Prostaglandins, Thromboxanes, PGX: Biosynthetic Products from Arachidonic Acid

By K. H. Gibson
ICI PHARMACEUTICALS DIVISION, MERESIDE, ALDERLEY PARK, MACCLESFIELD, CHESHIRE

1 Introduction

During the two years since the last review on prostaglandins (PG's) appeared in this series[1] significant advances have been made in this field. The identification of thromboxane A_2 (TXA_2; 5) and prostacyclin (PGX or PGI_2; 6) and the unprecedented potencies of these compounds in their effects on blood platelets and blood vessels has given additional impetus to the rapidly expanding area of prostaglandin research and, in addition, revealed new areas in which to search for therapeutically useful drugs.

The intention of this review is to consider the biosynthesis of prostaglandins, thromboxane A_2, and prostacyclin, and, in addition, it is hoped to convey some impression of, (i) the wide range of biological processes in which these compounds have important physiological roles, (ii) the possible ways in which control is exerted over the levels of prostanoid material involved in these processes, (iii) the pathological involvement of prostaglandins and related compounds in various disease states, and (iv) the potential uses which can be envisaged, regarding the manipulation of prostaglandin levels or the use of synthetic analogues or antagonists. The major biosynthetic transformations which will be considered in detail are shown in Figure 1.

The natural prostaglandins are a family of 'hormone-like' substances nominally described as oxidized derivatives of prostanoic acid (7) and characterized by a five-membered ring with two side chains containing, in total, twenty carbon atoms. The nomenclature is derived from the oxidation pattern and is depicted in Figure 2. It appears that prostaglandins are not stored in mammalian tissue but are elaborated in response to various stimuli and virtually every mammalian tissue examined has been shown to have some capacity to synthesize prostaglandin-related material. Their ubiquitous occurrence, wide range of biological effects, and high potency, have stimulated much research into the natural materials and major pharmaceutical effort has been devoted to the search for analogues which are more selective in their actions and which may have longer-lasting biological effects than the rapidly-metabolized natural prostaglandins.

2 History and Development

The biological actions of prostaglandins were first reported in the early 1930's

[1] E. W. Horton, *Chem. Soc. Rev.*, 1975, **4**, 589.

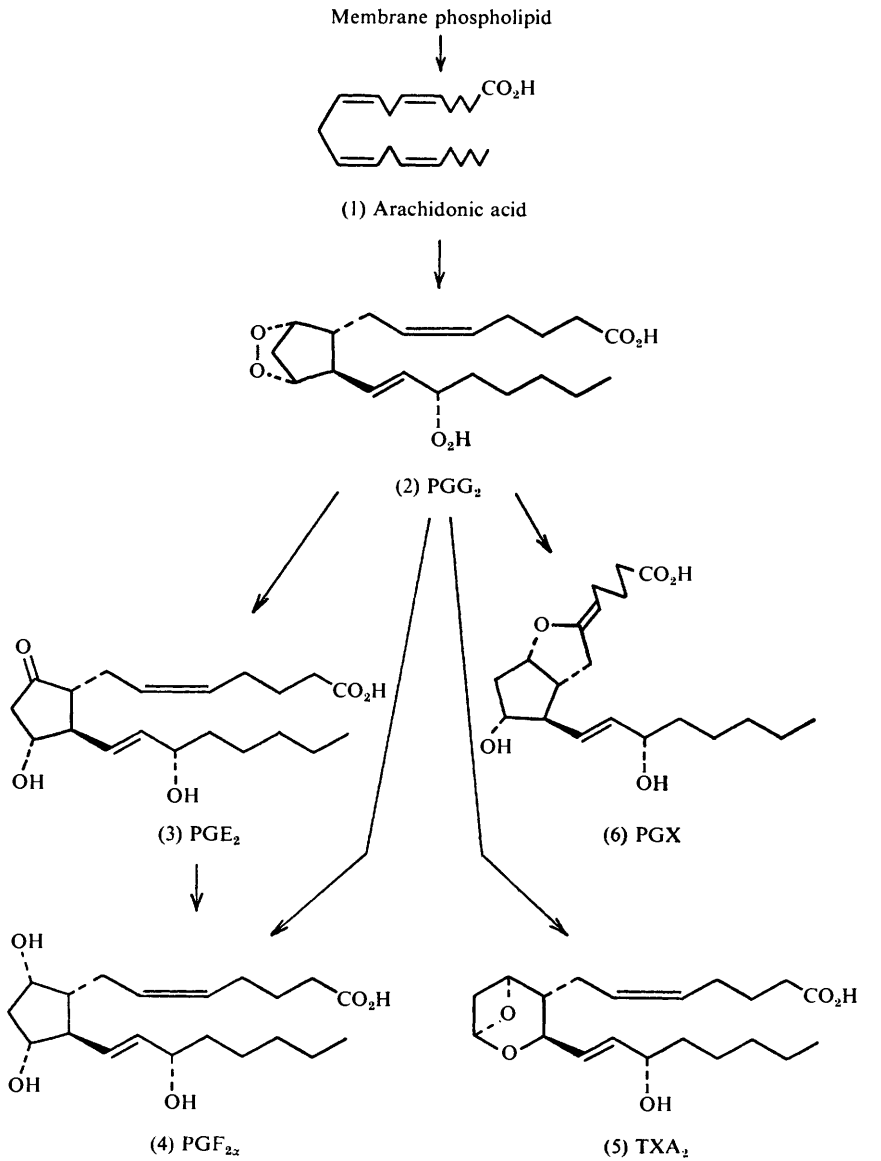


Figure 1 *Major pathways of arachidonic acid metabolism*

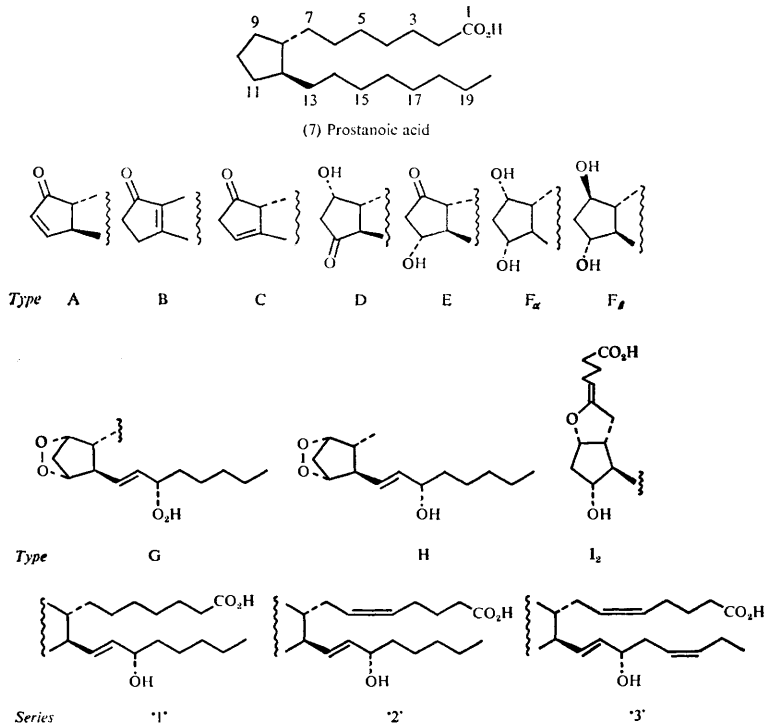


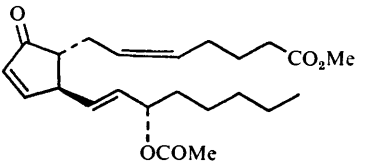
Figure 2 *Prostaglandin nomenclature*

when it was shown that a constituent of seminal plasma had the properties of lowering blood pressure and stimulating smooth muscle. Because it was thought to derive from the prostate gland the active principle was named prostaglandin by von Euler.[2] In fact, most seminal prostaglandin probably comes from the seminal vesicles and although virtually all tissues contain prostaglandin synthesizing enzymes, the misnomer 'prostaglandins' has now been universally accepted for this class of compound. However, it was not until 1962 that the first isolation, purification, and structural identification of a natural prostaglandin were reported by the Swedish biochemist Professor Sune Bergström and his colleagues.[3] Subsequently, he and fellow workers at the Karolinska Institute in Stockholm have played a leading role in the rapid increase in the volume of knowledge relating to the prostaglandins, their biosynthesis and metabolism, and their biological properties.

The structures of the prostaglandins soon prompted the speculation that they

[2] U. S. von Euler, *Klin. Wochensch.*, 1935, **14**, 1182.
[3] S. Bergström, R. Ryhage, B. Samuelsson, and J. Sjövall, *Acta Chem. Scand.*, 1962, **16**, 501.

were derived biosynthetically from the naturally-occurring, polyunsaturated, 20-carbon atom fatty acids (*e.g.* arachidonic acid). This was indeed shown to be correct and during the early years after the first structural identifications, research quantities of prostaglandins were provided by using enzyme systems to convert polyunsaturated fatty acids into the corresponding prostaglandin products.[4] However, the discovery of another remarkable natural source of prostaglandin material has provided valuable quantities for research purposes. A species of Caribbean coral, *Plexaura homomalla*, was found to contain relatively vast amounts of prostaglandins, the major component being either the 15-acetate methyl ester of PGA_2 (8) or its 15-epimer (9) depending, apparently, on the exact location of the coral colonies.[5] The prostaglandin content can be as much as 3% of the dry weight of the coral and commercial exploitation of this source is being investigated. The reason why the coral should produce such vast amounts of prostaglandin is still unknown but the enzyme system responsible will only function in solution of marine-like ionic strength.



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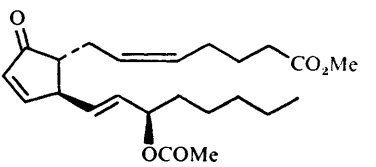


(9)

In 1964 the first papers on prostaglandin biosynthesis appeared[6] and subsequently Hamberg and Samuelsson[7] proposed a radical mechanism leading to the endoperoxide intermediate PGG_1.

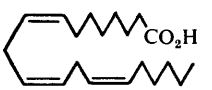
Knowledge of PG involvement in disease processes can be considered to date from 1971 when Vane[8] and others showed that aspirin-like drugs inhibit PG synthesis and Vane suggested that this was the biochemical mechanism of their anti-inflammatory activity. As well as PG involvement as mediators in a wide range of very diverse disease processes there may also be disorders which are very specifically attributable to defects in PG metabolism. The Karolinska workers[9] have identified a blood-clotting disorder which is caused by a deficiency of blood platelet cyclo-oxygenase, one of the PG biosynthesizing enzymes. A

[4] D. I. Weisblat, *Drug Cosmet. Ind.*, 1972, **111**(1), 34.
[5] W. P. Schneider, R. D. Hamilton, and L. E. Rhuland, *J. Amer. Chem. Soc.*, 1972, **94**, 2122.
[6] D. A. van Dorp, R. K. Beerthius, D. H. Nugteren, and H. Vonkeman, *Biochim. Biophys. Acta*, 1964, **90**, 204; S. Bergström, H. Danielsson, and B. Samuelsson, *Biochim. Biophys. Acta*, 1964, **90**, 207.
[7] M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, 1967, **242**, 5336.
[8] J. R. Vane, *Nature New Biol.*, 1971, **231**, 232; J. B. Smith and A. L. Willis, *Nature New Biol.*, 1971, **231**, 235.
[9] C. Malmsten, M. Hamberg, J. Svensson, and B. Samuelsson, *Proc. Nat. Acad. Sci., U.S.A.*, 1975, **72**, 1446.

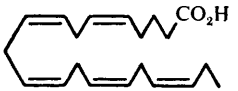
disorder known as Bartter's syndrome characterized by hypokalaemia, hyperreninaemia, hyperaldosteronism, juxtaglomerular hyperplasia, normotension with resistance to the pressor effects of angiotensin II seems to be caused by a primary lesion which is the overproduction of prostaglandin by the kidney.[10]

Evidence is now emerging of PG involvement in many normal physiological processes. The most widely known example is the natural luteolytic effect of $PGF_{2\alpha}$ in certain species[11] but effects on the circulation, on respiration, neurotransmission, immune response, temperature control, pain receptors, *etc.*, are now being reported and evaluated. There is also a certain amount of speculation[12] as to how they function and what role they take on, *e.g.* are they local hormones or bioregulators?

Regardless of where PG's occur, the biosynthetic route appears to be basically the same in all mammalian tissues. Thus, for example, PGE_2 (3) is derived from the endoperoxide intermediate PGG_2 (2) which in turn is derived from arachidonic acid (1). Although it is possible that the enzymes of different tissues may be different, or present in different concentrations, and their co-factor requirements may be different, thereby giving rise to the differing proportions of the various prostanoid products which are characteristic of any one particular tissue, the biosynthetic chain of events leading to prostaglandins provides us with a common theme. Furthermore, although we are here concerned principally with metabolites of arachidonic acid, the biosyntheses of prostaglandins of the PG_1 and PG_3 series have a number of close analogies with the corresponding conversions in the PG_2 series. The former are derived from dihomo-γ-linolenic acid (10) and the latter from 5,8,11,14,17-eicosapentaenoic acid (11).



(10) Dihomo-γ-linolenic acid



(11) 5,8,11,14,17-Eicosapentaenoic acid

3 Availability of Arachidonic Acid

The major source of arachidonic acid is membrane phospholipid, principally lecithin (Figure 3). It is claimed, for example, that of the arachidonic acid released from platelets stimulated by thrombin, 70% derivatives from phosphatidyl choline (lecithin), 25% from phosphatidyl inositol, and 5% from phosphatidyl serine.[13] There does seem to be some possibility that in special cases some arachidonic acid may derive from triacyl glycerols (adipose tissue) or cholesterol esters (adrenal and ovarian tissue).

[10] L. Norby, R. Leutz, W. Flamenbaum, and P. Ramwell, *Lancet*, 1976, II, 604.
[11] E. W. Horton and N. L. Poyser, *Physiol. Rev.*, 1976, **56**, 595.
[12] E. W. Horton, *J. Pharm. Pharmacol.*, 1976, **28**, 389.
[13] J. B. Smith and M. J. Silver, *Biochim. Biophys. Acta*, 1976, **424**, 303.

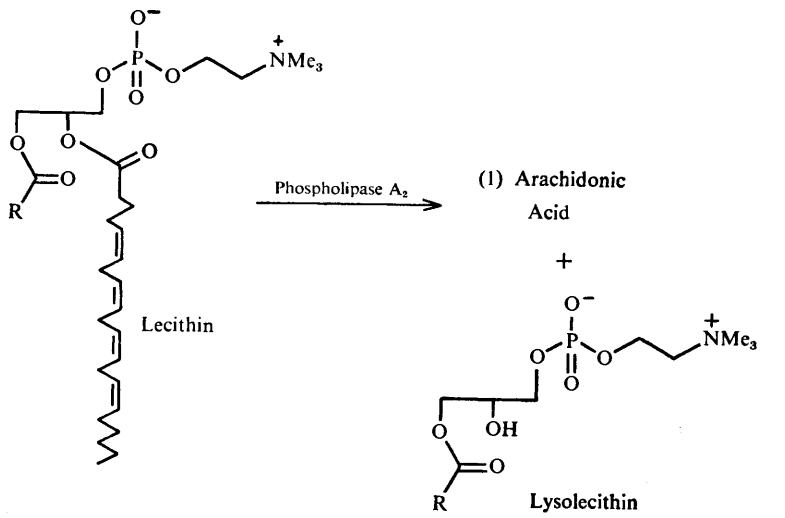


Figure 3 *Phospholipase A_2 action*

The enzyme responsible for cleaving the arachidonic acid from the 2-position of lecithin is phospholipase A_2. Many different phospholipase A_2 enzymes have been obtained from various sources and quite a lot is known about them.[14] Roughly they fall into two types. Type I is membrane bound, is stimulated by Ca^{2+}, has optimum pH 7—8, and its activity is very closely related to membrane stability. Type II phospholipase A_2 enzymes are soluble enzymes inhibited by Ca^{2+} and with pH optimum in the range 4—6. These enzymes are found in lysosomes in adrenals, spleen, macrophages and similar enzymes are also found in the venoms of bees, wasps, snakes, scorpions, and gila monsters. The active form of soluble (Type II) phospholipase A_2 is formed from an inactive pre-phospholipase A_2 and the porcine pancreatic prephospholipase A_2 has been obtained in a crystalline form and its structure determined by *X*-ray crystallographic analysis.[15] It has 132 amino-acids and can be activated by trypsin which removes the first seven amino-acids to give the active enzyme. A possible mechanism of action is proposed in which the enzyme binds the lecithin by the phosphate to a Ca^{2+} atom and Arg-108 and the fatty acid side-chains sit on a lipophilic area of the enzyme. The reacting parts of the enzyme are probably the carboxylate of Asp-56 which functions as the nucleophile, His-55 which stabilizes the tetrahedral intermediate, and Tyr-35 which provides a proton for the lysolecithin.

[14] W. E. M. Lands and L. H. Rome, in 'Advances in Prostaglandin Research. Prostaglandins: Chemical and Biochemical Aspects', ed. S. M. M. Karim, M.T.P. Press, Lancaster, 1975, p. 87.
[15] J. Drenth, C. M. Enzing, K. H. Kalk, and J. C. A. Vessies, *Nature*, 1976, **264**, 373.

It is still necessary, however, to explain why mammalian phospholipase A_2 is unable to attack lecithin within the tightly packed structure of the native membrane, although it can attack lecithin in the form of monomeric units or in loosely packed micelles. In this respect it is interesting to note that phospholipase A_2 from snake venom can attack lecithin in native membrane to release fatty acid and lysolecithin (Figure 3) which is a very potent haemolytic agent and will destroy both red and white blood cells.[16] Some of the toxic effects of venom may be the result of haemolysis caused by the lysolecithin released by phospholipase A_2 activity. However, the arachidonic acid released also warrants consideration as some of the toxic effects of venom[17] are not unlike effects which are attributable to arachidonic acid metabolites. (An intravenous injection of only 1.4 mg/kg body wt of arachidonic acid is lethal in the rabbit,[18] the cause of death being intravascular platelet aggregation leading to blockage of pulmonary arteries). The mammalian phospholipase A_2 may need, however, some assistance before it can release arachidonic acid from lecithin within the membrane and this may be where other activating factors are implicated.

Vane[19] has reported on an RCS-releasing factor (*R*abbit Aorta *C*ontracting *S*ubstance [RCS] activity is mainly due to thromboxane A_2-*vide infra*) and RCS-RF is probably a peptide of less than 10 amino-acid units. It is released from sensitized guinea pig lung upon immunological challenge and causes the release of arachidonic acid from lung tissue. It is apparently not stored in the lung tissue but is elaborated in response to the immunological stimulus. Somehow it is involved with the stimulation of phospholipase A_2 activity but whether this is stimulation of active phospholipase A_2 from prephospholipase A_2, or action as a cofactor, or is in some way related to making phospholipid in the membrane more available for phospholipase A_2 action remains to be determined. Interestingly, the action of RCS-RF is inhibited by the anti-inflammatory steroids with relative potency very similar to their relative anti-inflammatory action but only in intact cells and the effect of steroids is not demonstrable on cell fragments. The naturally occurring vasodilator peptide, bradykinin, will also release arachidonic acid from lung tissue but this action is not sensitive to inhibition by the anti-inflammatory steroids.

Slow reacting substance of anaphylaxis (SRS-A), another compound released from lung tissue during immunological challenge, is apparently similar to, but not identical with, RCS-RF. SRS-A also has the ability to release prostanoid material from lung tissue and furthermore the release of SRS-A is apparently subject to negative feedback control by product prostaglandin because blockage of the formation of prostaglandin causes potentiation of the release of SRS-A.*[20]

* *Note added in proof:* evidence has been recently presented (M. K. Bach, J. R. Brashler, and R. R. Gorman, *Prostaglandins*, 1977, **14**, 21) that SRS-A is itself a biosynthetic product from arachidonic acid.

[16] A. T. Tu, R. B. Passey, P. M. Toom, *Arch. Biochem. Biophys.*, 1970, **140**, 96.

[17] P. R. Larson and J. Wolff, *Biochem. Pharmacol.*, 1968, **17**, 508.

[18] M. J. Silver, W. Hoch, J. J. Kocsis, C. M. Ingerman, and J. B. Smith, *Science*, 1974, **183**, 1085.

[19] F. P. Nijkamp, R. J. Flower, S. Moncada, and J. R. Vane, *Nature*, 1976, **263**, 479.

[20] D. M. Engineer, P. J. Piper, and P. Sirois, *Brit. J. Pharmacol.*, 1976, **57**, 460P.

Clearly the availability of the precursor arachidonic acid is subject to complicated biological control mechanisms.[21] It may also be the locus from which hypersensitivity conditions (*e.g.* certain types of asthma) arise, a thesis supported by the apparent relationship between the activity of anti-inflammatory steroids and their effects on arachidonic acid release.

4 Prostaglandin Synthetase

A. Cyclo-oxygenase.—The conversion of arachidonic acid into prostaglandins is referred to loosely as a process brought about by PG synthetase (PGS) although it is clearly a complicated process involving a number of enzymes. In 1967 Hamberg and Samuelsson[7] proposed a radical mechanism (Figure 4) leading to an endoperoxide intermediate and the appropriate labelling studies have shown that it is specifically the pro-S proton at C-13 (labelled as ^3H in Figure 4) which is removed and studies[22] with isotopically-labelled oxygen have shown that one molecule of oxygen gives rise to both oxygen functions at C-9

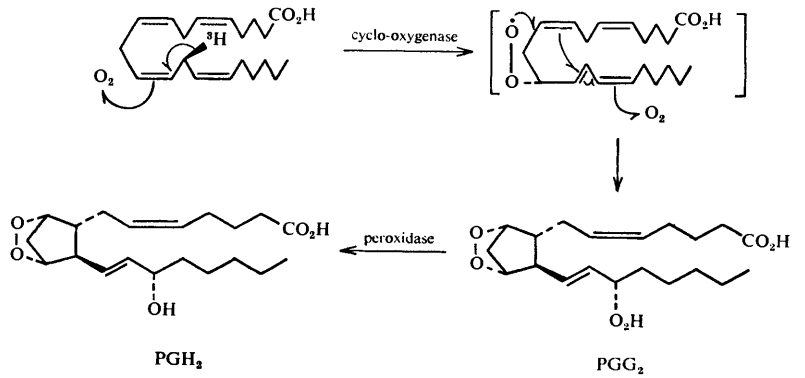


Figure 4 *Cyclo-oxygenase activity*

and C-11. Subsequently, both endoperoxide intermediates PGG_2 and PGH_2 have been isolated.[23] Although the PG synthetase activity is membrane bound, it can be solubilized and resolved into two fractions.[24] Fraction I, referred to as cyclo-oxygenase (it also contains peroxidase activity) because it leads to the formation of the cyclic endoperoxides PGG_2 and PGH_2, has the following properties: it uses haeme as a cofactor; the peroxidase activity requires tryptophan (or sero-

[21] R. J. Flower and G. J. Blackwell, *Biochem. Pharmacol.*, 1976, **25**, 285.
[22] B. Samuelsson, *J. Amer. Chem. Soc.*, 1965, **87**, 3011.
[23] D. H. Nugteren and E. Hazelhof, *Biochim. Biophys. Acta*, 1973, **326**, 448; M. Hamberg, and B. Samuelsson, *Proc. Nat. Acad. Sci., U.S.A.*, 1973, **70**, 899; M. Hamberg, J. Svensson, T. Wakabayashi, and B. Samuelsson, *Proc. Nat. Acad. Sci., U.S.A.*, 1974, **71**, 345.
[24] T. Miyamoto, N. Ogino, S. Yamamoto, and O. Hayaishi, *J. Biol. Chem.*, 1976, **251**, 2629; *ibid.*, 1977, **252**, 890.

tonin, or epinephrine, *i.e.* a hydrogen donor); it is irreversibly inhibited by peroxides; it is reversibly inhibited by glutathione peroxidase; the action of the enzyme appears to catalyse its own destruction and in this way it is similar to fatty acid dioxygenase, *e.g.* from soybeans; there is some need for an 'essential activator' which is produced during the oxygenation, *i.e.* some form of positive feedback;[25] it is inhibited by non-steroidal anti-inflammatory agents and also by poly-ynoic acids such as eicosatetraynoic acid. Fraction II, the isomerase part of the PG synthetase complex, causes the isomerization of the endoperoxide moiety of PGG_2 or PGH_2 to the β-hydroxyketone moiety present in PGE_2 (*vide infra*). This fraction is fairly unstable ($t_{\frac{1}{2}} \sim 30$ min at ambient temperature) although it can be stabilized by thiol groups and it actually uses glutathione as a cofactor.[24]

The cyclo-oxygenase part of the biosynthetic pathway has been investigated in some detail but no complete definitive picture has yet emerged. Lands and Rome[14] have proposed detailed kinetics to fit the observed data and O'Brien[26] has proposed that peroxidase activity in the synthase gives rise to hydroperoxides and then eventually the peroxy radical intermediate of the Hamberg and Samuelsson mechanism. O'Brien[27] has also proposed that singlet oxygen (as a metal complex) is involved as the initiator of the synthase. Singlet oxygen may also be responsible for the PGS inactivation because bilirubin, a singlet oxygen scavenger, inhibits the synthase reaction and also protects the synthase both from inactivation by peroxides and from self-catalysed destruction. These ideas are combined schematically in Figure 5.

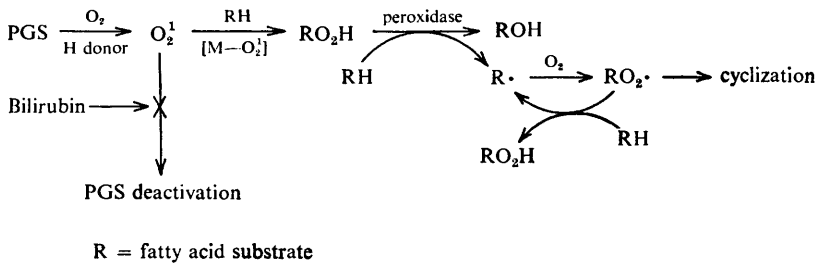


Figure 5 *Possible involvement of singlet oxygen and peroxidase mechanism for prostaglandin biosynthesis*

Peroxidation at C-11 is not the only pathway by which arachidonic acid is metabolized. For example, in blood platelets a large proportion or arachidonic acid is metabolized by an alternative pathway[28] which gives rise to 12-hydroperoxyeicosatetraenoic acid (HPETE) and 12-hydroxyeicosatetraenoic acid

[25] W. L. Smith and W. E. M. Lands, *Biochemistry*, 1972, **11**, 3276.
[26] P. J. O'Brien and A. Rahimtula, *Biochem. Biophys. Res. Comm.*, 1976, **70**, 832.
[27] A. Rahimtula and P. J. O'Brien, *Biochem. Biophys. Res. Comm.*, 1976, **70**, 893.
[28] M. Hamberg and B. Samuelsson, *Proc. Nat. Acad. Sci., U.S.A.*, 1974, **71**, 3400.

(HETE) (Figure 6). The conversion into 15-hydroperoxyarachidonic acid (15-HPAA) which is a potent inhibitor of the formation of prostacyclin may be of great importance to the vasculature (*vide infra*).

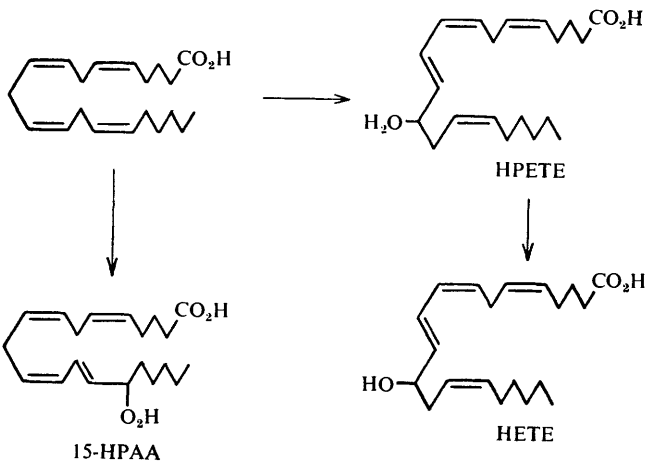
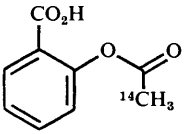


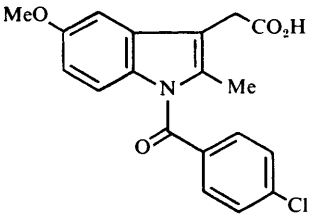
Figure 6 *Alternative metabolic oxidations of arachidonic acid*

B. Cyclo-oxygenase Inhibitors.—As mentioned earlier, in 1971 Vane, and Smith and Willis,[8] showed that aspirin-like drugs inhibit PG synthesis and Vane suggested that this is probably the biochemical mechanism by which they exert their anti-inflammatory activity. In the PG synthase pathway it is the cyclo-oxygenase step which is most sensitive to inhibition by this type of anti-inflammatory agent and a brief consideration of cyclo-oxygenase inhibitors may be worthwhile (Figure 7).[14]

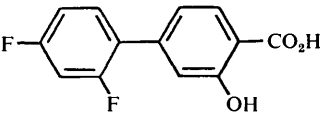
In the case of aspirin itself there is evidence to show that some of the inhibition is caused by irreversible acetylation of the cyclo-oxygenase enzyme; radio-labelling studies support this. With indomethacin, although an analogous *p*-chlorobenzoylation is mechanistically feasible, the available evidence[29] does not support this and indomethacin must be included within a large number of acidic anti-inflammatory compounds of salicylic acid types (*e.g.* diflufenisal), the fenamates (*e.g.* flufenamic acid), and the acetic acid derivatives (*e.g.* sulindac), where one can merely indicate a vague similarity of size and shape between the inhibitor and arachidonic acid or the hydroperoxy radical intermediate (Figure 4) and thereby suggest some interaction which can lead to inhibition of the cyclo-oxygenase. There may be some ability within the non-steroidal anti-inflammatory agents to react with radicals and a mechanism has been proposed along these

[29] N. Stanford, G. J. Roth, T. Y. Shen, and P. W. Majerus, *Prostaglandins*, 1977, **13**, 669.

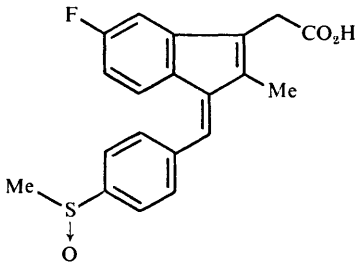
I Aspirin:  + Enz·NH$_2$ ⟶ Enz·NH·CO^{14}CH$_3$ (+SA)



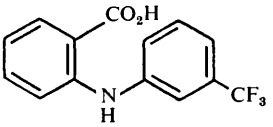
Indomethacin



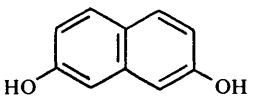
Diflufenisal



Sulindac

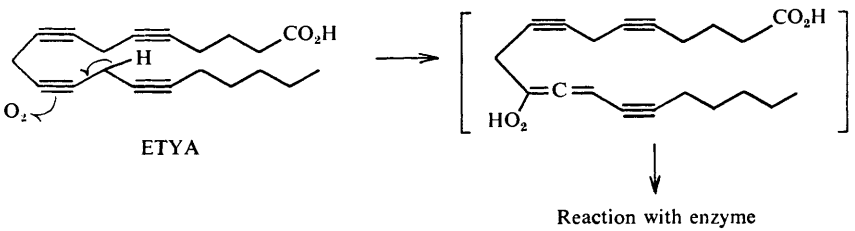


Flufenamic acid



2,7-Dihydroxynaphthalene

III



ETYA

Reaction with enzyme

IV Glutathione peroxidase ⟶ Removes radicals?

Figure 7 *Cyclo-oxygenase inhibitors*

lines.[30] 2,7-Dihydroxynaphthalene is an extremely potent inhibitor of cyclo-oxygenase.[31]

Acetylenic acids, *e.g.* eicosatetraynoic acid (ETYA), will inhibit the cyclo-oxygenase activity.[32] These acetylenic acid inhibitors may be acting as k_{cat} inhibitors[33] whereby the enzyme accepts them as a substrate and converts them into a highly reactive allene (Figure 7). This allene is formed at the active site of the enzyme and rapidly reacts with some nearby function and thereby renders the enzyme inactive. This is a mechanism well characterized for a number of other enzyme inhibitors. The enzyme is also inhibited by glutathione peroxidase, possibly by lowering the concentration of activator (radical?) and recent evidence indicates that this may be a natural inhibitor of PG synthesis.[34]

C. Reactions of PGG_2.—From PGG_2 there are two steps required in order to obtain PGE_2 or $PGF_{2\alpha}$, *i.e.* a peroxidase step to convert the 15-hydroperoxy into a 15-hydroxy function and an isomerase step to isomerize the endoperoxide moiety to a β-hydroxyketone, or alternatively a reductase step to directly cleave the endoperoxide to a 1,3-diol (Figure 8). In the 8000 × g supernatant from sheep seminal vesicles and in the absence of added glutathione the major products are 15-hydroperoxy-E_2 and 15-keto-E_2 and this is part of the evidence which leads Samuelsson[35] to suggest that the isomerase followed by the peroxidase is probably the preferred pathway in sheep seminal vesicles; both pathways may be operative.[24] With the 8000 × g sheep vesicular gland supernatant system and added glutathione $PGF_{2\alpha}$ is the major product.

Mechanistically, the isomerization of PGH_2 to PGE_2 requires only the loss of the proton from C-9 and the opening of the endoperoxide bridge as shown by the arrows. The analogous isomerization involving loss of the C-11 proton and the opening of the endoperoxide bridge in the alternative manner leads to PGD_2 (Figure 8) which is a very potent inhibitor of aggregation of human platelets.[36] An enzyme which converts PGG_2 into PGD_2 is present in human serum.

Not only can $PGF_{2\alpha}$ be formed by direct reduction of PGG_2 but it can also be formed by the reduction of PGE_2 by the action of an enzyme 9-keto-reductase and the widespread importance of this enzyme is now being recognized.[37–39] In certain situations PGE_2 and $PGF_{2\alpha}$ have antagonistic effects, *e.g.* PGE_2 is a vasodilator whereas $PGF_{2\alpha}$ is a vasoconstrictor. 9-Keto-reductase enzymes can

[30] D. W. Cushman and H. S. Cheung, *Biochim. Biophys. Acta.*, 1976, **424**, 449.
[31] C. Takeguchi and C. J. Sih, *Prostaglandins*, 1972, **2**, 169.
[32] D. T. Downing, D. G. Ahern, and M. Bachta, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 218.
[33] R. R. Rando, *Science*, 1974, **185**, 320.
[34] H. W. Cook and W. E. Lands, *Nature*, 1976, **260**, 630.
[35] M. Hamberg, J. Svensson, and B. Samuelsson, in 'Advances in Prostaglandin and Thromboxane Research', eds. B. Samuelsson and R. Paoletti, Raven Press, New York, 1976, vol. 1, p. 19; B. Samuelsson, *ibid.*, p. 1.
[36] J. B. Smith, M. J. Silver, C. M. Ingerman, and J. C. Kocsis, *Thromb. Res.*, 1974, **5**, 291.
[37] P. Y-K. Wong, D. A. Terragno, N. A. Terragno, and J. C. McGiff, *Prostaglandins*, 1977, **13**, 1113.
[38] K. J. Stone and M. Hart, *Prostaglandins*, 1976, **12**, 197.
[39] Y. Friedman, S. Levasseur, and G. Burke, *Biochim. Biophys. Acta*, 1976, **431**, 615.

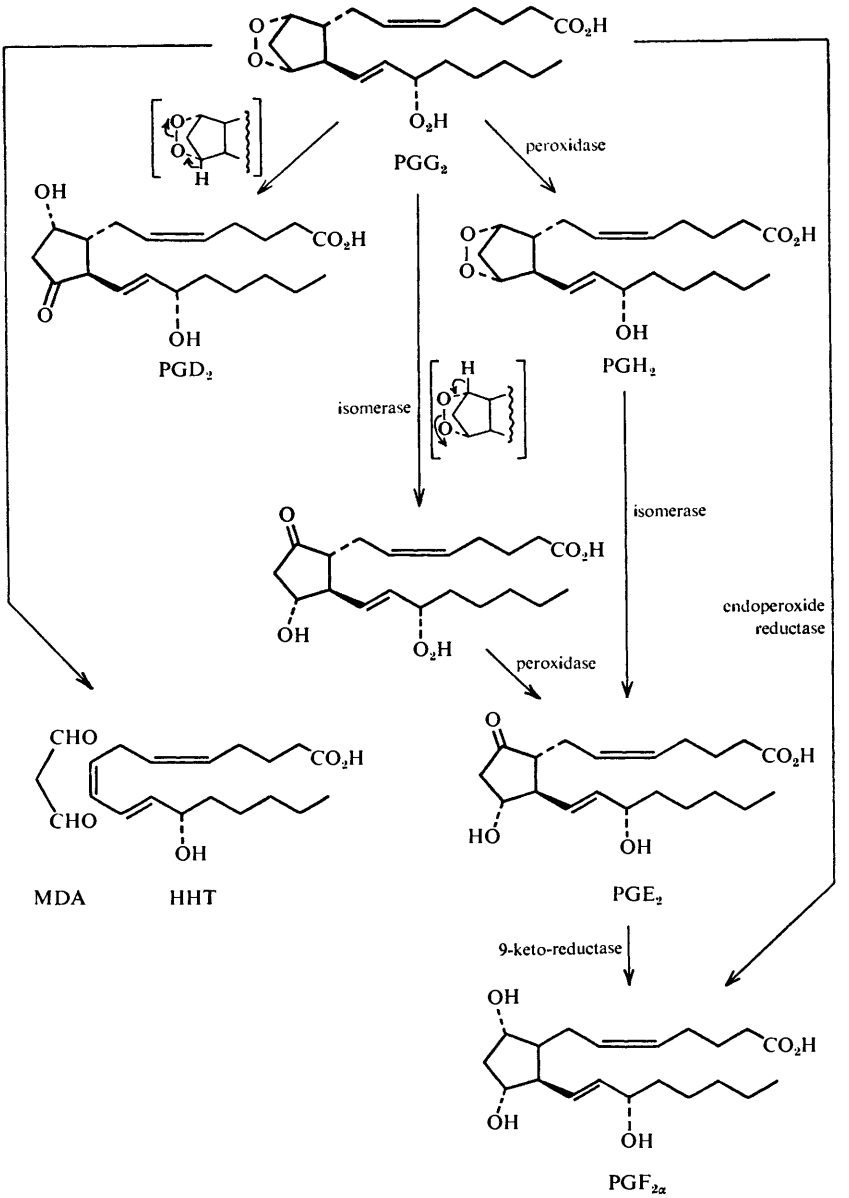


Figure 8 *Reactions of PGG_2*

be obtained from arterial and veinous tissues and both enzymes are activated by cyclic-GMP. However, the enzyme from veinous tissue is much more sensitive to cyclic-GMP stimulation than is the enzyme from arterial tissue. Kinins also stimulate the 9-keto-reductase and as they also stimulate the availability of arachidonic acid so they have tremendous potential to affect the amount and the ratio of the E and F prostaglandins.[37] The 9-keto-reductase from kidney is reported to be inhibited by non-thiazide diuretics (furosemide, chlorthalidone); other inhibitors of this enzyme are hydralazine (vasodilator), phentolamine (α-blocker), indomethacin (anti-inflammatory) and ethacrynic acid (diuretic).[38] It seems likely that the control of the PGE:PGF ratio has physiological importance and it is possible that some pharmacological effects are manifestations of the ability of certain drugs to cause variations in this ratio.

PGG_2 can also decompose to malondialdehyde (MDA) and hydroxyheptadecatrienoic acid (HHT, Figure 8). The formation of MDA has been widely used as a quantitative measure of the formation of PGG_2 but the reaction may simply be a thermal decomposition and biologically it is of dubious significance.

5 Thromboxanes

The biosynthetic products formed from endogenous arachidonic acid, upon treatment of washed human platelets with thombin are shown in Figure 9.[35]

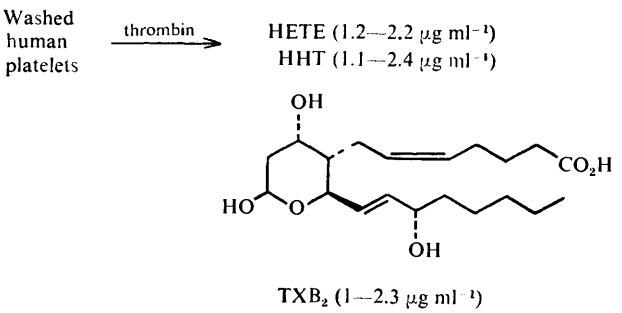


Figure 9 *Arachidonic acid metabolites from human platelets*

Only very small amounts of PGE_2 and $PGF_{2\alpha}$ are formed in this reaction. Some of the arachidonic acid is converted *via* hydroperoxyeicosatetraenoic acid (HPETE) into hydroxyeicosatetraenoic acid (HETE; Figure 6). Hydroxyheptadecatrienoic acid (HHT) is formed by retro-Diels–Alder reaction which splits off malondialdehyde (MDA) from the endoperoxide (Figure 8), and a similar amount of material is found as the novel compound, originally referred to as PHD, now known as thromboxane B_2 (TXB_2), in which the cyclopentane ring has been converted into a tetrahydropyranyl ring. It was reasoned that TXB_2 was derived by rearrangement of PGG_2 (or PGH_2) with subsequent addition of a molecule of water and in accord with this hypothesis it was found that the inter-

mediate could be trapped by added nucleophiles such as MeOH, EtOH, or N_3^- (Figure 10). These products were identified and this led to the proposal that the intermediate was an oxetane and it was named thromboxane A_2 (TXA_2) because of its extreme potency in inducing platelet aggregation.

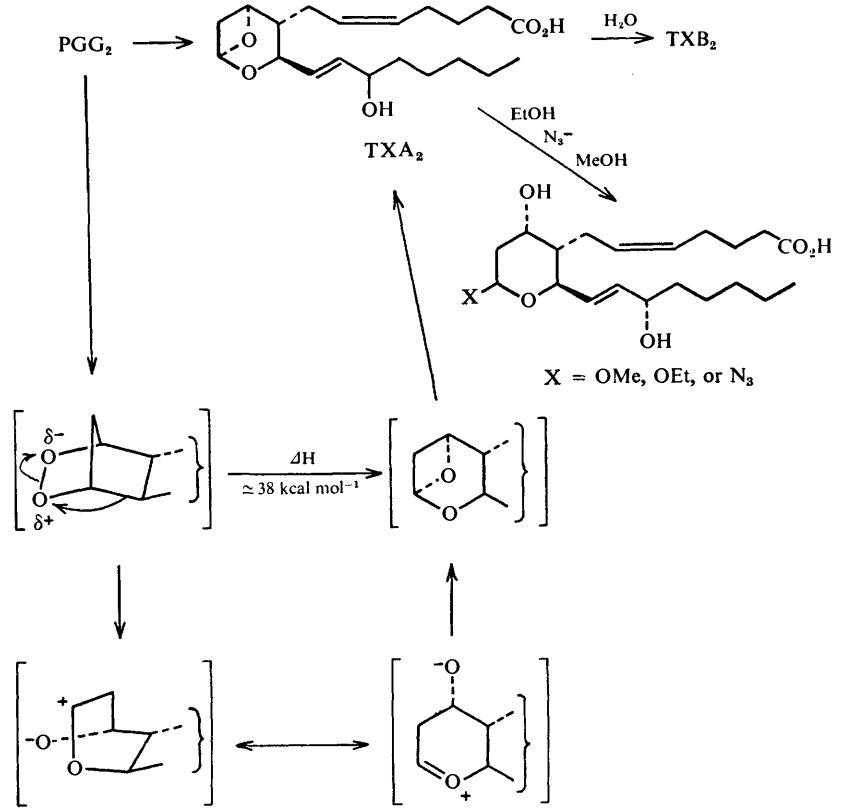
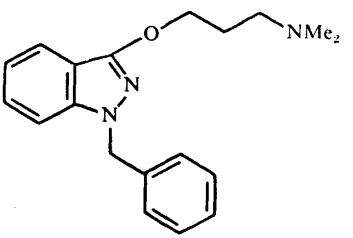


Figure 10 *Formation of thromboxane* A_2

Thromboxane A_2 can be obtained by very brief incubations of arachidonic acid with human platelets or by incubation of preformed PGG_2 with horse platelet microsomes. It is extremely labile and hydrolysis to form TXB_2 proceeds with a half-life of about 36 s in water at 37 °C. TXA_2 is a highly potent stimulant of the contraction of certain smooth muscle preparations, *e.g.* it is many times more potent than $PGF_{2\alpha}$ in the stimulation of guinea-pig trachea and in its effect on guinea pig lung insufflation pressure. The effect of TXA_2 on certain vascular tissue was known long before its structural identification by Samuelsson. As

early as 1969 Piper and Vane[40] had described the release of RCS from guinea pig lung during anaphylaxis. Vane subsequently showed that aspirin-like drugs inhibit PG synthesis and also inhibit the release of RCS and it was suggested that RCS might be an endoperoxide intermediate.[41] However, when the pure endoperoxides became available, Samuelsson showed that although they were active on rabbit aorta they were too long lived and of insufficient potency to be identical with RCS. It now transpires that RCS is in fact a mixture of TXA_2 together with some endoperoxides, but the majority of the biological activity is due to the TXA_2.[35] Thromboxanes have now been found in platlets, lung, leucocytes, umbilical artery, spleen, brain, kidney, and seminal vesicles; indeed, in some of these tissues this is the major route of arachidonic acid metabolism.[42] Their widespread occurrence is the subject of much speculation regarding their physiological importance, particularly that of TXA_2 in cardiovascular incidents, because of its ability to aggregate platelets and to cause vasoconstriction. TXA_2 production by anaphylactically shocked lung tissue is highly suggestive of a possible involvement in lung dysfunction. The search for selective inhibitors of thomboxane synthase (the enzyme responsible for the production of TXA_2 from endoperoxide) is underway and the effects of inhibitors of this enzyme are being evaluated.[43] It has been reported[44] that benzydamine (12) is a more selective inhibitor of thomboxane synthase (inhibitory at 100 $\mu g\ ml^{-1}$) than of cyclo-oxygenase (inhibitory at 250 $\mu g\ ml^{-1}$). By comparison indomethacin inhibits cyclo-oxygenase at 5 $\mu g\ ml^{-1}$ and thomboxane synthase at 100 $\mu g\ ml^{-1}$.



(12) Benzydamine

TXB_2 is reported to be chemotactic for leucocytes and this may be significant to inflammatory processes.[45]

Mechanistically, the conversion of the endoperoxide to TXA_2 can be considered as a polarization of the peroxide bridge,[46] as shown in brackets in Figure 10, leading to a carbon to oxygen migration with the formation of a secondary

[40] P. J. Piper and J. R. Vane, *Nature*, 1969, **223**, 29.
[41] R. Gryglewski and J. R. Vane, *Brit. J. Pharmacol.*, 1971, **43**, 420.
[42] C. R. Pace-Asciak, *Prostaglandins*, 1977, **13**, 811.
[43] *Scrip*, 1976, **237**, 21.
[44] S. Moncada, P. Needleman, S. Bunting, and J. R. Vane, *Prostaglandins*, 1976, **12**, 323.
[45] J. R. Boot, W. Dawson, and E. A. Kitchen, *J. Physiol. (London)*, 1976, **257**, 47P.
[46] Discussion by G. Fried and G. Just, in *Prostaglandins*, 1976, **11**, 431.

carbonium ion (stabilized as the oxonium ion canonical form) which then collapses by formation of the oxetane ring to give TXA_2. Due to the relatively weak oxygen—oxygen single bond, the endoperoxide to oxetane transformation is energetically quite favourable because a simple summation of the bond energies gives an enthalpy change of about -38 kcals mole^{-1}.

It is interesting to note that in the 1-series, from dihomo-γ-linolenic acid, the corresponding conversion of PGH_1 into TXA_1 does not occur in platelet microsomes.[47]

6 Prostacyclin (PGX or PGI_2)

In 1971 Pace–Asciak[48] reported that incubation of arachidonic acid with rat stomach homogenate gave the Δ^7-6(9)-oxy-$PGF_{1\alpha}$ (13) shown in Figure 11. When the incubation was performed with 5,6,8,9,11,12,14,15-octadeutero-arachidonic acid only six of the dueterium atoms were retained in the Δ^7-product which could infer that the Δ^5-compound (14) is an intermediate to the Δ^7-compound. During 1975 and 1976 6-keto-$PGF_{1\alpha}$, which can also exist in the lactol form (Figure 11), was reported from several sources: Pace–Asciak[49] showed it to be present in the products from incubation of arachidonic acid with homogenate of rat stomach fundus; Poyser[50] claimed that it was the major prostaglandin present in the uterus of the five day pseudopregnant rat and it was also claimed to be present in the sheep uterus; Dawson[51] showed it to be released from guinea pig lung during anaphylaxis; and it was also shown to be produced by bovine seminal vesicles[52] and microsomes of human and rabbit kidney cortex.[53] In late 1976 Vane and co-workers[54] described an unidentified unstable substance, PGX, formed by incubation of PGG_2 or PGH_2 with microsomes from rabbit or pig aorta. PGX, unlike TXA_2, does not contract rabbit aorta; also unlike TXA_2, it relaxes rabbit mesenteric and coeliac artery. PGX will contract rat stomach strip, chick rectum, guinea pig ileum and trachea but with very much reduced potency relative to PGG_2 or PGH_2. But the most remarkable property of PGX is its potent ability to inhibit platelet aggregation[55] and even to bring about the reversal of platelet aggregation.[56] Collaboration between Vane's and Upjohn's workers[57] resulted in the determination of the structure of PGX (14). This is, of course, the putative intermediate to Pace–Asciak's Δ^7-6(9)-oxy-$PGF_{1\alpha}$ (13). It is

[47] P. Needleman, M. Minkes, and A. Raz, *Science*, 1976, **193**, 163.
[48] C. Pace-Asciak and L. S. Wolfe, *Biochemistry*, 1971, **10**, 3657.
[49] C. Pace-Asciak, *Biochim. Biophys. Acta*, 1976, **424**, 323.
[50] R. L. Jones, N. L. Poyser, and N. H. Wilson, *Brit. J. Pharmacol.*, 1977, **59**, 436P.
[51] W. Dawson, J. R. Boot, A. F. Cockerill, D. N. B. Mallen, and D. J. Osborne, *Nature*, 1976, **262**, 699.
[52] W. C. Chang and S. Murota, *Biochim. Biophys. Acta*, 1977, **486**, 136.
[53] A. R. Whorton, M. Smigel, J. A. Oates, and J. C. Frolich, *Prostaglandins*, 1977, **13**, 1021.
[54] S. Moncada, R. J. Gryglewski, S. Bunting, and J. R. Vane, *Nature*, 1976, **263**, 663.
[55] R. J. Gryglewski, S. Bunting, S. Moncada, R. J. Flower, and J. R. Vane, *Prostaglandins*, 1976, **12**, 685.
[56] S. Moncada, R. J. Gryglewski, S. Bunting, and J. R. Vane, *Prostaglandins*, 1976, **12**, 715.
[57] R. A. Johnson, D. R. Morton, J. H. Kinner, R. R. Gorman, J. R. McGuire, F. F. Sun, N. Whittaker, S. Bunting, J. A. Salmon, S. Moncada, and J. R. Vane, *Prostaglandins*, 1976, **12**, 915.

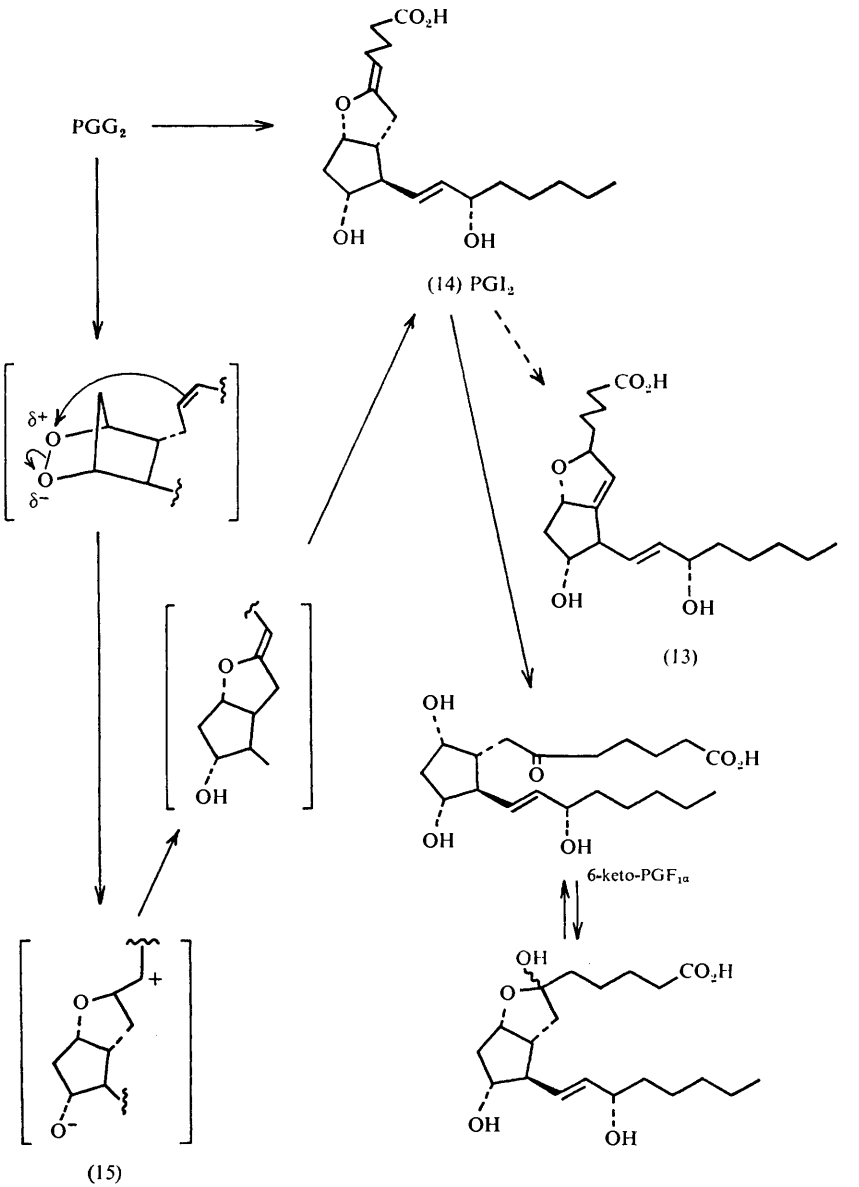


Figure 11 *Formation and hydrolysis of* PGI_2

also an enol ether obviously readily hydrolysed to 6-keto-$PGF_{1\alpha}$ (Figure 11) and quite probably in the situations in which 6-keto-$F_{1\alpha}$ has already been found, the true active principle may be PGX. With the structure established, PGX was renamed prostacyclin and then given the notation PGI_2 in the alphabetically-based semi-systematic nomenclature.

The formation of prostacyclin is the major metabolic pathway in vascular tissue in man and other species[58] and is no less a subject for widespread speculation[59] than is the formation of TXA_2. In the vasculature we have the remarkable possibility that the common intermediate endoperoxides, produced by the platelets, are converted by the platelets into the potent vasoconstricting and platelet aggregating TXA_2, but that the vessel walls convert the endoperoxides into the potent vasodilating and platelet aggregation inhibiting prostacyclin. Thus the balance of the antagonistic properties of TXA_2 and PGI_2 may maintain blood vessel tone, platelet functionality and thereby be critical for thombi formation. Prostacyclin is the major prostaglandin produced by the isolated perfused heart of rabbit or rat, apparently without the necessity for preformed endoperoxide from platelets, and it may function to protect the coronary circulation against the formation of blood platelet aggregates which could occlude the vulnerable coronary microvasculature.[60] Similarly, the generation of the PGI_2 by human placenta may be important in the maintenance of placental circulation;[61] pulmonary circulation could be influenced by lung prostacyclin production; implantation of the fertilized ovum into the uterus may require protection from platelet aggregation; possible involvement in bone resorption has been suggested,[62] and so has the protection of the stomach against ulcer formation.[55]

The conversion of PGG_2 to PGI_2 is brought about by the enzyme prostacyclin-synthetase and this enzyme is inhibited by 15-hydroperoxyarachidonic acid (15-HPAA; Figure 6) at a very low concentration.[55] The possible implication of this to diseases of the cardiovascular system has been speculated upon by Vane. Regarding the mechanism of the formation of PGI_2, polarization of the endoperoxide in the opposite sense to that required for TXA_2 formation could lead to participation of the 5,6-double bond thus leading to the secondary carbonium ion (15) which by loss of the proton from position-6 gives PGI_2.[46] The isomerization of PGI_2 to Δ^7-6(9)-oxy-$PGF_{1\alpha}$ is somewhat baffling at present, both from the mechanistic point of view and also from the point of view of the biological reason for such an isomerization.

7 Prostaglandin Metabolism

Transformations of PGE_2 into PGC_2 and PGB_2 and the 19-hydroxy compounds[63]

[58] G. J. Dusting, S. Moncada, and J. R. Vane, *Prostaglandins*, 1977, **13**, 3.
[59] J. L. Marx, *Science*, 1977, **196**, 1072.
[60] E. A. M. de Deckere, D. H. Nugteren, and F. Ten Hoor, *Nature*, 1977, **269**, 160.
[61] L. Myatt and M. G. Elder, *Nature*, 1977, **268**, 159.
[62] L. G. Raisz, J. W. Dietrich, H. A. Simmons, H. W. Seyberth, W. Hubbard, and J. A. Oates, *Nature*, 1977, **267**, 532.
[63] P. L. Taylor and R. W. Kelly, *Nature*, 1974, **250**, 665; R. W. Kelly, P. L. Taylor, J. P. Hearn, R. V. Short, D. E. Martin, and J. H. Marston, *Nature*, 1976, **260**, 544.

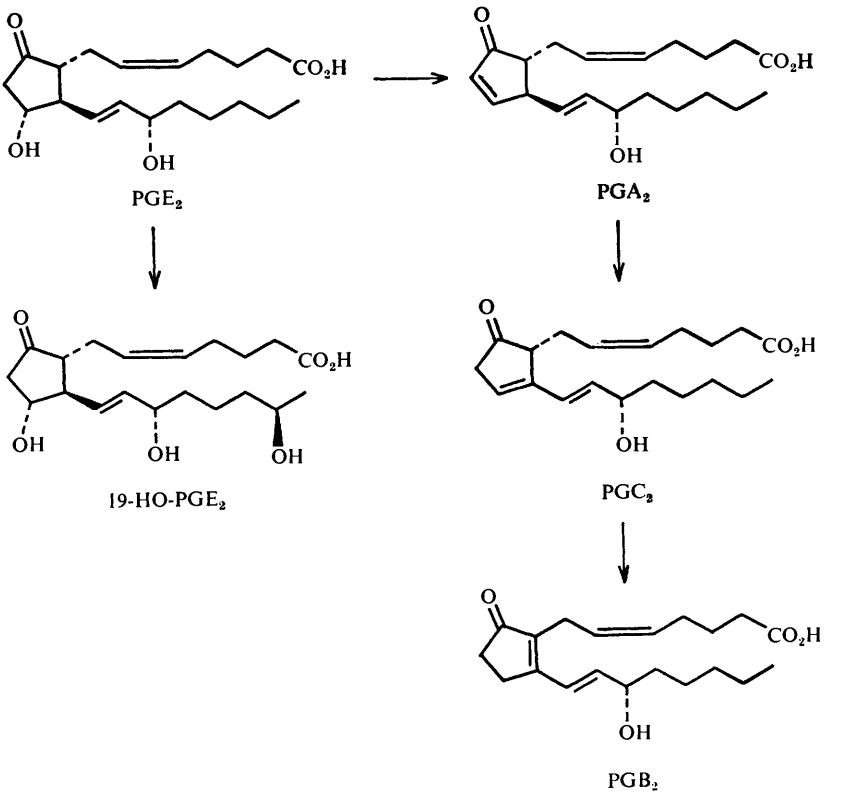


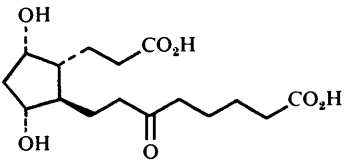
Figure 12 *Other PGE_2 transformations*

are conceptually simple reactions (Figure 12). However, *in vivo* these transformations are probably enzymically controlled and therefore of importance either as giving products with a different range of activities or as deactivation mechanisms. As mentioned in the previous review, prostaglandins are very rapidly metabolized by degradative enzymes. The primary deactivation step is the oxidation of the 15-hydroxy function by the enzyme prostaglandin-15-dehydrogenase which is particularly active in the lungs: greater than 95% of $PGF_{2\alpha}$ present in the blood stream is deactivated by this enzyme on one passage through the lungs.[64] Subsequent stages of metabolism involve reduction of the 13,14-double bond, two sequences of β-oxidation of the acid (top) side chain, and ω-oxidation of the alkyl side chain leading eventually to the major human urinary metabolite (16) which thus represents the end product of arachidonic acid

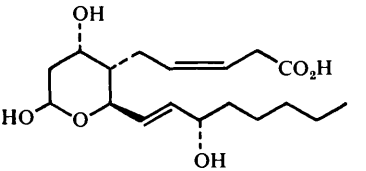
[64] B. Samuelsson, *Federation Proc.*, 1972, **31**, 1442.

metabolism *via* this pathway.[65] PGE_2 undergoes similar metabolism but with a keto function at C-9 instead of the hydroxy function of $PGF_{2\alpha}$.

In monkeys the major urinary metabolite of TXB_2 is reported to be the product (17) from one sequence of β-oxidation.[66]



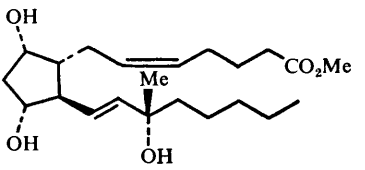
(16)



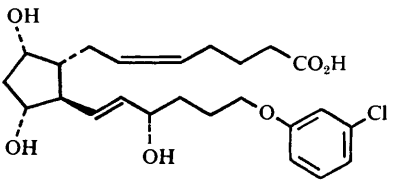
(17)

8 Concluding Remarks

The wide variety of physiological activities associated with the compounds within this field offers potential therapeutic use in the many areas which have been mentioned. The very short biological half-lives of natural PG's are a severe restriction on their mode of administration and modified prostaglandins have been produced which are much less susceptible to the degradation processes. An obvious modification such as a 15-methyl group, as in 15-methyl-$PGF_{2\alpha}$ methyl ester (18) renders the molecule no longer susceptible to the prostaglandin-15-dehydrogenase enzyme, and this compound (18) has been used extensively for the induction of abortion in women. Synthetic analogues can also offer greater selectivity in their actions, for example cloprostenol (19) is a highly selective luteolytic agent on sale for the induction of luteolysis in cattle.[67]



(18)



(19) Cloprostenol

The recent identification of TXA_2 and PGI_2 means that biological processes which have previously been considered only in terms involving the classical

[65] B. Samuelsson, E. Granström, K. Gréen, M. Hamberg, and S. Mammarström, *Ann. Rev. Biochem.*, 1975, **44**, 669.

[66] H. Kindahl, *Prostaglandins*, 1977, **13**, 619; L. J. Roberts, B. J. Sweetman, J. L. Morgan, N. A. Payne, and J. A. Oates, *Prostaglandins*, 1977, **13**, 631.

[67] M. J. Cooper and A. L. Walpole, in 'Advances in Prostaglandin Research. Prostaglandins and Reproduction', ed. S. M. M. Karim, M.T.P. Press, Lancaster, 1975, p. 309.

prostaglandin types must now be re-evaluated in the light of the possible involvement of these potent but labile molecules.

The unstable nature of TXA_2 and PGI_2 is a strong incentive for the production of stable analogues as potential therapeutic agents. Alternatively, selective inhibitors of thomboxane synthase or prostacyclin synthase can also be envisaged as potentially useful drugs. The involvement of TXA_2 and PGI_2 in the functioning of the vasculature and their effects on blood platelets opens up a whole new area of great interest in the field of cardiovascular disease. However, their probable involvement in pulmonary and reproductive tissues and their possible involvement in many other tissues will require a high order of selectivity to be achieved for analogues to become clinically successful. It is a fact that the impact of the prostaglandins in human clinical practice is, as yet, meagre considering the vast amount of effort which has been expended in the area. This failure is largely due to the lack of sufficient selectivity in those analogues which have undergone clinical trials.