*Biochemical Pharmacology*, Vol. 31, No. 5, pp. 633–638, 1982.
Printed in Great Britain.

0006-2952/82/050633-06 $03 00/0
Pergamon Press Ltd.

# INACTIVATION OF PROSTAGLANDINS IN THE PERFUSED RAT LUNG

C. ROBINSON and J. R. S. HOULT

Department of Clinical Pharmacology, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12 0HS, U.K. and Department of Pharmacology, King's College, Strand, London WC2R 2LS, U.K.

(*Received* 24 *April* 1981; *accepted* 17 *August* 1981)

**Abstract**—Inactivation in the isolated perfused rat lung of prostaglandins (PG) $D_2$, $E_1$, $F_{2\alpha}$, $I_2$ and the metabolites 6-keto $PGF_{1\alpha}$ (= $6KF_{1\alpha}$) and 13,14-dihydro-15-keto $PGF_{2\alpha}$ (= $KH_2F_{2\alpha}$) was studied using 5 min perfusions of 7–10 ng/ml PG in Krebs' solution containing 0.02 $\mu$Ci/ml tritiated PG and 4.5% bovine serum albumin (BSA). The parameters measured were (a) extent of inactivation ($F_{2\alpha} > E_1 > D_2 > 6KF_{1\alpha} > I_2$; $KH_2F_{2\alpha}$ unchanged), (b) the accumulation of PG within the lung measured as tissue to medium ratio ($F_{2\alpha} = D_2 > E_1 > 6KF_{1\alpha} > I_1 = KH_2F_{2\alpha}$), and (c) rate of equilibration of PG within the lung measured as "wash-in $t_{\frac{1}{2}}$" ($D_2 > F_{2\alpha} > E_1 > I_2 = 6KF_{1\alpha} = KH_2F_{2\alpha}$). Removal of sodium ions produced a small decrease in $PGD_2$ and $PGE_1$ breakdown but not of $PGF_{2\alpha}$, whereas breakdown of all PGs was markedly inhibited at 5°. Removal of BSA enhanced $PGE_1$ and $PGI_2$ breakdown but not that of $PGF_{2\alpha}$. Addition of 10% BSA inhibited $PGE_1$ breakdown but not that of $PGF_{2\alpha}$. Binding of PGs to 4.5% BSA was $PGE_1 = KH_2F_{2\alpha} > D_2 > F_{2\alpha}$, and increased at 10% BSA or after removal of sodium ions. These data support the view that PGs must be taken up into pulmonary cells by a transmembrane carrier process as a prerequisite for enzymatic breakdown. The metabolites are then released back into the pulmonary circulation.

The ability of lungs to accumulate and metabolise prostaglandins and other vasoactive substances has been widely studied (for reviews, see Refs 1–7). Prostaglandins (PGs) are inactivated *in vivo* and *in vitro* on passage through the lungs of all species tested, although the extent depends upon a number of factors. Thus E and F prostaglandins are extensively broken down to the corresponding 15-keto and 13,14-dihydro-15-keto metabolites [9–12], but prostacyclin and A-series prostaglandins are largely resistant to pulmonary breakdown both *in vivo* [13–16] and *in vitro* [17–19].

Since the enzymes which metabolise prostaglandins (15-hydroxyprostaglandin dehydrogenase and $\Delta^{13}$-reductase) are cytoplasmic [8, 9, 20], and as far as is known cell membranes are impermeable to prostaglandins [21], it has been suggested that inactivation occurs as a two-step process in which "carrier-mediated transmembrane transport" (i.e. "uptake") of prostaglandins precedes enzymatic transformations within the cell [4, 6, 12, 22]. Little is known, however, about how the metabolites are released back into the circulation.

There is other evidence for carrier-mediated uptake of prostaglandins. Several tissues [23–26], including lung, appear to take up prostaglandins against a concentration gradient, as evidenced from tissue to medium ratios of greater than unity. In lungs perfused *in vitro* there is also a measurable delay before the appearance of prostaglandin metabolites when compared with the efflux of extracellular space markers [12, 26, 27]. Moreover, substances which are known to inhibit certain membrane transport processes reduce prostaglandin breakdown in perfused lungs [26]. Finally, there is the apparent paradox that A-series prostaglandins and prostacyclin, whilst being good substrates for the 15-hydroxyprostaglandin dehydrogenase [18, 20, 28, 29], appear to escape pulmonary inactivation. This could be explained if they do not possess all the structural requirements for the carrier or are unavailable for transport due to protein binding [19, 22, 30, 31].

To date there is no single detailed comparative survey of the pulmonary inactivation of a series of prostaglandins; nor have the previous studies of individual prostaglandins generally been extended to include analysis of parameters likely to yield information about the proposed uptake process, such as tissue-to-medium ratios or the time profile of appearance of prostaglandin in the effluent. Moreover, $PGD_2$ and biologically inactive prostaglandin metabolites have not been investigated in this manner.

Thus the work presented here concerns the inactivation and disposition of several parent compounds (prostaglandins $D_2$, $E_1$, $F_{2\alpha}$ and $I_2$) as well as of two metabolites (13,14-dihydro-15-keto $PGF_{2\alpha}$ and 6-keto $PGF_{1\alpha}$). We have used radiolabelled prostaglandins of high specific activity such that isolated rat lungs could be perfused with suitably low concentrations to apparent steady state, thereby allowing measurement not only of the extent of metabolism, but also of the tissue to medium ratios and the time course of appearance of radioactivity.

We have also tested lungs under control conditions and after certain manipulations such as at low temperature, removal of sodium and altered albumin content as these are factors which have an important

bearing on pulmonary prostaglandin uptake and inactivation. Some of these results have been presented in preliminary form elsewhere [32].

## MATERIALS AND METHODS

*Materials.* [5,6(n)-$^3$H]-$PGE_1$, sp. act. 40 Ci/mmole; [9-$^3$H]$PGF_{2\alpha}$, sp. act. 20 Ci/mmole; 13,14-dihydro-15-keto-[5,6,8,9,11,12,14(n)-$^3$H]-$PGF_{2\alpha}$, sp. act. 80 Ci/mmole and inulin-[$^{14}$C]carboxylic acid MW 5175, sp. act. 5.2 mCi/mmole were purchased from the Radiochemical Centre, Amersham. [5,6,8,9,12,14,15-(n)-$^3$H]-$PGD_2$, sp. act. 100 Ci/mmole, was purchased from New England Nuclear GmbH. [11$\beta$-$^3$H]Prostacyclin, sp. act. 8 Ci/mmole, was synthesized by Dr. I. A. Blair, Department of Clinical Pharmacology, Royal Postgraduate Medical School, London.

Cohn fraction V bovine serum albumin was from Sigma. Other reagents used were of analytical grade. Unlabelled prostacyclin and all other prostaglandins were generous gifts of Wellcome Research Laboratories, Beckenham, and Dr. J. E. Pike of the Upjohn Co., Kalamazoo, respectively.

*Lung perfusion.* Male Wistar rats (150–250g) were anesthetised with pentobarbitone (60 mg/kg, i.p.), tracheostomy performed and then artificially ventilated with humidified room air (30 strokes/min, tidal volume 2–5 ml). The thorax was opened and a heparinised polyethylene cannula inserted into the left ventricle to remove the pulmonary venous effluent. The pulmonary artery was then cannulated and the lungs removed and placed in a perspex perfusion chamber.

Lungs were perfused at 7 ml/min with Krebs solution (37°) gassed with 5% $CO_2$/95% $O_2$ and containing 4.5% w/v Cohn fraction V bovine serum albumin. After two minutes equilibration the lungs were perfused with prostaglandin solutions (7–10 ng/ml containing 0.02 $\mu$Ci/ml of the corresponding tritiated prostaglandin) for a total period of 5 min. 0.02 $\mu$Ci/ml [$^{14}$C]inulin was co-perfused in some experiments as an extracellular marker. Fractions (15 sec) were collected for further analysis. After 5 min infusion the experiment was terminated and the lungs processed as described below; there was little if any visible evidence of oedema after the perfusion, even in lungs perfused with albumin-free Krebs. This was confirmed in preliminary experiments in which wet:dry ratio was found not to differ between perfused and freshly-exsanguinated lungs.

*Equilibration of prostaglandins within the lung.* Radioactivity in 250 $\mu$l aliquots of each effluent sample was expressed as a percentage of total activity present in the inflow perfusate. Thus a profile of equilibration of prostaglandin within the lung was built up (see Fig. 1) and empirically quantified in terms of the time taken for 50 per cent to appear in the effluent ("wash-in $t_{\frac{1}{2}}$"). This was estimated by regression analysis of a double reciprocal plot of percentage recovery versus time using a Digital PDP-8 computer, for which purposes we assumed the profile to be a rectangular hyperbola.

*Pulmonary inactivation of prostaglandins.* Pooled 10 ml perfusate samples were acidified to pH 3.5 with hydrochloric acid (2M) and loaded on to Clin Elut CE 1010 columns (Analytichem International). After 5–10 min the prostaglandins were eluted from the column with 40 ml ethyl acetate. After removal of solvent, prostaglandin breakdown was quantitated by a thin-layer radiochromatography on 5 × 20 cm Merck silica gel 60 plates. Authentic prostaglandin standards were run as reference markers. Prostaglandins were eluted from appropriate 0.5 cm sections into methanol and the radioactivity measured by liquid scintillation counting (Packard Tricarb 2650). Three t.l.c. systems based on chloroform/methanol/acetic acid were used: (a) 90:5:5 v/v for $PGF_{2\alpha}$, $PGD_2$ and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ experiments [33]; (b) 95:1:5 v/v for $PGE_1$ [35]; (c) 90:9:1 plus water 0.75 v/v [35] for prostacyclin experiments.

*Tissue to medium ratio.* After perfusion the lungs were dissected rapidly, blotted, weighed and homogenised in 2 vol. of Krebs solution at 4° with three 15 sec bursts of a Polytron PT10-35 homogeniser at maximum speed. Triplicate 100 and 300 mg aliquots of homogenate were solubilised overnight at room temperature in 1 or 2 ml, respectively, of Soluene 350 (Packard). The samples were then counted after addition of 10 ml Dimilume-30 (Packard) and corrected for quenching by the external standard ratio method. Results are expressed as:

$$\text{tissue to medium ratio} = \frac{\text{dpm/g lung}}{\text{dpm/ml inflow perfusate}}$$

*Equilibrium dialysis.* The degree of albumin-binding of prostaglandins in the perfused medium was determined by 3½ hr dialysis at 37° against albumin-free Krebs solutions (both pH 7.4) in a Dianorm macrocell dialysis system (Diachema AG, Zurich) running at 10 rpm. Each half-cell contained 1 ml of solution separated by Visking membrane (24–25 Å pore). There was negligible adsorption of prostaglandin to the dialysis membranes.

*Partition coefficients.* The water:chloroform partition coefficients of $PGE_1$, $PGF_{2\alpha}$ and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ were determined by vortexing 5 ml of aqueous solutions of these prostaglandins (10 ng/ml, containing 0.02 $\mu$Ci/ml label) with 5 ml chloroform for one minute. The mixtures were then left to stand for 24 hr at 20° before taking samples from each phase for radioactivity determinations.

## RESULTS

### *Perfusion of lungs with labelled prostaglandins*

Isolated rat lungs were perfused for 5 min with prostaglandins so as to achieve apparent steady-state conditions of pulmonary degradation. After switching to the prostaglandin-containing solution, radioactivity rapidly appeared in the pulmonary venous effluent and reached plateau levels within 60–150 sec (Fig. 1). The rate at which apparent steady-state conditions were achieved depended upon the prostaglandin (e.g. Fig. 1) and could be altered by various experimental manipulations. Estimates of the rate of equilibration (the "wash-in $t_{\frac{1}{2}}$") are shown in Table 1. Similar profiles were also obtained for two prostaglandin metabolites and for the extracellular space

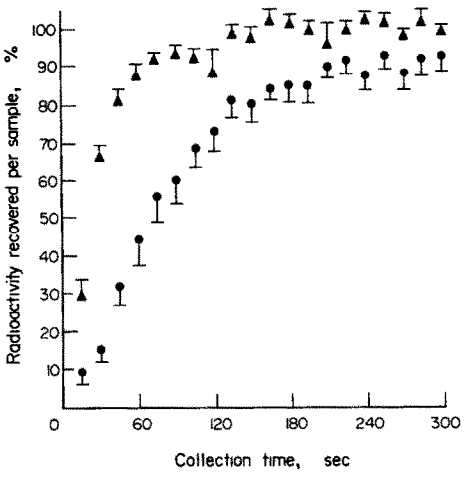


Fig. 1. Profiles in lung effluent of radioactivity following perfusion of the isolated rat lung with 7 ng/ml prostaglandin $F_{2\alpha}$ (●) or 10 ng/ml 13,14-dihydro-15-keto $PGF_{2\alpha}$ (▲). Calculated wash-in $t_{\frac{1}{2}}$ values for these prostaglandins were 109.9 ± 16.2 sec and 21.4 ± 3.2 sec respectively (Table 1). Perfusion conditions: Krebs solution containing 4.5% bovine serum albumin; results show mean ± S.E.M. for results obtained using 11 and 5 lungs respectively.

marker inulin, although all substances appeared more rapidly.

Table 1 shows that the wash-in $t_{\frac{1}{2}}$ parameter serves as a qualitative index for the pulmonary disposition of the prostaglandins. The rapid appearance of prostacyclin, 6-keto $PGF_{1\alpha}$ and 13,14-dihydro-15-keto $PGF_{2\alpha}$ along with inulin suggests that there is little net accumulation of these prostanoids into lung cells. Prostaglandins $F_{2\alpha}$, $E_1$ and $D_2$ are metabolised by the lung, presumably following uptake, and have longer wash-in $t_{\frac{1}{2}}$ values. At 5° metabolism and uptake are reduced and the wash-in $t_{\frac{1}{2}}$ values correspondingly shortened. In general, for a given prostaglandin, there is a correlation between wash-in $t_{\frac{1}{2}}$ and the extent of metabolism.

*Pulmonary inactivation of prostaglandins*

There was a marked difference in pulmonary breakdown of the four prostaglandins tested (Table 1), the descending order of breakdown being prostaglandin $F_{2\alpha} > E_1 > D_2 > I_2$. However, these prostaglandins are all effective substrates for types I or II 15-hydroxyprostaglandin dehydrogenase and our results are best explained in terms of selectivity of the proposed transmembrane carrier. Metabolic transformation of two biologically inactive prostaglandins was also studied. As expected, 13,14-dihydro-15-keto $PGF_{2\alpha}$ was not transformed during passage through the pulmonary circulation. However, 6-keto $PGF_{\alpha}$, which is a poor substrate for 15-hydroxyprostaglandin dehydrogenase [28] although it still retains a hydroxyl group at position 15, was broken down by 16.2 per cent (Table 1).

Metabolism of prostaglandins $D_2$, $E_1$, $F_{2\alpha}$ and $I_2$ was inhibited almost totally at 5° (Table 1).

Replacement of sodium ions by iso-osmolar sucrose in the perfusate produced a small but significant decrease in the metabolism of $PGE_1$ and $PGD_2$ but did not affect $PGF_{2\alpha}$ (Table 1). Prosta-

Table 1. Inactivation of prostaglandins by the isolated perfused rat lung

| PG | Concn (ng/ml) | Treatment | $n$* | %PG metabolism† | T/M ratio‡ | Wash-in $t_{\frac{1}{2}}$§ (sec) |
|---|---|---|---|---|---|---|
| $PGD_2$ | 10 | Control (4.5% albumin) | 11 | 55.0 ± 1.3 | 2.25 ± 0.13 | 126.3 ± 21.3 |
| | | Sodium-free | 3 | 46.3 ± 3.7¶ | 1.46 ± 0.17¶ | 17.3 ± 1.8¶ |
| | | 5° | 6 | 9.9 ± 4.5¶ | 0.31 ± 0.02¶ | 26.0 ± 3.7¶ |
| $PGE_1$ | 10 | Control (4.5% albumin) | 8 | 68.7 ± 1.6 | 1.22 ± 0.07 | 44.9 ± 9.1 |
| | | Sodium-free | 3 | 56.7 ± 3.9¶ | 1.31 ± 0.08 | 41.9 ± 7.7 |
| | | 5° | 3 | 4.6 ± 1.6¶ | 0.60 ± 0.04¶ | 29.1 ± 5.6 |
| | | Albumin-free | 5 | 78.0 ± 1.9¶ | 1.22 ± 0.06 | 64.0 ± 10.9 |
| | | 10% Albumin | 4 | 33.0 ± 8.8¶ | 0.83 ± 0.07¶ | 23.9 ± 3.1 |
| $PGF_{2\alpha}$ | 7 | Control (4.5% albumin) | 11 | 90.9 ± 2.1 | 2.25 ± 0.16 | 109.9 ± 16.2 |
| | | Sodium-free | 3 | 89.6 ± 3.8 | 1.46 ± 0.06¶ | 73.7 ± 14.0 |
| | | 5° | 3 | 3.2 ± 0.7¶ | 0.58 ± 0.14¶ | 72.3 ± 15.0 |
| | | Albumin-free | 4 | 89.8 ± 2.9 | 1.78 ± 0.06¶ | 83.0 ± 13.0 |
| | | 10% Albumin | 4 | 85.3 ± 3.4 | 1.68 ± 0.09¶ | 79.2 ± 12.9 |
| $PGI_2$ | 10 | Control (4.5% albumin) | 5 | 12.8 ± 1.9 | 0.19 ± 0.02 | 19.7 ± 4.2 |
| | | Albumin-free | 5 | 18.2 ± 1.7¶ | 0.55 ± 0.03¶ | 20.4 ± 4.6 |
| | | 5° | 4 | 4.8 ± 0.5¶ | 0.16 ± 0.02 | 17.8 ± 4.0 |
| $6KF_{1\alpha}$‖ | 10 | Control (4.5% albumin) | 3 | 16.2 ± 2.2 | 0.82 ± 0.09 | 14.1 ± 2.9 |
| $KH_2F_{2\alpha}$‖ | 10 | Control (4.5% albumin) | 4 | 0 | 0.17 ± 0.01 | 21.4 ± 3.2 |
| | | Albumin-free | 4 | 0 | 0.31 ± 0.03¶ | 17.7 ± 2.1 |
| | | Inulin | 16 | | 0.10 ± 0.07 | 21.7 ± 4.0 |

* $n$ = number of lungs perfused.
† Per cent inactivation of prostaglandin measured by radiochromatography, number of determinations = 2 × $n$.
‡ T/M = tissue to medium ratio (see Methods); number of determinations = 6 × $n$.
§ Wash-in $t_{\frac{1}{2}}$ values obtained by regression analysis of $n$ profiles of effluent radioactivity (see Fig. 1).
‖ $KH_2F_{2\alpha}$ = 13,14-dihydro-15-keto $PGF_{2\alpha}$; $6KF_{1\alpha}$ = 6-keto $PGF_{1\alpha}$.
Values show mean ± S.E.M.; significance of differences with respect to control treatment, ¶ $P < 0.05$–$0.001$.

cyclin metabolism was not studied under sodium-free conditions.

Prostaglandins are known to bind to albumin [30, 31] and this may have an important effect on pulmonary inactivation, particularly in these experiments because the perfusing solution routinely contained 4.5% bovine serum albumin. Removal of albumin significantly enhanced $PGE_1$ inactivation, whereas addition of albumin at 10% w/v decreased it by 51% (Table 1). These manipulations did not affect inactivation of $PGF_{2\alpha}$. Removal of albumin caused a significant increase in breakdown of prostacyclin (Table 1). Effects of altering albumin concentration on $PGD_2$ metabolism were not tested.

The $PGE_1$ wash-in $t_{\frac{1}{2}}$ values altered in a manner consistent with the effects of the albumin manipulations on metabolism, but less effect was seen with $PGF_{2\alpha}$ and prostacyclin.

*Pattern of metabolites formed by pulmonary breakdown*

Radiochemical assay showed that the predominant metabolite of $PGE_1$ in all conditions tested was 13,14-dihydro-15-keto-prostaglandin $E_1$; it exceeded the amount of 15-keto-prostaglandin $E_1$ by 1.7 to 3.8 times. In these experiments a suitable correction was applied for conversion (approx. 5%) of E-series to A-series prostaglandins resulting from the acid extraction used. The predominant metabolite of $PGF_{2\alpha}$ was 15-keto-prostaglandin $F_{2\alpha}$ which exceeded the amount of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ by 1.1 to 1.7 times.

Three apparent products were formed after infusion of $PGD_2$ through the isolated rat lung, but their identity has not yet been assigned. Of the three products (M-I, $R_f$ 0.58; M-II, $R_f$ 0.72 and M-III, $R_f$ 0.93; cf. $PGD_2$ $R_f$ value 0.38) M-II was the most abundant in all three experimental conditions tested and M-III least abundant. In some cases M-III was not detectable. M-II accounted for 66–74 per cent of the total metabolite radioactivity. Analysis of $PGD_2$ metabolites is complicated by the fact that the enedione system of 15-keto $PGD_2$ gives rise to tautomers under acid conditions [36] and because dehydration of the ring system of $PGD_2$ may also occur. In our experiments dehydration of parent $PGD_2$ was less than 10 per cent as measured after acid extraction and chromatography.

Prostacyclin was not substantially broken down in the perfused rat lung. The radiochromatographic quantitation of its metabolism was performed after acid extraction. Two radioactive peaks were seen; the major peak ($R_f$ 0.27) co-chromatographed with authentic 6-keto-$PGF_{1\alpha}$ and probably reflects the recovery of unchanged prostacyclin in the venous effluent. The other radioactive peak ($R_f$ 0.43) corresponded to the positions of both 6,15-diketo-prostaglandin $F_{1\alpha}$ and 6,15-diketo-13,14-dihydro-prostaglandin $F_{1\alpha}$ which could not be further resolved in the solvent systems used. As noted above, 6-keto $PGF_{1\alpha}$ was also transformed after passage through the lung; the sole radioactive metabolite peak appeared at $R_f$ 0.43, indicating oxidation of the hydroxyl at position 15.

Special precautions were taken to minimise breakdown of prostacyclin during these experiments. Solutions were made up in pH 8.5 50 mM Tris buffer containing 4.5% w/v bovine serum albumin and maintained at 4° until required. Aliquots of these solutions were then allowed to equilibrate to room temperature (5–7 min) before infusion into the arterial inflow using a Sage model 355 syringe pump coupled to a purpose-built mixing chamber (volume 1 ml). The pH of the resulting inflow perfusate was pH 8–8.3 and entry into the lung occurred within 2 sec after mixing. In separate experiments in which prostacyclin solutions were tested for their capacity to inhibit ADP-induced aggregation of human platelets we found that in this buffer prostacyclin was stable for up to 130 min at 4° and 40 min at room temperature. We are therefore confident that the concentration of prostacyclin available to the lung was the same as that added.

*Tissue-to-medium ratios*

Accumulation of prostaglandins against a concentration gradient has been inferred from studies of the tissue-to-medium ratios obtained when tissue pieces are incubated with, or lungs perfused with, labelled prostaglandins [23, 24, 26].

Tissue-to-medium ratios in lung achieved after 5 min perfusion to apparent steady state conditions illustrate that, in general, the greater the extent of metabolism of a given prostaglandin, the greater the tissue-to-medium ratio achieved (Table 1). Reductions of the ratio to values less than unity occurred after perfusion at 5°, suggesting marked inhibition of the capacity to take up and retain prostaglandin. Substantial exclusion of prostaglandins from lung tissue under control conditions was seen with prostacyclin and 13,14-dihydro-15-keto $PGF_{2\alpha}$, as evidenced by the similarity of the ratio to that seen with inulin (0.10). However, the ratio for 6-keto $PGF_{1\alpha}$ (0.82) was higher than that found for prostacyclin,

Table 2. Percentage binding of prostaglandins to albumin and their water/chloroform partition coefficients

| Conditions* | $PGE_1$ | $PGF_{2\alpha}$ | $KH_2F_{2\alpha}$† | $PGD_2$ | $n$‡ |
|---|---|---|---|---|---|
| 4.5% BSA (control) | 77.3 ± 1.8 | 47.0 ± 3.5 | 76.1 ± 0.8 | 60.2 ± 0.8 | 6–7 |
| 10% BSA | 83.2 ± 1.4§ | 61.9 ± 3.1§ | 81.6 ± 2.2§ | n.d. | 3–6 |
| Sodium free + 4.5% BSA | 79.3 ± 2.0 | 62.5 ± 1.7§ | n.d. | 68.0 ± 0.5§ | 5 |
| Water/chloroform partition coefficient (20°) | 2.6 ± 0.2 | 15.1 ± 2.2 | 2.2 ± 0.2 | n.d. | 8–10 |

* All binding experiments at 37°, pH 7.4 in Krebs solution.
† $KH_2F_{2\alpha}$ = 13,14-dihydro-15-keto $PGF_{2\alpha}$.
‡ $n$ = number of determinations; results show mean ± S.E.M; differences with respect to control.
§ $P < 0.05$–$0.001$.
n.d. = not determined. BSA = bovine serum albumin.

suggesting that it can to a limited extent be taken up by the carrier, thus enabling it to be broken down even though it is a poor substrate.

*Binding of prostaglandins to albumin*

This study confirms previous work showing that albumin alters the pulmonary metabolism of certain prostaglandins [19]. Thus $PGE_1$, but not $PGF_{2\alpha}$, breakdown was enhanced in the absence of albumin (Table 1).

Dialysis experiments showed that under these perfusion conditions $PGE_1$ and 13,14-dihydro-15-keto $PGF_{2\alpha}$ bind extensively to albumin (Table 2), whereas binding of $PGF_{2\alpha}$ and $PGD_2$ is not so marked. These results are to be predicted from the known association constants for prostaglandin–albumin interactions [30]. We could not perform similar equilibrium dialysis experiments using prostacyclin because of its chemical instability, but it is known that its biological effects are prolonged in plasma [37–39] or albumin solutions [40], perhaps because direct protein binding confers resistance to hydrolysis (as well as to pulmonary uptake).

Replacement of sodium ions with iso-osmolar sucrose afforded a small increase in the binding to albumin of the three prostaglandins tested (Table 2).

The water/chloroform partition coefficients of $PGE_1$, $PGF_{2\alpha}$ and 13,14-dihydro-15-keto $PGF_{2\alpha}$ showed that, as expected, the degree of albumin binding was inversely related to the polarity of the prostaglandin (Table 2). The similar coefficients obtained for $PGE_1$ and the $PGF_{2\alpha}$ metabolite suggest that these two compounds are equally lipophilic.

## DISCUSSION

These experiments provide a detailed comparison of the metabolism of four prostaglandins and two metabolites in the isolated perfused rat lung. Using three measured parameters (extent of enzymatic breakdown; tissue to medium ratio; wash-in $t_{\frac{1}{2}}$) and by varying the conditions under which the lungs were perfused (alteration of albumin concentration; removal of sodium ions; cooling to 5°), and studying binding to albumin *in vitro*, we hoped to provide a detailed survey of the relationships of enzymatic inactivation and prostaglandin uptake.

Our results are consistent with the concept that transport of prostaglandins into the cells which contain the prostaglandin metabolising enzymes is a necessary prerequisite for inactivation of the prostaglandin.

Summarised briefly this evidence is as follows. Compared to the 'classical' prostaglandins, prostacyclin was not substantially inactivated although it is an effective substrate for 15-hydroxyprostaglandin dehydrogenase [18, 28, 29]; moreover, its resistance to metabolism cannot solely be due to binding to albumin (Table 1). Prostaglandins which were substantially inactivated appeared to accumulate within the lung (measured as tissue-to-medium ratio), and their appearance in the venous effluent (measured as wash-in $t_{\frac{1}{2}}$) was retarded. Both facts suggest that the volume of distribution of these prostaglandins was larger (viz. into an intracellular compartment) than for prostaglandins which were not extensively metabolised. The values for the unmetabolised prostaglandins were closer to those obtained for the extracellular space marker inulin.

Cooling to 5° reduced breakdown and accumulation of prostaglandins tested and shortened the wash-in $t_{\frac{1}{2}}$ as expected for a temperature-sensitive carrier-mediated pulmonary uptake process. Hypothermia has been shown by others to inhibit prostaglandin metabolism reversibly [11, 27, 41] and the effect may be due not only to reduced enzyme activity but also to an increase in membrane viscosity or reduced pulmonary perfusion due to vasoconstriction. While removal of sodium had only a very modest effect on extent of metabolism, it reduced the tissue to medium ratio (for $PGD_2$ and $PGF_{2\alpha}$) and shortened the wash-in $t_{\frac{1}{2}}$ (for all three prostaglandins tested). These results suggest that in distinction to the pulmonary uptake of other substances such as catecholamines [4], carrier-mediated transport of prostaglandins is not to a large extent dependent on $Na^+K^+$-ATPase activity. A similar conclusion was drawn from experiments showing that ouabain does not inhibit rabbit lung $PGA_1$ metabolism [27]. We were not able to use ouabain in these experiments since rats are insensitive to this drug.

As noted previously [19], the interaction of prostaglandins with albumin has an important bearing on pulmonary prostaglandin metabolism. $PGE_1$ is highly bound to albumin (Table 2), and removal or addition of albumin considerably alters its pulmonary pharmacokinetics, both in terms of its enzymatic inactivation and accumulation and equilibration within the lung. Alterations in albumin concentration have less effect on $PGF_{2\alpha}$ metabolism since it is less bound to albumin. The increase in binding of prostaglandins to 4.5% bovine serum albumin observed when sodium ions are replaced by iso-osmolar sucrose (Table 2) may partly explain the modest reductions in their pulmonary breakdown under these conditions (Table 1). Binding of prostaglandins to albumin is dependent *inter alia* on their lipophilicity as illustrated by the partition coefficient measurements in Table 2.

The data for the 13,14-dihydro-15-keto metabolite offers support for the notion [42] that passive diffusion of prostaglandins across biological membranes is very restricted. This metabolite is as lipophilic and as highly bound to albumin as $PGE_1$ (Table 2) but does not markedly accumulate within the lung (and has a short wash-in $t_{\frac{1}{2}}$), even in the absence of albumin (Table 1). It was surprising that the stable chemical degradation product of prostacyclin, 6-keto $PGF_{1\alpha}$, was transformed to a greater extent than prostacyclin itself, even though it is a poorer substrate for 15-hydroxyprostaglandin dehydrogenase [28]. The tissue to medium ratio data suggests that this might be because 6-keto $PGF_{1\alpha}$ is more effectively taken up by the lung than prostacyclin.

How are prostaglandin metabolites released after their formation within pulmonary cells? This study and others show that both 15-keto and 13,14-dihydro-15-keto metabolites are released. It is tempting to speculate that they might be taken outwards on a carrier in a manner analogous to their inward transport: certainly the evidence described

above suggests that it is unlikely that they diffuse out passively, even though the concentration gradient may be favourable. The increased lipophilicity and binding to albumin of these metabolites compared to the parent prostaglandins (e.g. Table 2) suggests also that albumin may facilitate the outward movement of the metabolite. This emphasises that plasma proteins and albumin may act as important influences on prostaglandin metabolism at several different stages.

*Acknowledgements*—CR gratefully acknowledges receipt of an MRC award. We thank Professor C. T. Dollery for helpful discussion.

## REFERENCES

1. J. R. Vane, *Br. J. Pharmac.* **35**, 209 (1969).
2. S. I. Said, *Fedn Proc.* **32**, 1972 (1973).
3. Y. S. Bahkle and J. R. Vane, *Physiol. Rev.* **54**, 1007 (1974).
4. C. N. Gillis and J. A. Roth, *Biochem. Pharmac.* **25**, 2457 (1976).
5. J. W. Ryan and U. S. Ryan, *Fedn Proc.* **36**, 2683 (1977).
6. A. A. Mathé, P. Hedqvist, K. Strandberg and C. A. Leslie, *New Engl. J. Med.* **296**, 850, 910 (1977).
7. A. L. Hyman, E. W. Spannhake and P. J. Kadowitz, *Am. Rev. Resp. Dis.* **117**, 111 (1978).
8. E. Ånggard, K. Gréen and B. Samuelsson, *J. biol. Chem.* **240**, 1932 (1965).
9. B. Samuelsson, *Fedn Proc.* **31**, 1442 (1972).
10. S. H. Ferreira and J. R. Vane, *Nature* **216**, (1967).
11. P. J. Piper, J. R. Vane and J. H. Wyllie, *Nature* **225**, 600 (1970).
12. M. W. Anderson and T. E. Eling, *Prostaglandins* **11**, 645 (1976).
13. J. M. Armstrong, N. Lattimer, S. Moncada and J. R. Vane, *Br. J. Pharmac.* **62**, 125 (1978).
14. G. J. Dusting, S. Moncada and J. R. Vane, *Br. J. Pharmac.* **64**, 315 (1978).
15. R. P. Robertson, *Am. J. Physiol.* **228**, 68 (1975).
16. E. W. Horton and R. L. Jones, *Br. J. Pharmac.* **37**, 705 (1969).
17. H. J. Hawkins, J. B. Smith, K. C. Nicolaou and T. E. Eling, *Prostaglandins* **16**, 871 (1978).
18. P. Y-K. Wong, J. C. McGiff, F. F. Sun and K. U. Malik, *Biochem. biophys. Res. Commun.* **83**, 731 (1978).
19. H. J. Hawkins, A. G. E. Wilson, M. W. Anderson and T. E. Eling, *Prostaglandins* **14**, 251 (1977).
20. H. S. Hansen, *Prostaglandins* **12**, 647 (1976).
21. L. Z. Bito and R. A. Baroody, *Am. J. Physiol.* **229**, 1580 (1975).
22. T. E. Eling, H. J. Hawkins and M. W. Anderson, *Prostaglandins* **14**, 51 (1977).
23. L. Z. Bito, *J. Physiol.* **221**, 371 (1972).
24. L. Z. Bito and E. V. Salvador, *J. Pharmac. exp. Ther.* **198**, 481 (1976).
25. L. Z. Bito, H. Davson and E. V. Salvador, *J. Physiol.* **256**, 257 (1976).
26. L. Z. Bito, R. A. Baroody and M. E. Reitz, *Am. J. Physiol.* **232**, E382 (1977).
27. K. B. Gross and C. N. Gillis, *J. Pharmac. exp. Ther.* **198**, 716 (1976).
28. J. C. McGuire and F. F. Sun, *Archs Biochem. Biophys,* **189**, 92 (1978).
29. P. Y-K. Wong, F. F. Sun and J. C. McGiff, *J. biol. Chem.* **253**, 5555 (1978).
30. J. L. Gueriguian, *J. Pharmac. exp. Ther.* **197**, 391 (1976).
31. A. Raz, *Biochem. J.* **130**, 631 (1972).
32. C. T. Dollery, J. R. S. Hoult and C. Robinson *Br. J. Pharmac.* **73**, 214 (1981).
33. R. Camp and M. W. Greaves, *Biochem. J.* **186**, 153 (1980).
34. J. E. Shaw and P. W. Ramwell, *Meth. Biochem. Anal.* **17**, 325 (1969).
35. C. R. Pace-Asciak, K. Morawska and L. S. Wolfe, *Biochim. biophys. Acta* **218**, 288 (1970).
36. R. L. Jones and N. H. Wilson, *J. Chem. Soc. Perkin* I, 209 (1978).
37. M. Steer, D. MacIntyre, L. Levine and E. Salzman, *Nature* **283**, 194 (1980).
38. M. F. Gimeno, L. Sterin-Borda, E. S. Borda, M. A. Lazzari and A. L. Gimeno, *Prostaglandins* **19**, 907 (1980).
39. D. Pifer, L. Chesney and L. Cagen, *Fedn Proc.* **39**, 295 (1980).
40. M. A. Orchard and C. Robinson, *Thrombos. Haemostas.*, in press.
41. R. Hook and C. N. Gillis, *Prostaglandins* **9**, 193 (1975).
42. R. A. Baroody and L. Z. Bito, *Prostaglandins* **21**, 133 (1981).