PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 414–421 (1975)

Actions of Prostaglandins E_1 and $F_{2\alpha}$ on Isolated Intrapulmonary Vascular Smooth Muscle[1] (39047)

PAUL D. JOINER, PHILIP J. KADOWITZ,[2] J. PRESTON HUGHES AND ALBERT L. HYMAN

Departments of Pharmacology and Surgery and the Surgical Cardiopulmonary Laboratory, Tulane University School of Medicine and Ochsner Foundation Hospital, New Orleans, Louisiana 70112

The lung is an important organ for both the synthesis and metabolism of prostaglandins. Prostaglandins of the E and F type have been shown to be released from the lung into the venous effluent. Moreover, these prostaglandins are readily inactivated by the lung (1, 2).

The roles prostaglandins may play in lung function are still unclear. A number of investigations have attempted to elucidate their effects on bronchomotor tone but fewer studies of their actions on the pulmonary circulation have been reported (1, 2). The present study sought to assess the actions of prostaglandins E_1 (PGE_1) and $F_{2\alpha}$ ($PGF_{2\alpha}$) on tone and contractile activity of isolated intrapulmonary arteries and veins from dog, sheep, swine and man.

Materials and Methods. Arteries and veins 2–5 mm in diam were dissected from the lung lobes of healthy dogs (n = 9), sheep (n = 6) and swine (n = 4) and from human lung tissue removed at surgery, usually for carcinoma (n = 9). The animals had been previously anesthetized lightly with pentobarbital and sacrificed by bleeding. Helical segments 4–5 mm wide (3) were mounted in standard muscle baths containing a physiological salt solution (PSS). The PSS was prepared with distilled water and contained: NaCl, 125 mM; KCl, 2.7 mM; $CaCl_2$, 1.8 mM, and glucose, 11 mM. It was buffered to pH 7.4 with Tris buffer, 23.8 mM, and HCl. The bathing medium was kept at 37° by a heated water jacket and bubbled continuously with 100% O_2. A few experiments were also performed using a Krebs' bicarbonate solution without any significant differences in results from those employing the Tris-buffered medium.

Vessel strips were attached at one end by means of silk ties to a fixed stainless steel aerator and at the other end to a Grass FT.03C force-displacement transducer. Isometric force changes were recorded on a Grass 79D polygraph. Segments were initially placed under stretching forces of 3 g (vein) and 4 g (artery). These loads were found to be optimal in a preliminary study measuring force developed as a function of resting load. Strips of dimensions similar to those of the present study generated approximately the same force upon exposure to 10^{-5} M serotonin when the resting load was varied from 2 to 8 g. Force elicited at resting loads <2 g or >8 g were less than that induced over the optimal 2–8 g range.

The resting loads were maintained throughout the experiments and resulted in tension generation approximating calculated *in vivo* wall tensions. To substantiate this, the distending pressure (P) which would be present *in vivo* were the resting load (L_r) wholly responsible for the wall tension (T) was calculated using (a) Lame's formula: $P = T\sigma/R$ where radius (R) and wall thickness (σ) were determined for each strip and (b) $T = L_r/W\sigma$ where W = width of the strip measured directly. The value of P calculated in this manner for arterial strips under L_r = 4 g was 24 mm Hg, in the range reported (4) for lobar arterial pressure in dog (18 mm Hg), sheep (22 mm Hg) and swine (30 mm Hg). The corresponding P value for venous strips was 18 mm Hg, somewhat higher than that reported (4) for lobar venous pressure: dog (12 mm Hg), sheep (11 mm Hg), swine (15 mm Hg).

After a 2 hr equilibration, strips were exposed briefly to norepinephrine (NE, 10^{-5} M) to elicit a test contraction. All the tissues contracted well when exposed to the

[1] Supported by U. S. Public Health Service Grant Nos. HL 18070, HL 11802, and HL 15580 and a grant from the National Cystic Fibrosis Foundation.

[2] Established investigator of the American Heart Association.

catecholamine. Strips were returned to normal PSS and allowed to remain undisturbed for 1 hr. During the first 15 min of this recovery period, tissues relaxed to the prestimulation state. After 1 hr in normal PSS, strips were exposed to $PGF_{2\alpha}$ or PGE_1 (10^{-8}–10^{-5} *M*) by a cumulative dose technique (5). The total volume of the drug solutions added to the bathing medium never exceeded 5% of the volume of the latter. Tissues were then returned to drug-free PSS for 1 hr and reexposed to the prostaglandin or to 10^{-5} *M* NE at hourly intervals. By allowing these 1 hr reequilibration periods between challenges, the possible influence of drug desensitization on the results was avoided. Thus, several dose–response curves for each PG and several NE contractions were obtained from the same strip. Since responses were reproducible, only one experiment per tissue for each PG and NE is reported.

The prostaglandins were kindly donated by the Upjohn Company, Kalamazoo, Mich. They were dissolved in absolute ethanol to prepare a concentrated stock solution of 5–10 mg/ml which was kept in a freezer. Aliquots of this stock solution were diluted in PSS each day prior to exposure to the tissues. Control experiments with equivalent concentrations of ethanol failed to reveal an effect of the alcoholic vehicle alone.

Median effective concentrations (ED_{50}) of each PG were estimated and indicated on the log dose-response curves. In order to better evaluate results from the different species, responses to the maximum PG concentration tested (10^{-5} *M*) were compared to the contractile effect in the same tissue of 10^{-5} *M* NE, a level of the catecholamine eliciting a near-maximal contraction. Mean force generation induced by 10^{-5} *M* PG and NE were compared by Student's *t* test. Probability values of $P < .05$ were accepted as significant (6).

Results. The effect of the prostaglandins on canine intrapulmonary artery and vein are illustrated in Figs. 1 and 2. $PGF_{2\alpha}$ was without significant action on arteries but contracted veins in a dose-related fashion. 10^{-5} *M* $PGF_{2\alpha}$ was slightly less potent than 10^{-5} *M* NE (Table I). PGE_1 elicited slight dose-dependent relaxation of both vessels. Canine tissues were more responsive to the relaxant effects of nitrite ion. For comparison, 10^{-5} *M* Na NO_2 induced relaxation of

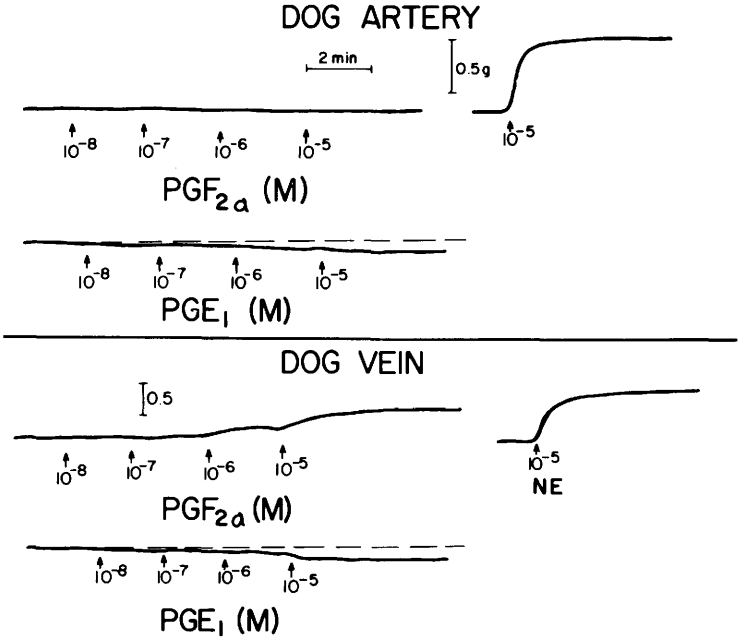

FIG. 1. Portions of polygraph tracing from experiments with dog isolated intrapulmonary artery and vein. Sufficient drug was added at each arrow to achieve the indicated concentration of drug in the bathing medium.

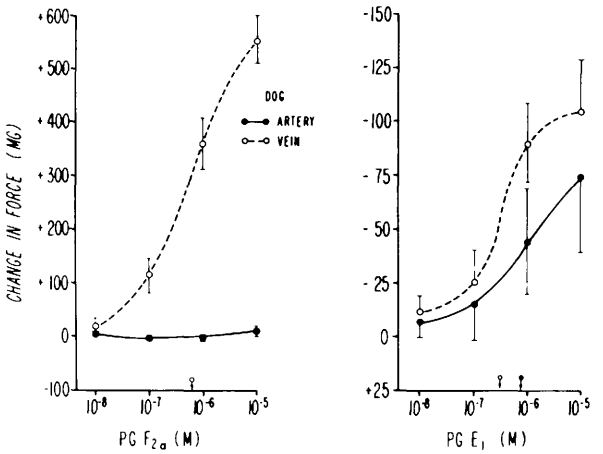

FIG. 2. Log concentration-response curves for $PGF_{2\alpha}$ and PGE_1 (10^{-8}–10^{-5} *M*) from experiments employing dog isolated intrapulmonary arteries and veins. + refers to contraction, − to relaxation. Estimated ED_{50} value for each curve whenever appropriate is indicated by arrow. Brackets denote SEM.

TABLE I. COMPARISON OF MEAN RESPONSES[a] TO PG'S AND NE ($10^{-5}M$).

Tissue	Source	*n*	Response (g)		Response (g)	
			$PGF_{2\alpha}$	NE	PGE_1	NE
Artery	Dog	9	.01 ± .01[b]	.71 ± .13	−.07 ± .03	.93 ± .19
	Sheep	6	−.02 ± .02[b]	1.60 ± .23	−.11 ± .03	1.59 ± .18
	Man	9	.69 ± .10[c]	.70 ± .16	.04 ± .03[b]	.70 ± .15
Vein	Dog	9	.55 ± .05	.79 ± .13	−.11 ± .02	.56 ± .13
	Sheep	6	.24 ± .04	.99 ± .13	−.20 ± .06	.85 ± .13
	Man	9	.48 ± .14[c]	.56 ± .11	.02 ± .04[b]	.56 ± .10

[a] As mean ± SEM; minus sign denotes relaxation.
[b] Mean response is not significant.
[c] Not significantly different from corresponding value for NE contraction.

140 mg ± 20 in arterial strips and 200 mg ± 30 in veins. Maximum relaxation was obtained with 10^{-3} *M* $NaNO_2$: 470 mg ± 20 (arteries) and 540 mg ± 50 (veins).

Since PGE_1 appeared to relax slightly the unstimulated vessels, we also determined the effect of PGE_1 added to vessel strips undergoing active contraction. The alpha adrenergic agent phenylephrine (10^{-6} *M*) was added to the bath to induce a sub-maximal level of contraction before exposure to PGE_1 (10^{-8}–10^{-5} *M*). This level of phenylephrine elicited a mean force increase of 270 mg ± 44 in six arteries and 230 mg ± 43 in nine venous strips. The magnitude of relaxation evoked by PGE_1 in these partially-contracted strips (Fig. 3) was not much different from that seen in the unstimulated strips (Fig. 2). Accordingly, further studies were done only on unstimulated tissues.

A pattern similar to the canine was generally seen in the sheep vessels except that sheep arteries relaxed slightly to $PGF_{2\alpha}$ (Figs. 4 and 5). While veins from sheep were less sensitive to $PGF_{2\alpha}$ than those of the dog, sheep vessels appeared to be somewhat more sensitive to the relaxant action of PGE_1 (Fig. 5, Table I). 10^{-5} *M* $PGF_{2\alpha}$ was only 0.25 as effective a contractile agent as an equivalent concentration of NE in sheep veins (Table I).

In vessels from man, $PGF_{2\alpha}$ exhibited a different pattern of activity, contracting both artery and vein from nine patients (Figs. 6 and 7). The prostaglandin was as effective as an equivalent level of NE (Table

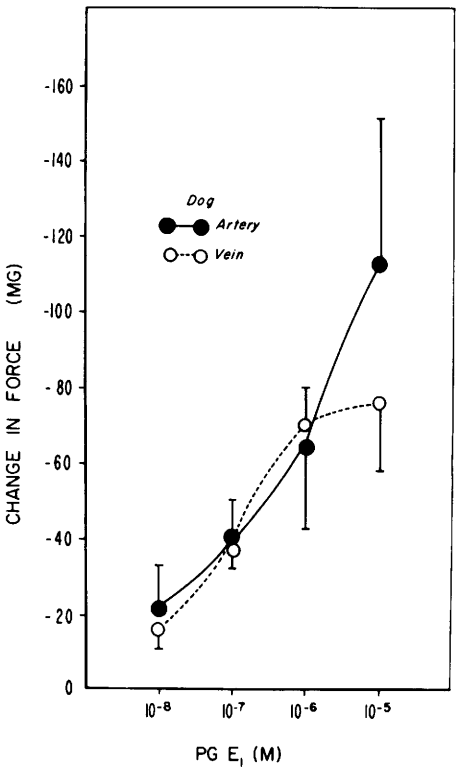

FIG. 3. Log concentration-response curves for PGE_1 (10^{-8}–10^{-5} *M*) from experiments employing dog isolated intrapulmonary arteries and veins. – refers to relaxation of phenylephrine (10^{-6} *M*)-induced contractions. Brackets denote SEM. See text for details.

I). PGE_1's effects were small and variable, slightly contracting arteries from four patients and relaxing slightly those from one patient. PGE_1 did not affect tone of arteries from four other patients although these tissues responded well to NE. Human veins from five patients relaxed slightly to PGE_1, while those from one patient contracted vigorously. Veins from three other patients failed to respond to PGE_1, although they contracted well in the presence of 10^{-5} *M* NE.

Arteries and veins from four swine did not respond significantly to PGE_1 or $PGF_{2\alpha}$ although NE (10^{-5} *M*) was an effective agonist (arteries: 1.02 ± 0.09; veins: 0.48 ± 0.13 g).

Discussion. $PGF_{2\alpha}$ has been shown to increase pulmonary resistance to blood flow in a number of animal species (7–13). In the dog, $PGF_{2\alpha}$ caused a rise in pressure in both pulmonary lobar artery and vein (11). In sheep and swine, $PGF_{2\alpha}$ appeared to affect primarily precapillary vessels (12, 13). In the human and dog, 15 methyl $PGF_{2\alpha}$, less readily metabolized than $PGF_{2\alpha}$, has been shown to induce pulmonary hypertension (14).

In the present study, $PGF_{2\alpha}$ was a powerful stimulant of isolated intrapulmonary veins from dog and man while sheep veins responded less and swine veins not significantly. Of the intrapulmonary arteries tested, only those from man contracted significantly when exposed to $PGF_{2\alpha}$. Indeed, in human vessels, $PGF_{2\alpha}$ was equivalent to NE in contractile effect. Sheep arteries appear to relax slightly to $PGF_{2\alpha}$. Comparison of extimated ED_{50} values indicates that human venous strips are about 10 times more sensitive to $PGF_{2\alpha}$ than are human arterial segments. Both canine and human veins are more sensitive to $PGF_{2\alpha}$ than sheep veins.

The actions of $PGF_{2\alpha}$ on the dog and sheep veins suggest that venoconstriction may play a large role in the pulmonary hypertensive effect observed in the intact animal (11, 13, 15). However, especially in sheep and swine, constriction of vessels smaller than those we studied *in vitro* may occur. Alternatively, factors present *in situ* but absent *in vitro* may enhance the contractile responses of the pulmonary vascular smooth muscle in these species. One such factor might be the presence of sympathetic nervous activity since it has been found that the cutaneous vasoconstrictor effect of prostaglandins in the canine hind-paw preparation is reduced by acute denervation and reserpine pretreatment (16).

The potent action of $PGF_{2\alpha}$ on the isolated vessels from man, coupled with the recent report of pulmonary hypertension in man and dogs induced by 15 methyl $PGF_{2\alpha}$ (14) suggests that extreme caution should be employed when $PGF_{2\alpha}$ and its derivatives are administered to humans.

PGE_1 has been shown to reduce pulmonary vascular resistance in intact dog, swine and sheep (8, 11–13). It acted primarily on the arterial side in the latter two species whereas in the dog, pressure was seen to fall in both lobar artery and vein.

In vitro, PGE_1 acted essentially the same on

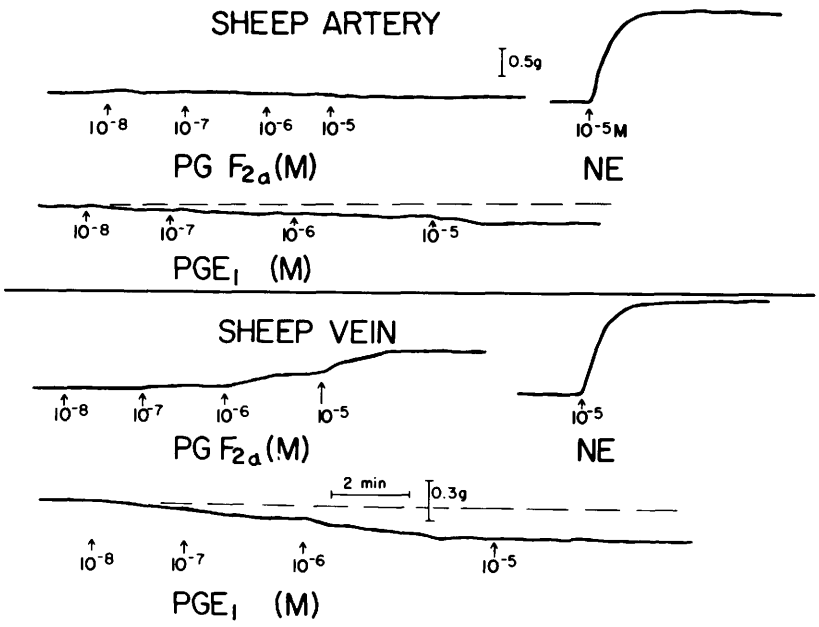

FIG. 4. Portions of polygraph tracing from experiment using sheep isolated intrapulmonary artery and vein. See legend, Fig. 1, for details.

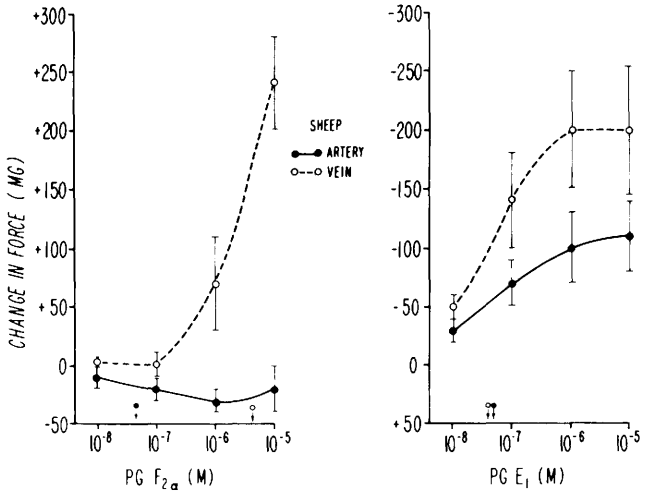

FIG. 5. Log concentration-response curves for $PGF_{2\alpha}$ and PGE_1 (10^{-8}–10^{-5} *M*) from experiments employing sheep isolated intrapulmonary arteries and veins. See legend, Fig. 2, for details.

dog and sheep vessels, relaxing slightly both artery and vein. Vessels from the sheep were more sensitive to PGE_1 than those from the dog. It may be that in the intact sheep, the rate of inactivation by the lung is so high that PGE_1 infused into the lobar artery fails to reach the venous bed in sufficient quantity to elicit a detectible effect. Some support for this postulate is provided by the observation that PGE_1 infused into the lobar artery of the intact dog resulted in a fall in systemic arterial pressure whereas no change was observed during PGE_1 infusion into the lobar artery of intact sheep (11, 13).

Comparison of Figs. 2 and 3 indicates that PGE_1 was about as effective in relaxing

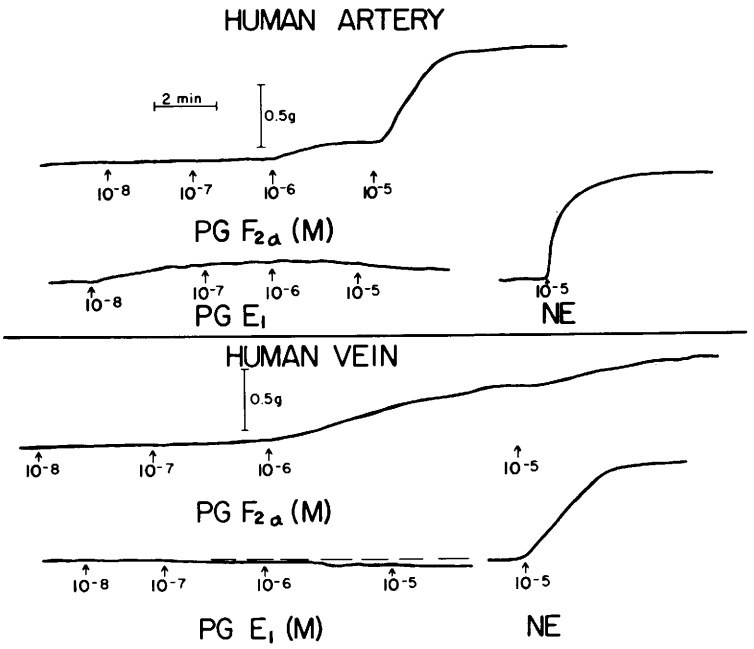

FIG. 6. Portions of polygraph tracing from experiment using human isolated intrapulmonary artery and vein. See legend, Fig. 1, for details.

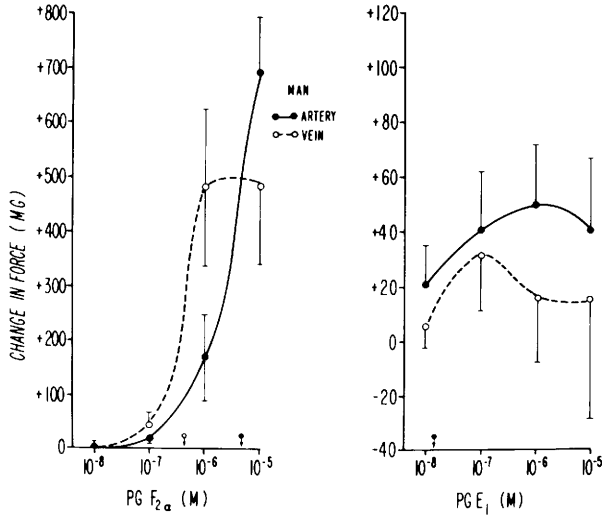

FIG. 7. Log concentration-response curves for PGF$_{2\alpha}$ and PGE$_1$ (10^{-8}–10^{-5} *M*) from experiments employing human isolated intrapulmonary arteries and veins. See legend, Fig. 2, for details.

unstimulated strips as tissues partially contracted by phenylephrine. Since alpha adrenergic blockade has little effect on pulmonary vascular pressure in the normal animal (17), sympathetic tone is likely to be low in the pulmonary circulation and thus assessing the actions of PGE$_1$ on unstimulated vessels is probably more physiologic than studying PG effects on phenylephrine-contracted strips.

The failure of PGE$_1$ to alter tone in swine arteries is at variance with the *in vivo* find-

ings (12) and may suggest that essential factors are present *in situ* but not *in vitro* in this species.

Responses of human vessels to PGE$_1$ varied with the individual donor. Of the veins responding, those from the majority of patients (5 of 6) relaxed slightly when exposed to PGE$_1$. Venous strips from one patient contracted so vigorously that the mean responses were not significant (Fig. 7). On the other hand, PGE$_1$ contracted slightly the responding arteries from the majority of donors (4 of 5). The aberrant arterial and venous strips were not from the same patient. Interestingly, the donor of the venous strips which contracted so well to PGE$_1$ was on antihypertensive therapy which included guanethidine and alpha methyl DOPA.

In general, the present results from isolated intrapulmonary vascular strips are in agreement with those obtained from *in vitro* studies using other vascular preparations. For example, PGF$_{2\alpha}$ has previously been shown to contract umbilical and placental vessels (18), calf pulmonary artery and vein (19), dog mesenteric vessels (20), bovine common dorsal digital vein and canine lateral saphenous vein (21). PGE$_1$ relaxed unstimulated umbilical and placental vessels (18), NE-contracted rabbit celiac artery (22), calf pulmonary vein and artery previously contracted submaximally by histamine, 5-HT or acetylcholine (19), canine mesenteric vessels (20) and canine renal, mesenteric and skeletal muscle small (400–1000 μm diam) arteries previously contracted by catecholamines, plasma contracting factor or KCl (23). Interestingly, in the latter study, high levels of PGE$_1$ (10^{-6}–10^{-5} g/ml) itself elicited contraction of these preparations. Moreover, PGE$_1$ consistently contracted rabbit aorta, dog coronary artery strips and cat carotid artery (23, 24), a finding similar to that seen in the majority of responding human intrapulmonary arteries (Figs. 6, 7).

It is apparent from our results that intrapulmonary arteries and veins from dog, sheep and man generally respond to PGE$_1$ and PGF$_{2\alpha}$. Inspection of the physiograph recordings (Figs. 1, 4, 6) reveal that the PG's induce a slower-developing response than does NE but, once developed, the PG responses are well-maintained. These acidic lipids are unlikely to be present to any great extent in circulating blood due to their rapid inactivation. However, PGF$_{2\alpha}$ and PGE$_1$ released locally in the lung could play major roles in the regulation of pulmonary blood flow by their actions on intrapulmonary venous smooth muscle.

Summary. The effects of PGE$_1$ and PGF$_{2\alpha}$ were studied on isolated strips of intrapulmonary arteries and veins from dog, sheep, swine and man. PGF$_{2\alpha}$ contracted human arterial strips in a dose-dependent fashion, relaxed slightly sheep arteries and had no effect on dog arteries. Canine, sheep and human venous strips were contracted by PGF$_{2\alpha}$. PGE$_1$ relaxed slightly both veins and arteries from dog and sheep. Human arteries usually contracted slightly and human veins usually relaxed slightly to PGE$_1$. In a limited number of experiments, swine arteries and veins failed to respond to PGF$_{2\alpha}$ or PGE$_1$. All the vascular strips contracted well when exposed to NE. These results suggest that the responses of intrapulmonary vessels to PGF$_{2\alpha}$ and PGE$_1$ are species-dependent. PGF$_{2\alpha}$ generally exhibits a contractile action, especially on veins. PGE$_1$ usually relaxes intrapulmonary vessels. With regard to vessels from man, PGF$_{2\alpha}$ is a powerful stimulant while PGE$_1$ produces only small, variable effects.

1. Kadowitz, P. J., Joiner, P. D., and Hyman, A. L., Annu. Rev. Pharmacol. **15**, 58 (1975).
2. Flower, R. J., Pharmacol. Rev. **26**, 33 (1974).
3. Furchgott, R. F., Methods Med. Res. **8**, 177 (1960).
4. Kadowitz, P. J., Joiner, P. D., and Hyman, A. L., Eur. J. Pharmacol. **31**, 72 (1975).
5. Van Rossum, J. R., Arch. Int. Pharmacodyn. Ther. **143**, 299 (1963).
6. Snedecor, G. E., "Statistical Methods," 5th ed. Iowa State Univ. Press, Ames (1956).
7. DuCharme, D. W., Weeks, J. R., and Montgomery, R. G., J. Pharmacol. Exp. Ther. **160**, 1 (1968).
8. Nakano, J., and Cole, B., Amer. J. Physiol. **217**, 222 (1969).
9. Giles, T. D., Quiroz, A. C., and Burch, G. E., Experientia **25**, 1056 (1969).
10. Anderson, F. L., Kralios, A. C., Tsagaris, T. J., and Kuida, H., Proc. Soc. Exp. Biol. Med. **140**, 1049 (1972).
11. Kadowitz, P. J., George, W. J., Joiner, P. D., and Hyman, A. L., Adv. Biosci. **9**, 501 (1973).

12. Kadowitz, P. J., Joiner, O. D., and Hyman, A. L., Proc. Soc. Exp. Biol. Med. **145,** 53 (1974).
13. Kadowitz, P. J., Joiner, P. D., and Hyman, A. L., Proc. Soc. Exp. Biol. Med. **145,** 1258 (1974).
14. Weir, E. K., Drogmueller, W., Greer, B. E., Smith, S. C., Reeves, J. T., and Grover, R. F., Circulation **50,** III-48 (1974).
15. Hyman, A. L., J. Pharmacol. Exp. Ther. **165,** 267 (1969).
16. Greenberg, S., Engelbrecht, J. A., and Wilson, W. R., Circ. Res. **34,** 491 (1974).
17. Kadowitz, P. J., Joiner, P. D., and Hyman, A. L., Proc. Soc. Exp. Biol. Med. **144,** 172 (1973).
18. Hillier, K., and Karim, S. M. M., J. Obstet. Gynaecol. Brit. Comm. **75,** 667 (1968).
19. Lewis, A. J., and Eyre, P., Prostaglandins **2,** 55 (1972).
20. Kadar, D., and Sunahara, F. A., Can. J. Physiol. Pharmacol. **47,** 871 (1969).
21. Dunham, E. W., Haddox, M. K., and Goldberg, N. D., Proc. Nat. Acad. Sci. U.S.A. **71,** 815 (1974).
22. Aiken, J. W., Pharmacologist **16,** 295 (1974).
23. Strong, C. G., and Bohr, D. F., Amer. J. Physiol. **213,** 725 (1967).
24. Khairallah, P. A., Page, I. H., and Turker, R. K., Arch. Int. Pharmacodyn. Ther. **169,** 328 (1967).

Received November 11, 1974. P.S.E.B.M. 1975, Vol. 150.