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The Influence of Environmental Agents on Prostaglandin Biosynthesis and Metabolism in the Lung

INHIBITION OF LUNG 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE BY EXPOSURE OF GUINEA PIGS TO 100 PER CENT OXYGEN AT ATMOSPHERIC PRESSURE

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Enzymes in the 100000*g* supernatant fraction of guinea-pig lungs, in the presence of NAD^+, converted $PGF_{2\alpha}$ (prostaglandin $F_{2\alpha}$) into a less-polar compound. The u.v. spectrum of this metabolite showed a strong absorption band at 230nm, which is characteristic of a carbonyl group in conjugation with a double bond. Reduction of this metabolite with $NaBH_4$ resulted in a compound that behaved like $PGF_{2\alpha}$ on t.l.c. and g.l.c. From this evidence we concluded that $PGF_{2\alpha}$ is metabolized *in vitro* to 15-oxo-$PGF_{2\alpha}$ by the NAD^+-dependent prostaglandin dehydrogenase system of guinea-pig lung. The effect of exposure of the animal to SO_2 and O_2 on the rate of prostaglandin biosynthesis and catabolism by lung fractions *in vitro* was studied. Exposure of guinea pigs to 500p.p.m. of SO_2 for 5h or to 50p.p.m. for 9 days (6h/day) did not alter the production or degradation of prostaglandins by lung fractions *in vitro*. In contrast, exposure of guinea pigs to 100% O_2 for 48h inhibited the rate of prostaglandin metabolism *in vitro* by 60–70% without significantly altering the rate of biosynthesis by lung fractions. Inhibition of prostaglandin dehydrogenase activity *in vitro* by lung fractions after exposure of the animal to O_2 was dependent on the duration of exposure. Glutathione *S*-aryltransferase and catechol *O*-methyltransferase activities of guinea-pig lung 100000*g* supernatant were unaltered by exposure of the animal to O_2. Thus it appears that inhibition of pulmonary prostaglandin dehydrogenase by exposure of the animal to O_2 is not the result of a general toxic response. It was postulated that the inhibition of prostaglandin dehydrogenase may occur after exposure of the animal to other oxidant gases.

Since the initial discovery by Kurzork & Lieb (1930) that a substance in fresh human semen could either contract or relax strips of human uterus, and the isolation of PGE_1† and $PGF_{1\alpha}$ by Bergstrom & Sjovall (1957), numerous laboratories have studied the chemistry, biochemistry, physiology and pharmacology of prostaglandins (Horton, 1969; Von Euler & Eliasson, 1967; Hinman, 1972; Weeks, 1972; Fanburg, 1973). Prostaglandins display potent effects on bronchial smooth muscle; PGE_2 is a bronchial dilator and $PGF_{2\alpha}$ is a bronchial constrictor (Oesterling *et al.*, 1972; Horton, 1969). Prostaglandins are released from the lung after various stimuli (Piper & Vane, 1969, 1971; Vargaftig & Dao, 1971; Fanburg, 1973), are involved in inflammatory processes (Horton, 1969), and have been implicated in the etiology of bronchial asthma (Mathe *et al.*, 1973).

The lungs of several animal species contain small amounts of prostaglandins, mainly $PGF_{2\alpha}$ (Samuelsson, 1967). Both PGE_2 and $PGF_{2\alpha}$, as well as several unknown 'prostaglandin-like' substances, are biosynthesized from arachidonic acid *in vitro* by lung tissue (Anggard & Samuelsson, 1965; Oesterling *et al.*, 1972; Parkes & Eling, 1974). In addition the lung appears to be a major site for detoxification of circulatory prostaglandins (Ferreira & Vane, 1967). Prostaglandins are metabolized in the lung by oxidation of the secondary alcohol group at C-15 and reduction of the Δ^{13} double bond (Anggard & Samuelsson, 1967; Granstrom, 1971).

Parkes & Eling (1974) described a method for the determination of prostaglandin synthetase *in vitro*

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† Abbreviations: PGE_2, prostaglandin E_2 (11α,15-dihydroxy-9-oxo-5-*cis*,13-*trans*-prostadienoic acid); $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$ (9α,13α,15-trihydroxy-5-*cis*,13-*trans*-prostadienoic acid); 15-oxo-$PGF_{2\alpha}$, 9α,11α-dihydroxy-15-oxo-5-*cis*,13-*trans*-prostadienoic acid; PGE_1, prostaglandin E_1 (11α,15-dihydroxy-9-oxo-*trans*-prostenoic acid); $PGF_{1\alpha}$, prostaglandin $F_{1\alpha}$ (9α,13α,15-trihydroxy-*trans*-prostenoic acid).

in guinea-pig lung and examined the influence of some vasoactive amines on the lung prostaglandin synthetase system. In the present paper we describe a method for the determination of 15-hydroxyprostaglandin dehydrogenase (EC 1.1.1.141) activity *in vitro* in the lung and the influence of exposure of guinea pigs to SO_2 and O_2 on both the prostaglandin synthetase and dehydrogenase systems of these lungs.

Materials and Methods

[5,6,8,11,12,14,15-^{3}H]Prostaglandin E_2 (5.38Ci/mmol) and [9-^{3}H]prostaglandin $F_{2\alpha}$ (14.3Ci/mmol) were purchased from New England Nuclear Corp., Boston, Mass., U.S.A. Prostaglandin standards were the generous gift of Dr. J. E. Pike, The Upjohn Company, Kalamazoo, Mich., U.S.A. NAD^+ was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. All solvents were freshly distilled before use.

Preparation of enzyme fraction

All procedures were performed at 4°C. Lungs from adult male guinea pigs (350–450g body wt., obtained from Carworth Breeders, Portage, Mich., U.S.A.) were freed of extraneous tissue, cut into small pieces and homogenized in 0.25M-sucrose (tissue/sucrose ratio, 1:4, w/v) in a Potter–Elvehjem-type homogenizer with ten strokes of the pestle at 6000rev./min. The homogenate was centrifuged at 100000*g* for 60min (in a 60-Ti rotor) in a Beckman L-3-50 ultracentrifuge and the resulting supernatant fraction was pipetted off and diluted with 0.25M-sucrose to a protein concentration of 10mg/ml. Protein was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Incubation procedures and assay for metabolism

Each incubation mixture contained the following: 50μg of $PGF_{2\alpha}$ (trimethamine salt) or PGE_2, 0.1μCi of [^{3}H]$PGF_{2\alpha}$ or [^{3}H]PGE_2, 250μl of 8mM-NAD^+, 100μl of 100000*g* supernatant containing 1mg of protein, 250μl of 0.25M-KH_2PO_4 adjusted to pH8.0 with NaOH, and sufficient water to give a total volume of 1.0ml. Incubations were carried out at 37°C in a Dubnoff metabolic shaker. After 15min, the samples were acidified to pH3.5 with 1M-HCl and extracted with 8vol. of ethyl acetate. The organic phases were transferred and evaporated to dryness under reduced pressure. Residues from evaporation were dissolved in 100μl of methanol and 20μl portions were applied to silica gel G (Analtech, Newark, Del., U.S.A., 250μm thick) thin-layer plates. These plates were developed in either acetone–methylene chloride–acetic acid (80:120:3, by vol.), or chloroform–methanol–acetic acid–water (90:8.5:1:0.65, by vol.), air dried, and scanned on a Packard Radiochromatogram Scanner. Zones corresponding to the radioactive peaks were scraped into counting vials containing 10ml of dioxan scintillation cocktail [214g of Fluoralloy (Beckman, Palo Alto, Calif., U.S.A.), 2 litres of scintillation-grade dioxan], and assayed for radioactivity in a Beckman LS-250 liquid-scintillation counter. The recovery of [^{3}H]$PGF_{2\alpha}$ (when added to boiled 100000*g* supernatant and taken through the entire procedure) was greater than 90%.

Prostaglandin synthetase activity was determined by incubation of [1-^{14}C]arachidonic acid (Applied Sciences Lab, State College, Pa., U.S.A.) with the microsomal fraction of guinea-pig lung as described by Parkes & Eling (1974). Guinea-pig lungs converted arachidonic acid into $PGF_{2\alpha}$ and an unknown prostaglandin-like compound, designated M-I. Prostaglandin synthetase activity was expressed as the rates of formation of $PGF_{2\alpha}$ and compound M-I. Catechol *O*-methyltransferase (EC 2.1.1.6) was measured by the method of Law *et al.* (1974). Glutathione *S*-aryltransferase (EC 2.5.1.13) was determined by the method of Booth *et al.* (1961).

Isolation and purification of $PGF_{2\alpha}$ metabolites for structural identification

Soluble fraction was prepared from the lungs of five adult male guinea pigs as described above. A 10ml portion of this supernatant was incubated in the presence of 500μg of $PGF_{2\alpha}$, 1μCi of [^{3}H]$PGF_{2\alpha}$ and 10ml of 0.25M-KH_2PO_4 (adjusted to pH8.0 with NaOH) containing 8mM-NAD^+. After incubation of the mixture for 60min at 37°C, it was acidified to pH4 with 1M-HCl and extracted with 8vol. of ethyl acetate. The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The crude extract was applied to a glass column (0.5cm internal diam.) which had been packed to a height of 2cm with a slurry of silicic acid (Mallinkrodt Chemical Works, St. Louis, Mo., U.S.A.; 100 mesh) in benzene–ethyl acetate (7:3, v/v). After a preliminary wash with 150ml of benzene–ethyl acetate (7:3, v/v) the $PGF_{2\alpha}$ metabolite was eluted with 100ml of benzene–ethyl acetate (11:9, v/v). The eluate was evaporated to dryness under reduced pressure and the residue from evaporation was applied to a second silicic acid column. The metabolite was eluted with a continuous linear gradient between 100ml of 100% benzene and 100ml of 100% ethyl acetate. Fractions (4ml) were collected and 0.1ml samples of the fractions were assayed for radioactivity by liquid-scintillation counting. Tubes containing the ^{3}H-labelled metabolite were pooled and evaporated to dryness under reduced pressure. The purity of the metabolite was established by t.l.c. in two systems: (1) silica-gel G t.l.c. plates developed in acetone–methylene chloride–acetic acid (80:100:3, by vol.); (2) silica-gel G t.l.c. plates impregnated with

10% $AgNO_3$ (Analtech) developed in the upper phase of ethyl acetate–hexane–acetic acid–water (11:2:1:10, by vol.).

Reduction with $NaBH_4$

A fraction of the purified metabolite was dissolved in 2ml of methanol, cooled to 0°C for 20min and then at room temperature (20°C) for 50min. The reaction mixture was diluted with 2ml of water, acidified to pH3.5 with 1M-HCl, and extracted with 8vol. of ethyl acetate. The organic phase was transferred and evaporated to dryness under reduced pressure.

Preparation of methyl ester

Diazomethane was freshly prepared from Diazlad by using a diazomethane-generating kit (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.). The purified metabolite, or its reduced derivative, was dissolved in 300μl of methanol, 2.7ml of the diazomethane was added, and the solution was placed in the dark for 60min. After this period, the solvent was evaporated to dryness under N_2.

Preparation of trimethylsilyl ethers

Methyl esters were diluted to a concentration of 1μg/ml with trimethylsilylimidazole–bis(trimethylsilyl)trifluoroacetamide (2:1, v/v) (Pierce Chemical Co., Rockford, Ill., U.S.A.). After 2h at room temperature, samples of these mixtures were injected directly into the gas chromatograph.

Gas–liquid chromatography

A Tracor MT 220 gas–liquid chromatograph equipped with dual-flame ionization detector was used. The column was a 182cm (6ft) U tube, 4mm internal diam., packed with 3% OV-225 on 80/100 Gas Chrom Q (Applied Sciences Lab, State College, Pa., U.S.A.). Temperatures of the inlet, column and detector were maintained at 240°, 210° and 240°C respectively. Carrier gas was helium; flow rate was 60ml/min.

Methods for exposure of animals to O_2

Exposure chambers were airtight polystyrene cages (37cm×47cm×41cm) with two 1.25cm openings at the top and a 12.7cm opening on the side. O_2 or compressed air was introduced through one of the small openings in the top of the chamber, with the other opening serving as an exhaust. The side opening was used to replace food and water and was sealed when not in use. Normally, three animals were continuously exposed to 100% O_2 introduced at a flow rate of 8 litres/min. Control animals were placed in the chamber and exposed to air at a flow rate of 8 litres/min. O_2 concentration was verified by measurement of pO_2 on a blood-gas analyser.

Conditions for exposure of animals to SO_2

Exposure of animals to controlled atmospheres of SO_2 was carried out in a stainless-steel chamber of the type described by Henners *et al.* (1968). The atmosphere of SO_2 was maintained by metering the SO_2 directly into the incoming air and was monitored by an iodometric procedure. The desired concentration of SO_2 was found to vary by less than 5% throughout the exposure. Control animals were placed in stainless-steel chambers and exposed to air.

Results

In the presence of NAD^+, the 100000*g* supernatant fraction from guinea-pig lung converted [^{3}H]$PGF_{2\alpha}$ into a less-polar metabolite (M-II) (Fig. 1). The use of a second solvent system (chloroform–methanol–acetic acid–water; 90:8.5:1:0.65, by vol.), which has been shown to separate 15-oxo-$PGF_{2\alpha}$, 15-oxo-13,14-dihydro-$PGF_{2\alpha}$ (Pace-Asciak & Miller, 1973), indicated the presence of only one metabolite of $PGF_{2\alpha}$.

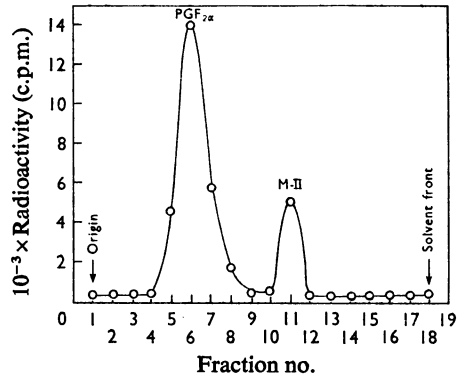

Fig. 1. *Thin-layer chromatogram of extract of incubation mixtures of [^{3}H]$PGF_{2\alpha}$ with the 100000g supernatant from guinea-pig lungs*

Incubation mixtures contained 0.1μCi of [^{3}H]$PGF_{2\alpha}$, 50μg of $PGF_{2\alpha}$ (trimethamine salt), 100μl of 100000*g* supernatant containing 1mg of protein, 250μl of 8mM-NAD^+ and sufficient water to make the final volume 1.0ml. Reaction was terminated after 15min incubation at 37°C. Metabolites were extracted as described in the Materials and Methods section. T.l.c. was carried out on silica gel G with the solvent system acetone–methylene chloride–acetic acid (80:120:3, by vol.). The t.l.c. plate was scraped in 0.5cm sections and the radioactivity determined.

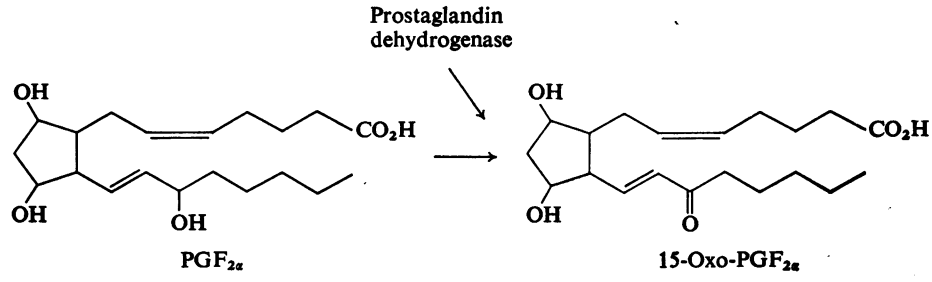

Fig. 2. *Schematic diagram for the metabolism of* [^3H]$PGF_{2\alpha}$ *by the* 100000*g supernatant from guinea-pig lungs*

For details see the text.

The metabolite (M-II) was isolated and purified by column chromatography in sufficiently large quantities for further analysis. The u.v. spectrum of purified metabolite M-II showed a strong absorption band at 232nm, which corresponds to that theoretically expected from an α,β-unsaturated ketone. Reduction of metabolite M-II with $NaBH_4$ produced a compound with chromatographic properties similar to those of $PGF_{2\alpha}$ and did not show a strong absorption at 232nm. The trimethylsilyl ethers of the methyl esters of $PGF_{2\alpha}$ and reduced metabolite M-II produced single peaks on g.l.c. (retention time on OV-225 = 7.6min). These data suggest that metabolite M-II is the 15-oxo derivative of $PGF_{2\alpha}$, produced from $PGF_{2\alpha}$ by 15-hydroxyprostaglandin dehydrogenase (Anggard & Samuelsson, 1966) (Fig. 2).

Conversion of [^{3}H]$PGF_{2\alpha}$ into metabolite M-II by guinea-pig lung 100000*g* supernatant fraction was examined to determine optimum conditions for assaying the rate of prostaglandin metabolism. The reaction rate was linear with time for 30min of incubation and linear with protein concentration to 1mg of supernatant protein/ml of incubation mixture. The K_m for NAD^+ was 0.05mM. A concentration of 1mM-NAD^+ was used as a routine in the incubation mixture of $PGF_{2\alpha}$ and 100000*g* supernatant fraction of guinea-pig lung. The K_m value for the conversion of $PGF_{2\alpha}$ into metabolite M-II was 20μM-$PGF_{2\alpha}$, with $V_{max.}$ 2.5nmol of metabolite M-II produced/min per mg of protein. This optimized assay system was used to assess the effect of exposure to O_2 and SO_2 on prostaglandin metabolism by lung fractions isolated from exposed animals.

Exposure of guinea pigs to 50p.p.m. of SO_2 (6h/day for 9 days) or to 500p.p.m. for 5h (single exposure) did not appear to alter prostaglandin biosynthesis by lung microsomal fractions or prostaglandin metabolism by lung supernatant fractions from such treated animals (Table 1). Lung weight, total microsomal and 100000*g* supernatant-fraction protein from homogenates of control and SO_2-exposed guinea-pig lungs were not significantly different (Table 1). Exposure of guinea pigs to higher concentrations of SO_2 for longer periods of time produced toxicity, as evidenced by gross oedema of lungs. In some experiments the metabolism of [^{3}H]PGE_2 by the 100000*g* supernatant of lungs from animals exposed to air or SO_2 was examined. PGE_2 and $PGF_{2\alpha}$ undergo metabolic conversion by the same enzyme in 100000*g* supernatant of lungs (Anggard & Samuelsson, 1967).

The rate of $PGF_{2\alpha}$ metabolism by the 100000*g* supernatant fraction of guinea-pig lungs from animals exposed to 100% O_2 for 48h was approx. 30% of the rate of metabolism by 100000*g* supernatant fraction from control animals' lungs (Table 2). Prostaglandin biosynthesis by microsomal fractions, as determined *in vitro* by the formation of $PGF_{2\alpha}$ and metabolite M-I from arachidonic acid, was unaltered by exposure of the animal to O_2. Lung weight, total microsomal protein and 100000*g* supernatant protein from homogenates of lungs from O_2-exposed animals were not significantly different from these parameters in control animals (Table 2). Thus loss of $PGF_{2\alpha}$ metabolic activity (per unit of protein) was not the result of dilution by oedema-fluid protein. Thin-layer chromatograms of the extracts from incubations of $PGF_{2\alpha}$ with the supernatant fraction from lungs of O_2-exposed animals indicated the presence of only 15-oxo-$PGF_{2\alpha}$. Thus exposure of animals to O_2 did not result in the production of other known metabolites of $PGF_{2\alpha}$ (Anggard & Samuelsson, 1967) by the 100000*g* supernatant fraction of lung.

Inhibition of prostaglandin metabolism *in vitro* by isolated lung fractions, as the result of exposure of the animal to O_2, was dependent on the duration of exposure to O_2 (Fig. 3). Exposure of animals to 100% O_2 for 8h produced approx. 12% inhibition of prostaglandin metabolism by supernatant fractions of lungs from exposed animals compared with lungs from control animals, whereas exposure of animals to O_2 for 68h produced approx. 90% inhibition of $PGF_{2\alpha}$ metabolism by 100000*g* supernatant fraction from exposed as compared with control animals.

Table 1. *Effect of exposure of the animal to SO_2 on prostaglandin biosynthesis and metabolism by fractions from lungs*

Guinea pigs were exposed to SO_2. Controls were handled in an identical manner but exposed to air. Conditions for these treatments and for biochemical and enzyme assays are described in the Materials and Methods section. To obtain sufficient tissue for analysis, lungs from three animals were pooled. Each value represents the mean ± S.E.M. of three such pools. PGE_2 was used as a substrate for prostaglandin dehydrogenase in the experiment involving exposure of animals to 50 p.p.m. SO_2, whereas $PGF_{2\alpha}$ was used as a substrate for prostaglandin dehydrogenase involving exposure of animals to 500 p.p.m. SO_2.

Parameter	Exposure to 50 p.p.m. of SO_2 at 6 h/day for 9 days		Exposure to 500 p.p.m. of SO_2 for 5 h	
	Control	Exposed	Control	Exposed
Lung wt. (g/organ)	3.8 ±0.64	3.7 ±0.52	3.2 ±0.4	3.4 ±0.57
Microsomal protein (mg/organ)	15.3 ±1.2	15.4 ±1.1	15.4 ±3.3	15.5 ±2.1
100000*g*-supernatant-fraction protein (mg/organ)	144.0 ±0.14	139.0 ±5.5	147.0 ±0.35	188.8 ±0.62
Prostaglandin synthetase (microsomal):				
(a) $PGF_{2\alpha}$ produced (nmol/min per mg of protein)	0.015±0.001	0.013±0.002	0.029±0.010	0.028±0.004
(b) Metabolite M-I* produced (nmol/min per mg of protein)	0.049±0.010	0.048±0.010	0.036±0.009	0.033±0.006
Prostaglandin dehydrogenase (100000*g* supernatant fraction):				
nmol of (*a*) PGE_2 or (*b*) $PGF_{2\alpha}$ metabolized/min per mg of protein	3.53 ±0.22	3.11 ±0.66	1.69 ±0.49	1.68 ±0.22

* M-I refers to an unknown prostaglandin material produced from [^{14}C]arachidonic acid by guinea-pig lung microsomal fraction. See Parkes & Eling (1974) for details.

Table 2. *Effect of exposure of the animal to O_2 on prostaglandin biosynthesis and metabolism by lung fractions in vitro*

Guinea pigs were exposed to 100% O_2 for 48 h. Control animals were housed in similar exposure chambers, but supplied with air. The numbers represent the means ± S.E.M. for three pools of tissue. Each pool contained the tissue from at least three animals. Analyses are described in the text.

Parameter	Control	Exposed
Lung wt. (g/organ)	2.96 ± 0.1	2.68 ± 0.18
Microsomal protein (mg/organ)	24.6 ± 3.3	25.3 ± 1.6
100000*g*-supernatant-fraction protein (mg/organ)	244.9 ±12.7	273.0 ±40.0
Prostaglandin synthetase (microsomal):		
(*a*) $PGF_{2\alpha}$ produced (nmol/min per mg of protein)	0.019± 0.002	0.020± 0.008
(*b*) Metabolite M-I* produced (nmol/min per mg of protein)	0.043± 0.025	0.038± 0.033
Prostaglandin dehydrogenase (100000*g* supernatant fraction):		
$PGF_{2\alpha}$ metabolized (nmol/min per mg of protein)	1.57 ± 0.29	0.56 ± 0.14†

* M-I refers to an unknown prostaglandin material produced from [^{14}C]arachidonic acid by guinea-pig lung microsomal fraction. See Parkes & Eling (1974).

† Significantly different from control; $P<0.05$ using a two-tailed Student's *t* test.

Since $PGF_{2\alpha}$ metabolism *in vitro* by supernatant fractions of guinea-pig lungs was inhibited by exposure of the animal to 100% O_2, whereas prostaglandin biosynthesis by the lung microsomal fractions was not inhibited, it is possible that exposure to O_2 may specifically affect only soluble-fraction enzymes or prostaglandin dehydrogenase. To investigate this possibility, guinea pigs were exposed to 100% O_2 for 60 h and $PGF_{2\alpha}$ metabolism, glutathione *S*-aryltransferase and catechol *O*-methyltransferase activities were measured in the 100000*g* supernatant fraction (Table 3). After the exposure to O_2, prostaglandin metabolism *in vitro* by 100000*g* supernatant was decreased to 25% of the activity of corresponding fractions of lungs of control animals, whereas glutathione *S*-aryltransferase activity of the same fraction was not inhibited.

Catechol *O*-methyltransferase activity in 100000*g* supernatant and lung weight were slightly elevated by exposure of the animal to O_2. Thus inhibition of

prostaglandin dehydrogenase activity *in vitro* of guinea-pig lung 100000*g* supernatant fractions, after exposure of the animal to 100% O_2, may be a specific inhibition. Prostaglandin dehydrogenase activity in the 100000*g* supernatant fraction from lung may therefore be a sensitive indicator of the toxic effects produced by exposure of animals to O_2 (Clark & Lambertsen, 1971).

Discussion

The influence of environmental agents on prostaglandin concentrations (amounts) in the lung could be examined directly by measuring the tissue contents of the various prostaglandins, or indirectly by studying the rates of biosynthesis and degradation of prostaglandins. Although methods exist for the determination of trace quantities of prostaglandins (Jouvenaz *et al.*, 1973), these methods may not give a true indication of prostaglandin concentrations in tissue, since prostaglandins have been shown to be released and metabolized during the tissue preparation procedures (Piper & Vane, 1971). Thus studying the rates of prostaglandin biosynthesis *in vitro* by microsomal fractions and rates of prostaglandin metabolism by 100000*g* supernatant fractions may give a better indication of the steady-state concentration of prostaglandins in a particular tissue.

Parkes & Eling (1974) described a method for the determination of prostaglandin synthetase activity in the microsomal fraction of guinea-pig lung. In the present paper we have described a similar procedure for the analysis of prostaglandin metabolism by the 100000*g* supernatant fraction. Granstrom (1971) studied the metabolism of $PGF_{2\alpha}$ by the supernatant fraction of guinea-pig lung and found that $PGF_{2\alpha}$ was converted into 15-oxo-dihydro-$PGF_{2\alpha}$, indicating metabolic conversion by the Δ^{13} reductase system as well as by the dehydrogenase system. This is in contrast with our findings that $PGF_{2\alpha}$ was converted into 15-oxo-$PGF_{2\alpha}$ by 100000*g* supernatant fractions of guinea-pig lung. However, variation in the methods of tissue preparation and incubation conditions could account for such difference. [Granstrom (1971) prepared tissue in a medium containing phosphate buffer, pH 7.4, 0.0276M-nicotinamide and 0.0036M-$MgCl_2$ and incubated for 60min without exogenous NAD^+.] The incubation conditions used by Granstrom (1971) gave both dehydrogenase and Δ^{13} reductase activity, whereas our incubation mixture possessed only dehydrogenase activity. Since the catabolism of prostaglandins primarily depends on the dehydrogenase system rather than reduction of the Δ^{13} double bond (Anggard, 1966; Samuelsson, 1970),

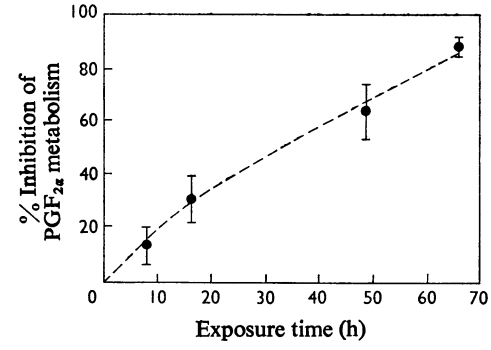

Fig. 3. *$PGF_{2\alpha}$ metabolism by the 100000g supernatant fraction obtained from lungs of guinea pigs exposed to O_2 for various times*

Animals were exposed to either O_2 or air at atmospheric pressure and killed at various times as described in the Materials and Methods section. Prostaglandin dehydrogenase activity was measured as described in the Materials and Methods section. Each point represents the mean (bars are ±S.E.M.) of duplicate determinations from three separate animals. Prostaglandin dehydrogenase activity of the 100000*g* supernatant from control guinea pigs varied from 1.7 to 1.9nmol/min per mg of protein.

Table 3. *Effect of exposure of the animal to O_2 on activity in vitro of several enzymes in 100000g supernatant of guinea-pig lung*

Animals were exposed to 100% O_2 for 60h. Controls were housed in similar exposure chambers but exposed to air. Numbers represent the means±S.E.M. for three animals.

Parameter	Control	Exposed
Lung wt. (g/organ)	2.83± 0.31	3.37± 0.83
100000*g*-supernatant-fraction protein (mg/organ)	192.0 ±28.0	181.0 ±52.0
Prostaglandin dehydrogenase (nmol of $PGF_{2\alpha}$ metabolized/min per mg of protein)	2.01± 0.54	0.50± 0.26*
Glutathione *S*-aryltransferase (nmol of 2-chloro-4-nitrophenyl-glutathione/min per mg of protein)	11.07± 0.32	10.17± 2.7
Catechol *O*-methyltransferase (nmol of *O*-methylisoproteranol/min per mg of protein)	0.09± 0.02	0.13± 0.01

* Significantly different from control; $P<0.05$, using a two-tailed Student's *t* test.

our studies on prostaglandin dehydrogenase appear to be applicable to the assay of prostaglandin catabolism by guinea-pig lung.

Several dehydrogenase systems of the lung have been shown to be inhibited by O_2 at high pressures, but not at atmospheric pressure, and these biochemical changes could not be directly related to O_2-induced lung toxicity (Clark & Lambertsen, 1971; Hauggaard, 1968). In contrast, our finding of inhibition of prostaglandin dehydrogenase after exposures to O_2 at 101 kPa (1 atm) pressure could be related to the pulmonary pathology found after treatment of the animal with O_2. Pathological changes in the lung after exposure to O_2 are characterized by oedema, decreased lung compliance, decreased vital capacity and decreased pulmonary blood flow (Clark & Lambertsen, 1971) all of which, in part, may be attributed to the action of prostaglandins (Fanburg, 1973). Since prostaglandin dehydrogenase activity in the 100000*g* supernatant fraction of lung was the only enzyme of those examined that was inhibited by exposure of guinea pigs to 100% O_2, exposure of guinea pigs to 100% O_2 at 10 kPa (1 atm) may produce a specific inhibitory effect on the prostaglandin dehydrogenase system as measured *in vitro*. However, additional evidence is needed to support this hypothesis further. Inhibition of prostaglandin metabolism *in vitro* by the 100000*g* supernatant fraction after exposure of guinea pigs to O_2 may be a direct effect of O_2 on the pulmonary enzyme system or an indirect effect mediated via a physiological process occurring distant from the lung. We have no evidence to distinguish between these alternatives.

It is possible that the inhibition of prostaglandin dehydrogenase activity may occur in lungs damaged by other oxidant gases such as ozone and NO_2. Exposure of guinea pigs (sensitized to bovine serum albumin) to NO_2 and ozone, but not SO_2, increased the symptoms of anaphylaxis induced by albumin (Matsumura, 1970; Matsumura *et al.*, 1972). Since prostaglandins are released during anaphylaxis (Piper & Vane, 1969), the increased symptoms of anaphylaxis in ozone- and NO_2-exposed animals may be the result of an inhibition of prostaglandin metabolism.

The lung, spleen and kidney possess significant prostaglandin dehydrogenase activity (Anggard *et al.*, 1971) and thus are major sites for the detoxification of circulating prostaglandins. Ferreira & Vane (1967) demonstrated that 90–95% of the PGE_2 and PGE_1 added to the pulmonary circulation was removed in a single passage through the lung. Inhibition of prostaglandin dehydrogenase by exposure of the animal to O_2 could result in prostaglandin remaining in the arterial circulation during and after passage through the lung, and this would elicit physiological effects in the lung as well as in other organs. In view of the potential clinical usage of prostaglandins as abortifacients, anti-asthmatics and hypotensive agents (Pike, 1971), inhibition of pulmonary prostaglandin metabolism by exposure to O_2 could produce deleterious effects in humans. In addition, Mathe *et al.* (1973) have reported that $PGF_{2\alpha}$ is 8000 times more active in patients with bronchial asthma, and they hypothesized that in asthmatic patients prostaglandin metabolism may be impaired. If O_2 therapy is used for treatment of such patients, the resultant inhibition of prostaglandin metabolism could aggravate rather than help the asthmatic condition.

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