

Pulmonary Biosynthesis and Metabolism of Prostaglandins and Related Substances

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On passage through the lung vascular bed, prostaglandins are removed from the circulation by a transport carrier and subsequently inactivated by intracellular enzymes. However, PGI₂ is not inactivated by the lung *in vivo*. Although PGI₂ is an excellent substrate for the intracellular enzymes *in vitro*, PGI₂ is not a substrate for the carrier system. Thus, the transport carrier determines which circulating prostaglandin is inactivated by the pulmonary vascular bed. Also, the lung has a high capacity for forming prostaglandins from arachidonic acid. Considerable differences exist between species in relation to amount and specific prostaglandin formed as determined by incubation of ¹⁴C-PGH₂ with pulmonary microsomes. The pulmonary biosynthesis and metabolism of these prostaglandins and related substances are discussed.

It has long been recognized that the lungs have a high capacity for inactivating circulating bioactive substances. The early work of Vane and his colleagues (1) indicated that the circulating prostaglandins (PGs) are extensively inactivated on single passage through the pulmonary circulation. It was subsequently shown (2) that the lung is particularly rich in two enzymes, 15-hydroxy prostaglandin dehydrogenase (PGDH) and 13,14-reductase, that degrade PGs. Thus, it is likely that the lung or its pulmonary vascular bed is a major site for the inactivation of PG present in the circulation.

Other studies have shown that the lung is also rich in enzymes that convert arachidonic acid into PGs and related substrates. In response to a variety of stimuli, both physiological as well as pathological, PGs and related substrates are released into the circulation. Recently, it has been proposed by Gryglewski et al. (3) that the vascular bed of the lung continuously secretes PGI₂ into the circulation and that the lung acts as a natural defense against development of intra-arterial thrombus. Thus, the lung appears to be a unique organ that controls or maintains the levels of a particular circulating prostaglandin.

Metabolism by Lung Subcellular Fractions *in Vitro*

Studies on the metabolism of prostaglandins in lung tissue indicate that PGs undergo metabolic transformation. First, the hydroxyl group at C-15 is oxidized to

a keto group by prostaglandin dehydrogenase (PGDH) followed by reduction of double bond at C-13 by Δ^{13} reductase (Fig. 1). This is accompanied by biological inactivation (4). The enzymes have been purified and extensively studied. PGE₁ is the best substrate having lowest K_m and highest V_{max} but PGE₂, PGF_{1 α} , PGA₁, and PGA₂ were also substrates with higher K_m values and lower V_{max} (5). With the discovery of PGI₂ and its nonenzymatic breakdown product 6-keto-PGF_{1 α} by Vane and his colleagues (6) and the potential biological importance of PGI₂, the question of the metabolism of this prostaglandin by PGDH arose. We have investigated the metabolism of PGI₂, 6-keto-PGF_{1 α} and PGF_{2 α} by rat lung 600 g supernatant fortified with NAD⁺.

Incubation of rat lung homogenates with ³H-PGF_{2 α} resulted in the formation of two distinct metabolite

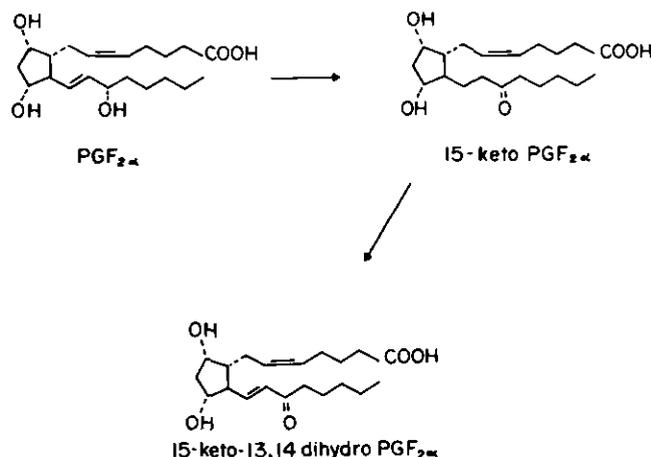


FIGURE 1. Metabolism of prostaglandin by lung prostaglandin dehydrogenase and reductase.

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peaks which cochromatographed with authentic 15-keto-PGF_{2α} and 13,14-dihydro-15-keto-PGF_{2α}. As found previously, the rat lung seems to lack the 15-keto-prostaglandin reductase that is necessary for formation of the 13,14-dihydro-PG metabolites (7).

Two chromatographic peaks resulted from the incubation of ³H-PGI₂ or ³H-6-keto-PGF_{1α} with rat lung homogenates. The peak remaining closest to the origin cochromatographed with both 6-keto-PGF_{1α}, the breakdown product of PGI₂, and 6-keto-13,14-dihydro-PGF_{1α}. The peak closest to the solvent front could be either 6,15-diketo-PGF_{1α} or 6,15-diketo-13,14-dihydro-PGF_{1α} or a mixture of these metabolites. Further attempts to separate these metabolites by means of other TLC systems were unsuccessful. However, Wong et al. (8) have recently used GC-MS to isolate and identify 6,15-diketo-PGF_{1α} as a major metabolite formed from incubation of PGI₂ with the cytoplasm of various blood vessels. The major metabolite formed in our incubation system was, therefore, most likely to be 6,15-diketo-PGF_{1α}. Since PGI₂ is relatively stable at pH used in the incubation, we conclude that the 6,15-diketo-PGF_{1α} found in this incubation system probably arose from nonenzymatic hydrolysis of 15-keto-PGI₂ during the workup procedure.

PGI₂ and PGF_{2α} appeared to be metabolized to comparable extents by rat lung homogenates over a 10-min incubation period (Fig. 2). After 4 min 16% of both the PGF_{2α} and PGI₂ was degraded, while after 10 min, 41% of the PGF_{2α} was metabolized compared to 35% of the PGI₂ (7). In contrast, 6-keto-PGF_{1α} was only minimally metabolized (6% at 10 min). Little or no metabolism (≤ 2%) of any PG was obtained with boiled homogenates.

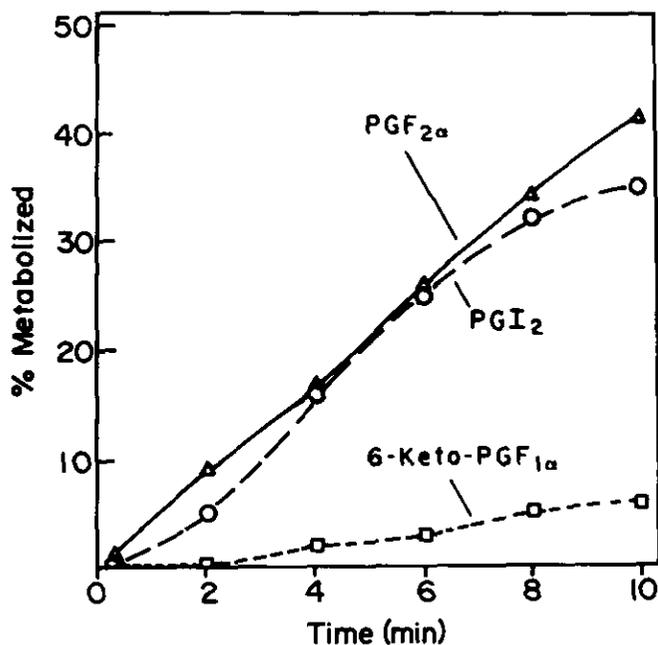


FIGURE 2. Metabolism of ³H-PGI₂, ³H-PGF_{2α} and ³H-6-keto-PGF_{1α} by rat lung homogenate.

McGuire and Sun (9) have investigated the metabolism of PGI₂ by a partially purified prostaglandin dehydrogenase obtained from rhesus monkey. These workers also found that PGI₂ but not 6-keto-PGF_{1α} was an excellent substrate for PGDH. PGI₂ was oxidized at 4 to 6 times faster than 6-keto-PGF_{1α}. The *k^m* for PGI₂ was found to be 7.4 μM compared to 14.5 μM for PGE₁ and 56 μM for PGF_{2α}. These data indicate that PGI₂ but not 6-keto-PGF_{1α} is the physiological substrate for pulmonary PGDH. The sequence of PGI₂ metabolism is the formation of 15-keto-PGI₂ followed by nonenzymatic breakdown to 6,15-diketo-PGI_{1α} (Fig. 3).

Studies on the Transport of PG by Pulmonary Tissue

The early work of Vane and his associates showed that prostaglandin E₁, E₂ and F_{2α} were rapidly and extensively inactivated in a single passage through the lung vasculature (1). Reports from various investigators reveal no species variation in the inactivation of PGE and PGF_{2α} from the circulation by the lung. Cat, dog, rabbit (1) and rat (10) lungs have all been shown to inactivate PGE and PGF_{2α} from the pulmonary circulation. However, guinea pig (11) and rabbit (12) isolated perfused lungs (IPL) have been shown to metabolize A-type PGs, while cat (13), dog (14), and rat IPL (10) have not. PGA₁ appears to be a substrate for the intracellular prostaglandin dehydrogenase (PGDH) (15). Thus, the species differences in metabolism may be the result of a species difference in accessibility to the intracellular degradative enzymes. These facts, together with the rapidity of the processes, suggested to us that a carrier or a transport system was responsible for the rapid removal of PGs from the circulation and that the transport system imparts selectivity to the pulmonary inactivation system.

In order to study the removal mechanism, measurement of the initial velocity or unidirectional flux of PGs from the perfusate into the intact lung cells was necessary. We therefore used an isolated perfused rat lung preparation which permitted measurement of the initial velocity at a constant concentration of PG in the arterial perfusate. Previously published work details the specifics of the measurements (10).

The initial velocity of PGE₁ uptake into the lung was saturated with increasing concentrations of PGE₁ in the

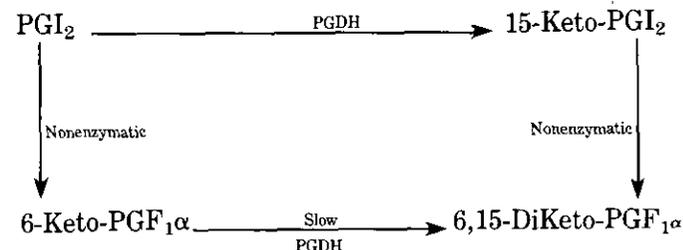


FIGURE 3. Pathway for the metabolism of PGI₂ and 6-keto PGF_{1α} by prostaglandin dehydrogenase.

perfusate (Fig. 4). This type of relationship between the initial velocity and the perfusate concentration suggests that a carrier-mediated or transport process was involved in the removal of PGE₁ by the lung. Diffusion or binding of the PG to lung tissue would give a linear relationship between the initial velocity and perfusate concentration.

We have also examined the rates of removal of PGF_{2α}, PGB₁, PGA₁ and 15-keto-PGF_{2α} from the vasculature to the lung. Both removal and metabolism was observed with PGF_{2α}. This suggests that definitive substrate specificity exist for the carrier molecule. PGA₁ is metabolized *in vitro* by lung PGDH. However, the PGA₁ metabolism is not observed *in vivo*. This may be explained by the fact that PGA₁ is not a substrate for the carrier molecule. Further support for the existence of a carrier or transport system in rat lung was obtained by inhibitor studies. The addition of a second PG substrate for example, PGF_{2α}, significantly inhibited the removal of the first substrate PGE₁. The addition of a nonsubstrate, i.e., PGA₁ did not inhibit the removal of the substrate PGE₁ for the transport system. Further studies showed that PGF_{2α} competitively inhibited the uptake of PGE₁. Thus, saturation of the initial velocity with respect to perfusate concentration, the inhibition of the removal of one PG by another, and the concentration dependence of this inhibition, support our hypothesis of a transport system.

The substrate specificity for the inactivation of circulating PGs appears to reside with the transport carrier since both PGE₁ and PGF_{2α} are removed, but PGA₁ and 15-keto-PGF_{2α} are not removed from the circulation. We have studied the structural requirement of the PG molecules essential for transport from the

circulation into the lung. As seen in Table 1 (16), PGE₁, PGE₂, PGF_{1α}, PGF_{2α}, PGF_{2β} and to some extent PGD₂ and PGD₁ were removed from the circulation into the lung. PGA₁, PGB₁ and various metabolites of the classical PGs were not removed. In addition, the methyl esters of PGs were not substrates for the transport system. With the discovery of PGI₂ and its potential importance in controlling intra-arterial thrombosis, it was important to determine if PGI₂ was a substrate for the transport system. Studies with *in vitro* incubation systems (see above) indicate that PGI₂ was an excellent substrate for pulmonary PGDH. However, using bioassay techniques, Dusting et al. have studied the disappearance of PGI₂ in the circulation of the dog (17). Their work indicated little or no inactivation of PGI₂ on passage through the lung, while extensive inactivation occurred in the liver and hind quarters. Similarly, the conclusion that PGI₂ escapes pulmonary metabolism has been reached by Bolger and co-workers while studying the renal actions of PGI₂ (18), and by Armstrong et al. in a study of hypotension induced by PGI₂ (19). The data suggest that PGI₂ is not a substrate for the carrier system. Since PGI₂ was not stable at pH 7.4, determining the removal of PGI₂ from the circulation into the lung required a different experimental approach than measurement of the initial velocity. Uptake was studied by indicator dilution techniques. The effluent from an isolated rat lung was collected in approximately 1-sec intervals after injection of a ³H-PG and the vascular marker ¹⁴C-dextran. For PGF_{2α} (a transport substrate), analysis of the effluent radioactivity showed a displaced tritium peak indicating that the PGF_{2α} was taken into the vascular cells (Fig. 5a). Analysis of the effluent indicated that the ³H-PGF_{2α} was extensively degraded. However, after bolus injection of ³H-PGI₂ or ³H-6-keto-PGF_{1α} mixed with ¹⁴C-dextran, analysis of the effluent indicated the mean transit times for both PGI₂ and 6-keto-PGF_{1α} (Fig. 5a) were identical to the vascular marker dextran. Furthermore, neither PGI₂ nor 6-keto-PGF_{1α} was metabolized during passage through the lung. Thus, PGI₂ and 6-keto-PGF_{1α} are not

Dependence of pulmonary removal of PG on the concentration of PG in the perfusate.

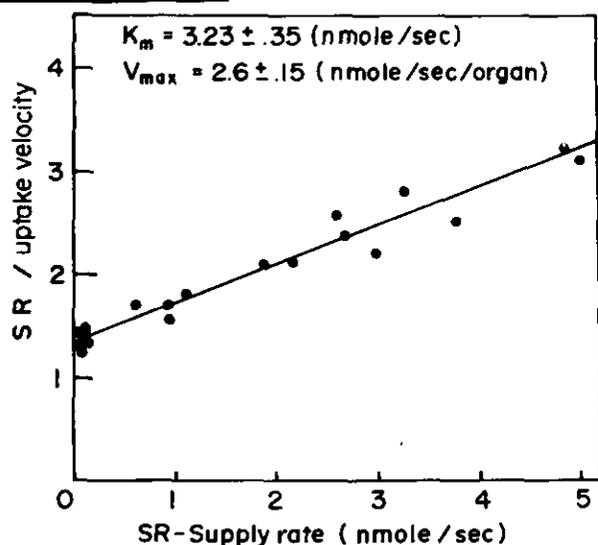


FIGURE 4. Saturation of PGE₁ uptake velocity with perfusate concentration.

Table 1. Uptake of PG by rat lung.

³ H-PG	Uptake velocity (relative to PGE ₁)
PGE ₁	1.0
PGE ₂	0.86
PGF _{1α}	0.68
PGF _{2α}	1.20
PGF _{2β}	1.0
PGA ₁	0
PGB ₁	0
PGD ₂	0.57
PGD ₁	0.25
15(S)methyl-PGF _{2α}	1.45
15(R)methyl-PGF _{2α}	0
15-keto-13,14-dihydro-PGE ₁	0
13,14-dihydro-PGE ₁	< 0.2
13,14-dihydro-PGF _{2α}	< 0.4
PG methyl esters	0
15-epi-PGE ₂	0
15-epi-PGF _{2α}	0

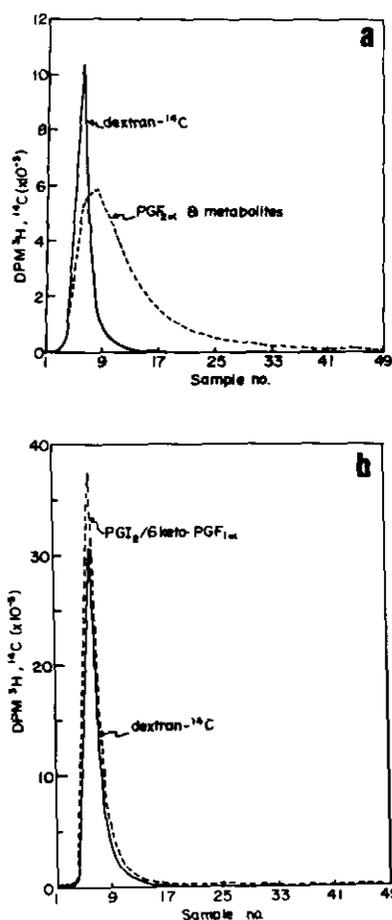


FIGURE 5. Appearance of radioactivity in the effluent of rat perfused lung after a bolus injection of ^3H -PG and ^{14}C -dextran: (a) (—) ^{14}C dextran, (---) ^3H -PGF $_{2\alpha}$ and metabolites; (b) (—) ^{14}C dextran, (---) ^3H -PGI $_2$ or ^3H -6-keto-PGF $_{1\alpha}$.

substrates for the transport system; thus, the biological half-life of PGI $_2$ is not controlled by passage through the pulmonary vascular bed.

The present studies (7,10,16) show there are apparently three critical portions (Fig. 6) of the PG molecule necessary for transport into lung tissue: the acid group at C-1, the oxygen function, particularly a hydroxyl group at C-11, and a hydroxyl group at C-15 in the *S* configuration. The steric relationship between these groups is important since reduction of the 13,14-double bond reduces or abolishes transport, and changing the C-15 hydroxyl group from *S* to *R* configuration abolishes transport.

Evidence for the importance of the acid group was derived from the observation that the methyl esters of PGE $_1$, PGE $_2$, and PGF $_{2\alpha}$ were not taken up into the perfused lung. In contrast, the methyl esters of PGs are substrates for lung prostaglandin dehydrogenase (PGDH) (15). The importance of an oxygen function at C-11 was illustrated by the lack of uptake observed for PGA $_1$ and PGB $_1$. The presence of a carbonyl group (PGD $_2$ and PGD $_1$) rather than a hydroxyl group reduces

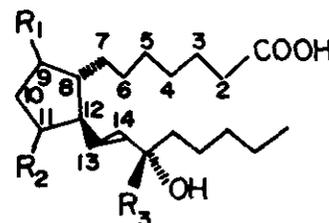


FIGURE 6. Structural requirement for pulmonary transport of prostaglandins: R $_1$ = hydroxyl, either α or β configuration, or carbonyl group; R $_2$ = hydroxyl or carbonyl group; R $_3$ = H or CH $_3$ in β configuration.

uptake by the rat isolated perfused lung. The presence of a hydroxyl group at C-15 appears to be an absolute requirement for removal of PGs from the circulation by the lung. Conversion of the hydroxyl group to a keto group abolishes uptake. Moreover, the configuration of the C-15 hydroxyl group is critical. 15(*R*) methyl-PGF $_{2\alpha}$ was not removed from the circulation by the lung, whereas approximately 90% of the 15(*S*) methyl-PGF $_{2\alpha}$ was removed. In addition, the 15-epi isomers of PGE $_2$ and PGF $_{2\alpha}$ did not inhibit the removal of PGE $_1$. Alteration of the functional groups at C-9 does not affect the removal. PGF $_{2\beta}$, which has a hydroxyl group at C-9 and PGE $_2$, with a keto group at C-9 are substrates for the transport system. Therefore, changing the hydroxyl group at C-9 from an α to a β configuration had little effect. However, since PGI $_2$ contains a ring structure between the 6 and 9 carbon atoms, the position of the side chain containing the carboxylic acid moiety is significantly altered in relation to the rest of the molecule. In 6-keto-PGF $_{1\alpha}$ a hemiketal ring between the keto group at C-6 and the hydroxyl at C-9 can be formed. The conditions that govern the equilibrium between the open and closed forms are not known. In the closed form of 6-keto-PGF $_{1\alpha}$ the hemiketal ring significantly alters the position of the carboxylic side chain. In the open form, the planar configuration of the carbonyl group may change the position of the side chain, modifying the PG hairpin structure. Another possible explanation for the lack of uptake of 6-keto-PGF $_{1\alpha}$ is that the presence of an additional oxygen molecule may change the electronic characteristics of this PG, subsequently altering the binding to the transport carrier. Thus, the geometric configuration of the C-1 carboxyl group in relation to the C-15 hydroxyl group and the C-11 oxygen function is different in PGI $_2$ and 6-keto-PGF $_{1\alpha}$ compared to that of other PGs. Our present results indicate that the three functional groups on the PG molecule that are necessary for transport

into lung tissue may need to form a precise geometric configuration in order for transport to occur.

An alternative explanation for the lack of pulmonary transport of PGI_2 and 6-keto- $\text{PGF}_{1\alpha}$ may involve the oxygen function at C-9. In PGI_2 and the hemiketal form of 6-keto- $\text{PGF}_{1\alpha}$, the oxygen function becomes part of a ring system. This points to the possibility that the presence of a free hydroxyl or carbonyl group at C-9 is an additional requirement for pulmonary transport of PGs. It could, therefore, be the difference in geometric configuration and/or the lack of a free oxygen function at C-9 that prevent PGI_2 and 6-keto- $\text{PGF}_{1\alpha}$ from being transported into lung tissue.

Thus, the pulmonary inactivation system consists of transport carrier and intracellular enzymes as shown in Figure 7. The selectivity of the transport system in the lung and hence the selectivity of the pulmonary inactivation of PGs may have important physiological significance. The pulmonary removal and inactivation of some PGs may serve to protect the arterial circulation from potentially deleterious effects of these PGs. Since PGI_2 presumably exerts beneficial effects including inhibition of platelet aggregation (19) and has been implicated in the prevention of arterial thrombosis (6), it would be disadvantageous for it to be removed during passage through the pulmonary bed. Recent studies (3) have, in fact, shown that the lung *in vivo* actually generates PGI_2 , and it has been proposed that PGI_2 may be a circulating hormone (20), although some evidence exists to the contrary. Thus, the lung may play an important role in the prevention of arterial thrombosis.

Inhibition of Pulmonary Inactivation

The addition of various chemicals or drugs to the perfusate altered the rate of removal and/or rate of PG metabolism by the lung. Several PG antagonists,

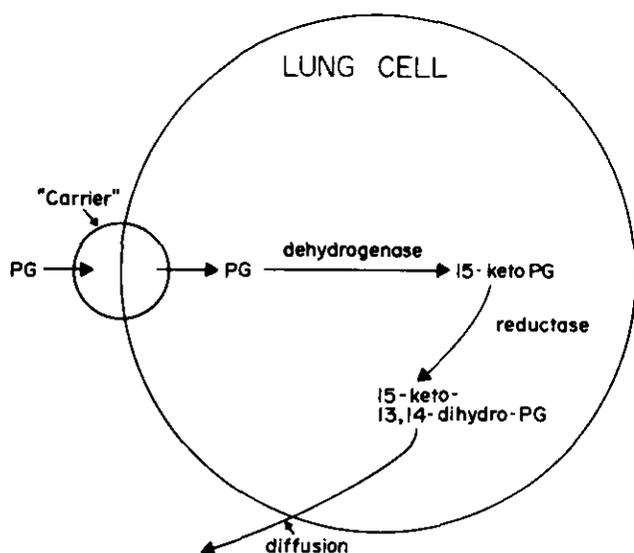


FIGURE 7. Schematic diagram of the pulmonary inactivation system.

polyphloretin phosphate (PPP) and diphloretin phosphate (DPP) were effective primarily by inhibiting the transport system (4). The organic acid transport inhibitor, bromocresol green, was a potent inhibitor of the transport system (16). The exposure of lung to various environmental pollutants significantly affected the pulmonary inactivation of circulating PGs. Bakhle et al. (21) recently investigated the effect of cigarette smoke on the metabolism of vasoactive hormones by the rat isolated lungs and reported that exposure to smoke decreased the inactivation of circulating PGE_2 .

We have examined the effect of exposure of guinea pig and rats to the environmental gases, NO_2 , SO_2 and O_2 (22). As measured by *in vitro* assay techniques, prostaglandin synthetase was not altered by the exposure. However, exposure to O_2 and NO_2 but not SO_2 significantly depressed PGDH activity. Exposure of animals to 100% O_2 depressed PGDH (*in vitro*) with the degree of inhibition dependent on the length of exposure. After 72 hr of O_2 exposure, PGDH was depressed by approximately 80%. Exposure of guinea pig to 100% O_2 did not apparently alter the transport carrier for PGs in the lung. Kinetic analysis suggested that exposure of the oxident gases resulted in destruction of PGDH (23).

Cyclo-oxygenase Metabolism of Arachidonic Acid by the Lungs

Prostaglandins have received the attention of pulmonary researchers and physicians for a number of reasons: their interaction by the lungs, their contractile effects on bronchial smooth muscle, and their mixed actions on the pulmonary circulation (24). In recent months other arachidonic acid metabolites, the leukotrienes, have begun to attract similar interests (25).

In the future a complete understanding of the interdependence and interactions among the prostaglandins, thromboxanes, and leukotrienes may provide new thoughts into the phenomena of immediate hypersensitivity reactions and could be of particular importance in the etiology of human asthma. In this section some aspects of lung prostaglandin biosynthesis, species variation and the use of microsomal preparation to study endoperoxide metabolism will be discussed. The metabolic pathway of arachidonic acid in mammalian cells is summarized in Figure 8, and the detailed biochemistry has been reviewed by Samuelsson et al. (26).

The precise biochemical or pathophysiological factors which lead to abnormal biosynthesis of prostaglandins, prostacyclin and thromboxane by lung tissue are not well understood. A wide variety of stimuli which presumably lead to membrane perturbation may initiate prostaglandin biosynthesis. These stimuli can be broadly categorized into anaphylaxis, chemical or environmental agents and physical stimuli (27).

There is an abundance of literature dealing with the production of thromboxane A_2 , SRS-A and prosta-

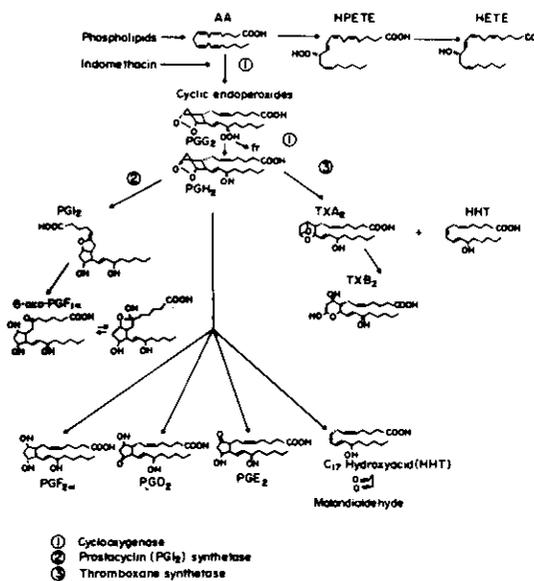


FIGURE 8. Pathways of arachidonic acid metabolism.

glandins in the guinea pig lung. However, the data for other species are sadly deficient. In 1965, Anggard (28) described the biosynthesis of PGs from endogenous substrate using lung homogenates of human, monkey, sheep and guinea pig by utilizing GC-MS analysis. Of these four species, human, rat and monkey produced significantly lower amounts of PGF_{2α} than guinea pig. A large number of compounds produced from endogenous arachidonic acid in guinea pig lung have been conclusively identified by Dawson et al. (29). These are TXB₂, PGE₂, PGF_{2α}, 15-oxo-PGE₂, 15-oxo-PGF_{2α}, 15-oxo-13,14-dihydro-PGE₂, and 15-oxo-13,14-dihydro-PGF_{2α} identified in the effluent from isolated perfused normal guinea pig lungs. In addition, 6-keto-PGF_{1α} (the spontaneous hydrolysis product of prostacyclin PGI₂; Fig. 8) and a novel oxodihydro derivative of TXB₂ were seen only in sensitized lungs during anaphylaxis. Human lungs have been reported to produce 6-keto-PGF_{1α}, *in vivo* (30) and PGE₁, PGE₂ and PGF_{2α} and RCS(TXA₂) *in vitro* (31). Recently, Palonek et al. (32) provided evidence for the spontaneous and angiotensin-induced release of PGI₂ from cat lungs, using PGI₂ antibodies and a PGI₂ tendon bioassay, while Veolkel et al. (33) described the release of PGI₂ during angiotensin stimulation of rat lung *in vitro*. Recently, using PGI₂ antibodies, Pace-Asciak et al. (34) obtained data *in vivo* that argued against the notion that blood PGI₂ exerts an antihypertensive action in spontaneous hypertension (29). Thus, it is still unclear whether PGI₂ is continuously secreted into the circulation in concentrations sufficient to exert biological effects. In sheep, Frolich et al. (35) found that lymph provided a better index of lung tissue prostaglandin biosynthesis. During endotoxin-induced pulmonary hypertension, blood prostaglandin and TXB₂ levels sampled from both the left atrium and the pulmonary artery were unchanged. In contrast, a

dramatic 400-fold increase in TXB₂ concentration was seen in lymph using both RIA and GC-MS analysis.

Enhanced lipid peroxidation has been shown to occur *in vivo* in the lung following exposure to oxidant gases. Exposure to ozone, nitrogen dioxide (36) and oxygen (37) leads to an increase in lung lipid peroxidation. These oxidant gases have been reported to inhibit pulmonary prostaglandin metabolism (23). Recently, Crutchley et al. (38) reported an increase in the release of TXB₂, 6-keto-PGF_{1α}, PGE₂ and PGF_{2α} as a result of inhibition of PGDH by exposure to 100% oxygen. These observations may be related to the phenomenon of increased lipid peroxidation reported by others. Oxygen (5–95%) has been shown to stimulate prostaglandin biosynthesis in tissue slices (39), which suggests a direct effect unrelated to the generation of reactive free radicals or inhibition of 15 hydroxyprostaglandin dehydrogenases.

Rat lung homogenates synthesize predominantly PGI₂ (40), while guinea pig lung homogenates synthesize primarily TXB₂ (41). The dominance of TXB₂ as the major arachidonate metabolite in the guinea pig has been established using lung homogenates (41) and isolated perfused lung (41–44). Earlier, Parkes and Eling (45) identified a novel arachidonic acid metabolite in guinea pig lung microsomes, which has now been named TXB₂ (46) and is formed by spontaneous hydrolysis of the parent TXA₂ (see Fig. 8).

In a comparative study of 1-¹⁴C-arachidonic acid metabolism in isolated perfused lung of guinea pig, rat and man, Al Ubaidi and Bakle (47) found that the rat lung provides a better model than guinea pig lung for arachidonic acid metabolism in human lung. A similar conclusion was reached in a study of slow reacting substance of anaphylaxis in rat, mouse, guinea pig and man (48).

There is no doubt that adult lungs biosynthesize TXB₂; it appears that fetal bovine and rabbit lungs produce little TXB₂ (49). In the third trimester, the biosynthesis of PGE₂ by fetal lung of both species increased steadily with little change in either TXB₂ or PGF_{2α}. Interestingly, a small amount of 6-keto-PGF_{1α} was seen in rabbit but not bovine fetal lung. In contrast, fetal lamb produced equal amounts of PGE₂, PGF_{2α} and TXB₂. In an earlier study, Pace-Asciak (50) found that in very young fetal lamb, PGF_{2α} was the dominant prostaglandin with PGE₂ increasing with gestational age until at term, it equaled PGF_{2α}. In this latter study, TXB₂ was not assayed. Therefore, it appears that fetal lung prostaglandin biosynthesis is a heterogeneous in terms of product and amounts as it is in adult animals. Thus, clear species and age differences exist in lung prostaglandin biosynthesis.

Prostaglandin Synthesis by Lung Microsomes

In experiments using lung microsomes, Tai et al. (51) found that ¹⁴C-arachidonic acid was metabolized by

sheep lung microsomes primarily to TXB₂ (14%) and 6-keto-PGF_{1α} (5%); with the inclusion of glutathione, PGE₂ became the major metabolite formed. Sun et al. (52) reported that rabbit lung microsomes synthesize both 6-keto-PGF_{1α} and TXB₂ from PGH₂. As the concentration of PGH₂ is increased, the production of 6-keto-PGF_{1α} plateaus while TXB₂ increases in a linear fashion. These data suggest that PGI₂ synthetase is readily saturated while thromboxane synthetase is not. Thus, if large quantities of endoperoxide are produced in pathophysiological states, TXA₂ may become the dominant product in species which normally produce small amounts of TXA₂, e.g., rat and man, detected as rabbit aorta contracting substance (46). In their studies with human lung microsomes, Sun et al. (52) were unable to demonstrate endoperoxide metabolism by thromboxane synthetase.

In other studies with porcine lung microsomes, Tai et al. (53) found that ¹⁴C-PGH₂ was metabolized to predominantly TXB₂. Pyridine (10mM) inhibited TXB₂ synthetase, resulting in an increase in the synthesis of both 6-keto-PGF_{1α} and PGE₂, without an overall decrease in total product formation. We have found that tranlycypromine (20 μg/mL) inhibits the production 6-keto-PGE₂ from the endoperoxide PGH₂ by porcine lung microsomes with a significant increase in the synthesis of both TXB₂ and PGE₂ (Ally and Eling, unpublished data). Thus, the inhibition of enzymes in the prostaglandin cascade produces a significant shift in product formation. It is possible that some form of negative feedback inhibition is responsible since PGE₂ has been reported to inhibit PGI₂ biosynthesis in rat liver endothelial cells (54).

The heterogeneity of lung arachidonic acid metabolism observed in different species (discussed above) is reflected at the subcellular level. As shown in Figure 9, the metabolic profile for 1-¹⁴C-PGH₂ in guinea pig lung microsomes analyzed by HPLC (55) is quite distinct from that in rat lung microsomes; the guinea pig produces little 6-keto-PGF_{1α} but greater than 40% of the total products is TXB₂, whereas rat appears to make equivalent amounts of 6-keto-PGF_{1α} and TXB₂. In experiments using 1-¹⁴C arachidonic acid and guinea pig lung microsomes, TXB₂ represented 28% and 6-keto-PGF_{1α} 6.1% of the cyclooxygenase products. Thus, in

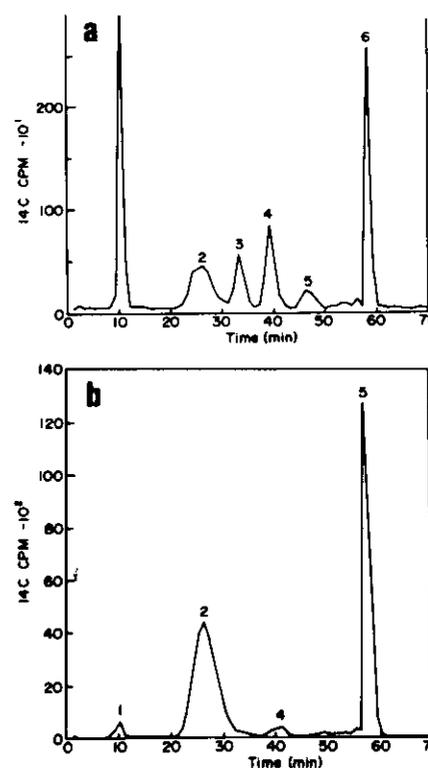


FIGURE 9. HPLC profile of metabolites of PGH₂ by (a) rat and (b) guinea pig lung microsomes: (a) rat, (1) 6-keto-PGF_{1α}, (2) TXB₂, (3) PGF_{2α}, (4) PGE₂, (5) PGD₂, (6) HHT; (b) guinea pig, (1) 6-keto-PGF_{1α}, (2) TXB₂, (4) PGE₂, (5) HHT.

our experiments the arachidonic acid metabolic profile is similar, both qualitatively and quantitatively, to that obtained using the endoperoxide PGH₂.

As shown in Table 2 human lung microsomes make equivalent amounts of 6-keto-PGF_{1α} and TXB₂ while PGE₂ is the major product. Of these five animal species, the guinea pig least resembles the human prostaglandin profile, while the rat and mouse appear quite similar. The 6-keto-PGF_{1α}/TXB₂ ratio in human lungs is close to unity and this ratio is seen in the porcine, bovine, rat and mouse lung. Interestingly, mouse lung makes as much PGE₂ from PCH₂ as does human lung.

These data, if representative of the extrapulmonary

Table 2. PGH₂ metabolism by lung microsomes.^a

Species	Product, % of total radioactivity ^b				
	6-Keto-PGF _{1α}	TXB ₂	PGF _{2α}	PGE ₂	PGD ₂
Human ^c	14.7 ± 1.4	17.9 ± 4.2	12.9 ± 0.9	23.1 ± 2.6	14.6 ± 1.2
Porcine	25.3 ± 2.8	21.9 ± 3.7	5.1 ± 1.7	6.3 ± 0.6	4.1 ± 1.1
Bovine	26.0 ± 2.0	26.0 ± 1.0	4.0 ± 1.0	3.4 ± 1.7	ND
Rat	23.0 ± 5.0	19.0 ± 3.2	9.0 ± 0.0	16.0 ± 1.0	5.0 ± 1.0
Guinea pig	3.0 ± 0.6	43.0 ± 2.0	2.0 ± 1.0	3.0 ± 1.0	ND
Mice	10.2 ± 1.2	7.1 ± 0.8	4.4 ± 0.5	23.4 ± 3.4	7.4 ± 0.3

^a Analysis by HPLC-radiometric procedure.

^b Values are means ± SD, N = 4; ND = not detectable.

^c Macroscopically normal lung samples obtained from patients undergoing resection for cancer.

prostaglandin profile in the lung, suggest that the bronchioles and other nonvascular lung elements are typically exposed to a wide range of prostaglandins, of which two (PGI_2 , PGE_2) have been shown to be bronchodilators and three (TXA_2 , PGD_2 and $\text{PGF}_{2\alpha}$) to be bronchoconstrictors. It is suggested that alterations in this balance together with abnormal levels of lipoxygenase products may be responsible for respiratory distress in lung disease (3).

These data discussed here suggest that lung parenchymal microsomes may provide a clear picture of biochemical changes in the prostaglandin and hydroxy fatty acid profiles in pathophysiological conditions of unknown etiology or exposure to airborne environmental pollutants. Of the five animal models examined, the rat appears to be a good choice for further studies since its prostaglandin profile is similar to man and it has the added convenience of being readily available, inexpensive and manageable.

Lung Thromboxane and Prostaglandin Secretion

Gryglewski and colleagues (3,56) have proposed that the lung continuously secretes PGI_2 into the circulation. The lung is highly vascularized and has large numbers of endothelial cells which are highly active in producing PGI_2 . Control of PGI_2 release from the lung is not understood. We have found (57) using the isolated perfused rat lung, that PGI_2 measured by RIA of 6-keto- $\text{PGF}_{1\alpha}$ and TXA_2 measured by RIA of TXB_2 , are continuously released into the perfusate. The concentration of TXB_2 was approximately 1/5 that of the PGI_2 detected by RIA of 6-keto- $\text{PGF}_{1\alpha}$ (6 ng/min TXB vs. 33 ng/min). Increasing the rate of respiration increased the release of both TXA_2 and PGI_2 , but PGI_2 release appear to be preferentially stimulated. At normal respiration rate (50/min) the ratio of 6-keto- $\text{PGF}_{1\alpha}$ to TXB_2 was 5:1, but at 100/min the rate increased to 11:1 (Fig. 10). Thus, measurement of PGI_2 in perfusate or blood could be highly variable dependent, in part, on the rate of respiration.

There is evidence for considerable species differences in the secretion of PGI_2 and TXA_2 by lung. Alabaster (42) found that guinea pig lung metabolized infused PGH_2 (800 ng) to primarily TXA_2 (101 ± 13 ng) and PGI_2 (10–16 ng), whereas in rabbit lung effluent only unmetabolized PGH_2 (46 ± 9 ng) and some PGE_2 (26 ± 10 ng) were detected. The PGE_2 seen may represent a spontaneous decomposition product from PGH_2 . Similarly, Boyd and Eling (62) reported that rat lung significant amounts of PGI_2 but guinea pig produces only very small amounts of PGI_2 from PGH_2 whereas guinea pig lung was found to make primarily, TXA_2 . However, guinea pig platelets appear to be very sensitive to PGI_2 .

It is now apparent that differences among the species

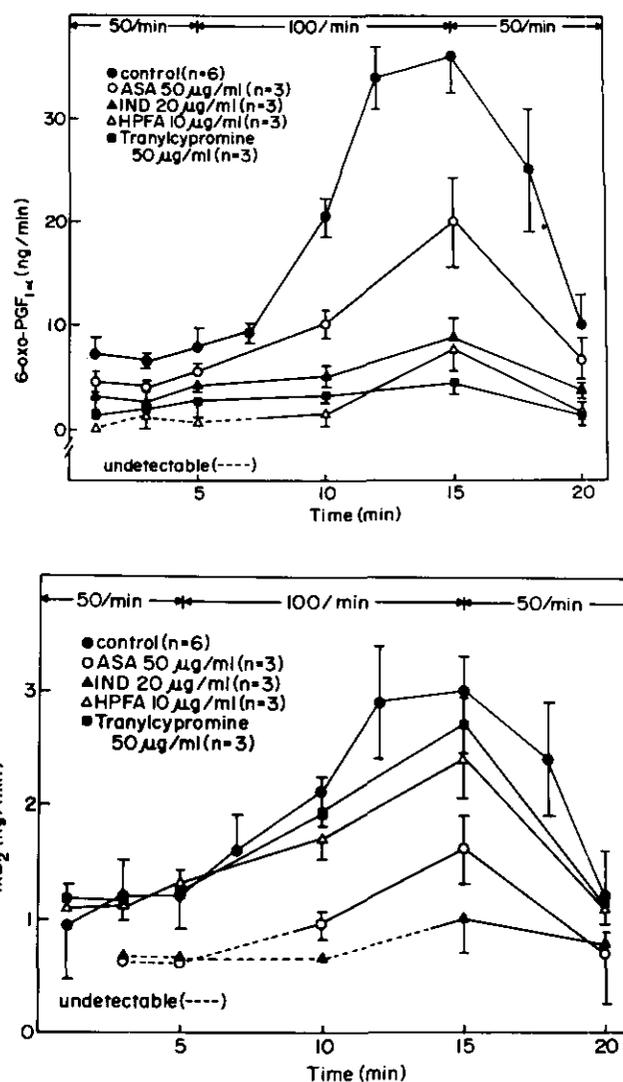


FIGURE 10. Effect of respiration rate on the release of TXB_2 and 6-keto- $\text{PGF}_{1\alpha}$ by rat perfused lung.

may be identified pharmacologically. Indomethacin infused into guinea pig and rabbit pulmonary vascular beds produced qualitatively dissimilar effects on bioassayable products of arachidonic acid metabolism (42). In guinea pig lung, low concentrations of indomethacin (10 mM) preferentially inhibited primary prostaglandin formation without affecting TXA_2 production. In rabbit lung, this same indomethacin concentration preferentially inhibited TXA_2 biosynthesis. In both guinea pig and rabbit, indomethacin at higher concentrations inhibited the biosynthesis of all prostaglandins. In these studies, Alabaster (42) found that rabbit thromboxane synthetase was very sensitive to inhibition by imidazole (50 μM). In contrast, guinea pig thromboxane synthetase required 50 to 100-fold higher concentrations of imidazole for an equivalent effect. However, inhibition studies must be viewed with caution since inhibitors

may have effects other than inhibition of the enzyme in question and the effect may not be as easily interpreted as presumed. For example, Hong et al. (58) found that the PGI₂ synthetase inhibitors, 15-HPAA and tranilcypromine, that 15-hydroperoxy arachidonic acid (15-HPAA) increased the release of arachidonic acid while inhibiting the biosynthesis of prostacyclin and PGE₂, whereas tranilcypromine decreased the release of arachidonic acid and the biosynthesis of prostacyclin in isolated endothelial cells.

The origin of thromboxane in the pulmonary vascular effluent is not clear. It is generally advocated that blood vessels do not synthesize thromboxane (26,46). It has been found that the rat mesenteric vascular bed secretes both 6-keto-PGF_{1α} (3–6 ng/min) and TXB₂ (0.09–0.15 ng/min) *in vitro* (59); other researchers have reported similar findings in experiments with arterial preparations of several species (59). Of particular interest is the recent data from Salzman and co-workers (60), who found that the rabbit intrapulmonary artery (IPA), including the lobular artery and the extrapulmonary artery (lying outside the lung mass), produces both TXB₂ and 6-keto-PGF_{1α} in ratios varying from 1:2 to 1:6. In these experiments ¹⁴C-arachidonic acid was converted by IPA to 9 ± 1% TXB₂ and 16 ± 2% 6-keto-PGF_{1α}. The synthesis of TXB₂ and 6-keto-PGF_{1α} from endogenous arachidonic acid was 100 to 200 ng/mg and 500 to 1000 ng/mg tissue, respectively, a ratio of 1:5. In their studies a cascade of biological detectors showed that TXA₂ produced by IPA dominated the biological responses, even though GC-MS analysis revealed five times more prostacyclin in the incubation mixture. The data highlight the much greater biological potency of TXA₂ and indicate that small changes in PGI₂ synthesis, such as in atherosclerosis, may have potentially dramatic biological consequences. Of particular interest is their statement that rabbit lung parenchyma did not produce detectable amounts of TXA₂. This is surprising, since Sun et al. (52) reported that rabbit lung microsomes synthesize both PGI₂ and TXA₂ from PGH₂.

It is probable that thromboxane detected in rabbit pulmonary vascular effluent arose primarily in the vascular compartment, since Salzman et al. (60) were unable to detect TXA₂ production by lung parenchyma, although as noted above rabbit lung parenchymal microsomes contain the necessary enzyme complex. Evidence to support the idea that nonvascular lung elements have the capacity to synthesize both TXA₂ and PGI₂ has been provided by Levine and Alam (61). Using a combined high pressure liquid chromatography–radioimmunoassay procedure these researchers found that endogenous arachidonic acid was metabolized to 65% PGE₂, 16% TXB₂, 16% PGF_{2α} and 4% PGI₂ in normal human lung cells, whereas rat Type II alveolar cells produced 78% PGE₂, 0.2% TXB₂, 18.2% PGF_{2α} and 0.7% PGI₂. Such studies culturing different cell types from the lung will in the future provide a better picture of arachidonic acid metabolism in the lung.

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