difference in the relaxant potencies of a prostacyclin analogue between the upper and the lower coronary arteries is used for calculation of the life span of the studied substances. Although it seems impossible, we have seen several prostacyclin analogues which combine prostacyclin-like, prostaglandin-like and TXA_2-like properties in one molecule (Figure 14).

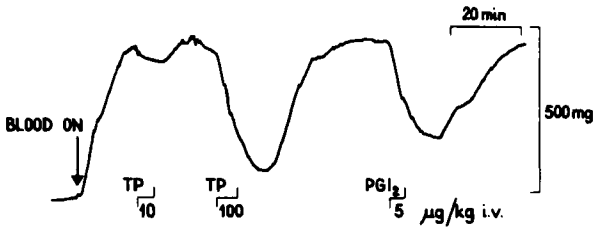


FIGURE 16. A comparison of the deaggregatory potency of a prostacyclin analog (6,9-thiaprostacyclin, TP)[303] with that of prostacyclin (PGI_2). The compounds were infused intravenously into anesthetized and heparinized cats, the mixed-venous blood of which superfused a collagen strip (blood on).[120,121] The preformed platelet clots which adhered to the blood-superfused strip were dissipated by the studied compounds. TP was 12 times less active than PGI_2

B. Assessment of Antiaggregatory Activity In Vitro

Citrated human platelet-rich plasma is aggregated with ADP (1.5 μM) in a Born aggregometer. Sixty seconds before instillation of ADP, various concentrations of an experimental analogue of prostacyclin or PGE_1 are added to platelet-rich plasma.[8] The ratio of the antiaggregatory potency of an analogue to prostacyclin and to PGE_1 is calculated. Prostacyclin inhibits the release of TXA_2 aggregated by arachidonic acid and collagen. Assessment of the inhibitory action of prostacyclin analogues on the platelet release of TXA_2 is studied by the method described previously.[72]

C. Assessment of Deaggregatory and Vasodepressor Activities In Vivo

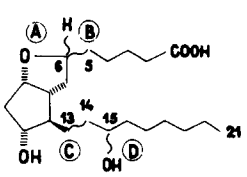
In vivo platelet clumps are formed on collagen fibers superfused with the blood of heparinized and anesthetized cats.[121] These clumps are dispersed by an intravenous injection of prostacyclin (Figure 4) and its analogues (Figure 16) but not by cyclooxygenase and thromboxane synthetase inhibitors which only prevent the formation of platelet clumps in vivo.[121] The deaggregatory and hypotensive actions of prostacyclin analogues are studied simultaneously.

This is a relatively simple way of looking for an "improved" prostacyclin among its analogues. Our approach does not exclude the detection of substances with prostaglandin-like or TXA_2-like activities, or their antagonists.

D. Structure-Activity Relationship

An example of a study on the relationship between chemical structure and biological activity within a series of prostacyclin analogues is presented in Figure 17. It may be seen that certain synthetic analogues of prostacyclin are more active biologically than the natural hormone.

The platelet receptors for prostacyclin are far more sensitive to structural variations in the molecule of prostacyclin than the smooth muscle receptors. This specificity of the platelet prostacyclin receptor may prove useful for designing a selective antiaggregatory drug, whose structure will be similar to that of prostacyclin.[307]



A POSITION	B BOND	C BOND	D POSITION	COMPOUND	ACTIVITIES ANTIPLATELET	ACTIVITIES VASODILATOR
	DOUBLE	DOUBLE		PGI_2(21-NOR)	100	100
	DOUBLE	DOUBLE		II	100	500
	DOUBLE	TRIPLE		I	400	800
	DOUBLE	TRIPLE	EPI	III	5	15
β	α - SINGLE	TRIPLE		VI	2	5
β	α - SINGLE	TRIPLE	EPI	X	0	0
β	α - SINGLE	DOUBLE		VIII	2	0
α	β - SINGLE	DOUBLE		IX	0	0
α	β - SINGLE	TRIPLE		VII	0	0

FIGURE 17. Structure-activity relationship within a series 21-methyl-PGI_2 analogs which were synthetized by C. A. Gandolfi, Carlo Erba, Milan. Their biological activity was assessed as described in Section X. In this figure antiplatelet and vasodilator activities refer to the in vitro tests. The following conclusions can be drawn.

1. Elongation of the aliphatic chain of PGI_2 by a methyl group increases vasodilator activity of prostacyclin (PGI_2 and II).
2. The desaturation of 13,14-double bond to an acetylenic bond renders a compound with a stronger antiplatelet and antiaggregatory activities than the material structure (I, II).
3. The transposition of 15-hydroxy group to 15-epihydroxy group weakens or abolishes biological activity (III, X).
4. The hydrogenation of the double the 5,6 double bond weakens biological activity when the hydrogen atom at the C6 is placed in the position beta (VI, VIII) or abolishes biological activity of PGI_2 when hydrogen atom at C6 is fixed in the position alpha (IX, X).[309]

XI. CONCLUSIONS

Blood platelets tend to adhere to and to aggregate on any surface but the intimal vascular lining. The endothelium repels platelets from its surface owing to the continuous generation of an antiaggregatory and vasodilatory local hormone prostacyclin. Prostacyclin may be also considered as a circulating hormone, since it is secreted by the lungs (pulmonary endothelium?) into arterial blood (Figure 18). Prostacyclin is biosynthetized from arachidonic acid via prostaglandin endoperoxides. These unstable intermediates in prostacyclin biosynthesis arise by the cyclooxygenation of arachidonic acid either in vascular walls or in platelets and from there are transferred to endothelial cells. This "feeding" of endothelium by the platelet prostaglandin endoperoxides could be the biochemical explanation of the morphologically detected "support" offered by platelets to endothelium.

Aggregating blood platelets convert their endogenous arachidonic acid through prostaglandin endoperoxides to a proaggregatory and vasoconstrictor local hormone, thromboxane A_2. A balance between enzymic generation of prostacyclin and thromboxane A_2 may be a principal factor responsible for maintaining homeostasis in the arterial segment of the circulation.

Prostacyclin synthetase is resistant to inactivation by a great number of trivial toxins with one exception: it is irreversibly inhibited by a low concentration of lipid peroxides. We believe that aging and atherosclerosis arise from the same common root, i.e., they

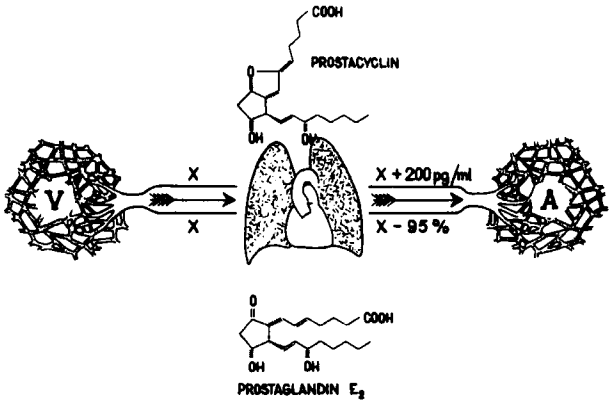


FIGURE 18. The lungs as the generator of prostacyclin. Some prostaglandins are removed from blood across pulmonary circulation (e.g., prostaglandin E_2). Prostacyclin is not removed by the lungs. On the contrary, its concentration in arterial blood (A) leaving lungs is higher than in venous blood (V). It has been proposed that the lungs act like an endocrine gland that continuously generates its hormone — prostacyclin.[60]

develop as a result of the pathological peroxidation of polyunsaturated fatty acids (Figure 19). Atherosclerosis is a consequence of an inability of endothelial prostacyclin synthetase to generate enough hormone. This biochemical defect of the arterial wall causes the loss of its unique antiplatelet properties. Platelets stick to the peroxide-contaminated patches of endothelial cells. Between a carpet of adherent platelets and a floor of defenseless endothelium a space exists where the substances released by platelets accumulate. These are proinflammatory mediators (prostaglandin endoperoxides, prostaglandin E_2, thromboxane A_2) and the destructive lysosomal enzymes (proteases, phospholipases). A local inflammatory reaction is followed by anatomical damage to arterial endothelium. The naked subendothelial layers amplify the aggregatory response of platelets and then the "myocyte migration factor" of Ross is released. The inflamed arterial wall proliferates in the form of an atherosclerotic plaque. The ulceration of an atherosclerotic plaque is the source of secondary intra-arterial thrombosis.

If the above concept gains further experimental and clinical support then prophylaxis and therapy of atherosclerosis and arterial thromboembolism need to be reformed. Hypocholesterolemic, anticoagulant, and fibrinolytic drugs should be replaced by se-lective antioxidants, thromboxane synthetase inhibitors, and prostacyclin analogues. Should these drugs be developed? Yes, since in several clinical studies, it has been shown that cyclooxygenase inhibitors (e.g., aspirin and sulfinpyrazone) are effective in the prevention of a second myocardial infarction. Cyclooxygenase inhibitors are not the best choice for antiplatelet therapy. Aspirin inhibits not only biosynthesis of thromboxane A_2 in platelets but also suppresses the generation of prostacyclin in arteries. Unlike prostacyclin, aspirin does not reverse, but only prevents, platelet aggregation. Cyclooxygenase inhibitors, however, are the only drugs available on the market which interfere with the "arachidonic acid cascade". If this nonselective interference in the platelet metabolism of arachidonic acid is successful in the prevention of thromboembolism, then it is necessary to undertake clinical trials with selective thromboxane synthetase inhibitors. An even better opportunity is present in the form of stable prostacyclin analogues which can deaggregate platelet clumps and dilate cor-

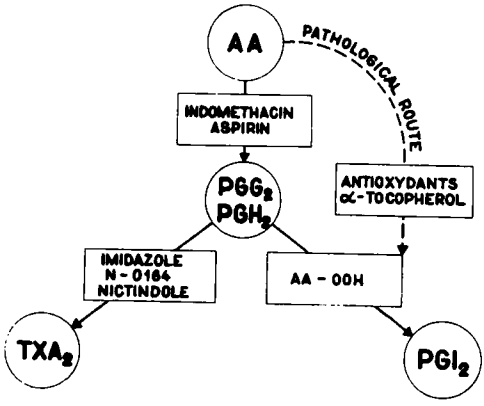


FIGURE 19. Possible interrelations within the arachidonic acid cascade. In circles there are shown major metabolites of arachidonic acid (AA) in circulation (see Figure 1). In frames there are shown the substances which inhibit the arachidonic acid metabolism at various stages. Nonsteroidal antiinflammatory drugs (e.g., aspirin, indomethacin, sulfinpyrazone) inhibit the conversion of AA to prostaglandin endoperoxides (PGG_2 and PGH_2) and thus abolish the generation of both TXA_2 and PGI_2. TXA_2 synthetase inhibitors (imidazole, N-1064 and nictindole) inhibit selectively TXA_2 formation in platelets, whereas prostacyclin synthetase is inhibited by lipid peroxide (AA-OOH) which may arise from AA due to its pathological peroxidation in the body. Activation of this pathological route of oxidation of polyunsaturated fatty acids is suggested to be responsible for the development of atherosclerosis. Vitamin E and some selective antioxidants might be helpful in blocking of this pathological route.

onary arteries. In advanced atherosclerosis, prostacyclin analogues may be considered to supplant the absent hormone and may be used as insulin is used in the treatment of diabetes mellitus.

Finally, when considering the risk factors of atherosclerosis, one should remember how dangerous lipid peroxides are. It may well be that a dietary supplement with selective antioxidants (cyclooxygenase pathway must remain open) will help us to defend ourselves against atherosclerosis, although the main problem is to spot biochemical deviations that cause pathological lipid peroxidation in the organism.

ADDENDUM

Recently there appeared papers[327,329,330] suggesting that a low incidence of myocardial infarction among the population of northwest Greenland Eskimos could be explained by high levels of eicosapentaenoic acid and low levels of arachidonic acid found in their plasma.

Most vegatable oils contain linoleic (18:2ω6), α-linolenic (18:3ω3) or oleic (18:1ω9) acids. Because of the presence of linoleic acid, the vegetable oils (e.g., sunflower oil) are recommended as the supplement to the European and American diets.[332] It is be-

lieved that linoleic acid through biotransformation to dihomo-γ-linoleic acid (20:3ω6) gives rise to a vasodilator and antiaggregatory and vasoconstrictor TXA_2.

Cod liver oil or other fish oils constitute the main dietary lipids for Eskomos. These oils hardly contain any linoleic acid but are rich in twenty and twenty-two carbon polyunsaturated fatty acids.[326] One of those acids, all-*cis*-5,8,11,14,17-eicosapentaenoic acid (20:5ω3, EPA) is the substrate for generation of prostaglandin endoperoxides, PGH_3 and PGG_3, for generation of prostaglandin endoperoxides, PGH_3 and PGG_3, which in turn are the precursors of PGE_3, PGI_3, and TXA_3.[331] It has been claimed that unlike TXA_2, TXA_3 has no proaggregatory activity on human blood platelets, while PGI_3 has antiaggregatory and vasodilator properties similar to those of prostacyclin (PGI_2).[330] Therefore, at least theoretically, high levels of EPA and loa levels of arachidonic acid in human tissues should lead to an antithrombotic state in which an active PGI_3 and nonactive TXA_3 are generated.[330]

Indeed, extensive epidemiological and analytical studies in northwest Greenland have shown that a low incidence of acute myocardial infarction, low levels of plasma cholesterol and triglycerides, and bleeding tendency among Eskimos[326-330] are not genetic in origin but are clearly a result of the Eskimo diet.[326,328] This diet causes a profound difference in the composition of plasma lipids between Eskimos and the Western population. In Eskimos the phospholipid plasma fraction has a very low content of arachidonic acid (0.8%) and a very high concent of EPA (7.1%), contrary to what is found in Caucasian Danish population (8.0% of arachidonic acid and 0.2% of EPA).[328,330] It seems that those two fatty acids compete for the same site of binding in phospholipids and therefore the composition of human phospholipids could be controlled by an appropriate diet.

Unlike arachidonic acid, EPA does not induce platelet aggregation; on the contrary it has an antiaggregatory effect.[327] There is little known about cardiovascular effects of EPA. The antithrombotic action of EPA in Eskimos is without doubt, although the mechanism of this action remains hypothetical. It may well be that dietary enrichment with EPA will be beneficial for the higly civilized societies suffering from atherosclerosis as it is beneficial for Eskimos.

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