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Prostaglandins A_1, A_2 and 19-hydroxy A_1; their actions on smooth muscle and their inactivation on passage through the pulmonary and hepatic portal vascular beds

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(With an addendum by JUDY M. HOPKIN AND E. W. HORTON)

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1. Prostaglandins A_1, A_2 and 19-hydroxy A_1 have qualitatively similar actions to prostaglandin E_1 on smooth muscle.

2. The prostaglandins A have little activity on gastrointestinal, respiratory and reproductive smooth muscle but are potent depressors of the systemic arterial blood pressure of the dog, cat and rabbit.

3. Our experiments support the view that the depressor action of the prostaglandins E and A is due to a direct dilator action on many peripheral vascular beds and not due to changes in nervous tone to these beds.

4. A single passage through the pulmonary circulation of the cat or dog causes substantial loss of the vasodilator activity of prostaglandin E_1 but little if any loss of the vasodilator activity of the prostaglandins A.

5. A single passage through the hepatic portal circulation of the cat causes substantial loss of the vasodilator activity of prostaglandins E_1, A_1 and A_2.

6. The cat blood pressure and rat fundal strip would be a suitable combination for the parallel biological assay of the prostaglandins A_1 and A_2.

Prostaglandins A_1, A_2 and their 19-hydroxy derivatives are natural constituents of human seminal plasma (Hamberg & Samuelsson, 1966). Information concerning the pharmacological actions of these compounds, especially the 19-hydroxy derivatives, is limited. It is known that prostaglandins A_1 and A_2 are less active than prostaglandin E_1 on certain isolated smooth muscle preparations but still retain the potent depressor activity of prostaglandin E_1 (Bygdeman, Hamberg & Samuelsson, 1966 ; Lee, Covino, Takman & Smith, 1965 ; Pike, Kupiecki & Weeks, 1967 ; Bygdeman & Hamberg, 1967 ; Weeks, Chandra Sekhar & DuCharme, 1969). Prostaglandin A_1 is a powerful inhibitor of food-induced gastric secretion in the dog (Robert, Nezamis & Phillips, 1967), but possesses only weak anti-lipolytic activity (Bergström, Carlson & Orö, 1967 ; Daniels, Hinman, Johnson, Kupiecki, Nelson & Pike, 1965) and is less active than prostaglandin E_1 as an inhibitor of platelet aggregation (Kloeze, 1967).

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In this study we have determined the activity of prostaglandins A_1, A_2 and 19-hydroxy A_1 relative to prostaglandin E_1 on a selection of non-vascular smooth muscle and cardiovascular preparations. We have also investigated the role of the lung and liver in terminating the vasodilator actions of these prostaglandins.

Methods

Non-vascular smooth muscle preparations

Contractions of the majority of preparations were recorded isometrically with a force-displacement transducer (Grass FTO3) and a Beckman-Offner Dynograph). For a few preparations an isotonic frontal writing lever and a smoked drum were used.

Rabbit jejunum. Proximal jejunum from rabbits weighing 1 to 2 kg was suspended in Tyrode solution at 37° C gassed with air.

Rat fundal strip. A strip of longitudinal fundal stomach muscle from rats weighing 200–300 g was suspended in Tyrode solution at 37° C gassed with oxygen. When bretylium tosylate (10 μg/ml.) was added to the bathing solution the fundal strip was left to equilibrate for at least an hour.

Guinea-pig seminal vesicles. Seminal vesicles from guinea-pigs weighing 0·5–1·0 kg were suspended in Tyrode solution at 37° C and gassed with 95% oxygen and 5% carbon dioxide.

Guinea-pig ileum. Ileum from guinea-pigs weighing 200–800 g was suspended in Tyrode solution at 37° C gassed with air.

Chick crop strip. One to six day old chicks were starved overnight and then deeply anaesthetized with ether. A transverse strip of crop, about 3 mm in width, was removed and suspended in Krebs–Henseleit solution at 32° C gassed with 95% oxygen and 5% carbon dioxide (Everett, 1964).

Cat and guinea-pig tracheal chains. Portions of trachea were removed from guinea-pigs killed by stunning and bleeding out and from cats under pentobarbitone or ether anaesthesia. The tracheal chains (two rings from cat and six rings from guinea-pig tracheas) were suspended in Krebs–Henseleit solutions at 37° C and gassed with 95% oxygen and 5% carbon dioxide.

Rat uterus. Uterine horns from rats weighing 140–300 g injected subcutaneously 18 hr previously with stilboestrol 0·5 mg/kg were suspended in de Jalon solution at 31°–33° C and gassed with air.

Guinea-pig uterus. Uterine horns from guinea-pigs weighing 300–400 g were suspended in Krebs–Henseleit solution at 37° C gassed with 95% oxygen and 5% carbon dioxide.

Rabbit oviduct in vivo. Rabbits weighing 2·0–3·3 kg were anaesthetized with urethane (1·75 g/kg) injected into a marginal ear vein. The trachea was cannulated. A femoral vein was cannulated for intravenous injections. Blood pressure was recorded from a carotid artery with a Statham pressure transducer.

The abdomen was opened with a midline incision and a polyethylene cannula was inserted into the uterine end of the oviduct through an incision in the uterine horn and tied in position. Using a Palmer slow-injection apparatus the oviduct was perfused with Tyrode solution at a rate of 0·05–0·27 ml./min. The oviduct intraluminal pressure was recorded with a Statham pressure transducer P23AA.

Cat blood pressure assay

Pentobarbitone anaesthetized cats. Cats weighing 0·6–1·25 kg were anaesthetized with pentobarbitone sodium, 40 mg/kg, injected intraperitoneally. To maintain a constant depth of anaesthesia a solution of pentobarbitone sodium 5 mg/ml. in 0·9% NaCl solution was infused into a femoral vein by means of a Palmer slow injection apparatus. The rate of infusion varied from 0·25 to 6·5 mg/kg per hr in different experiments.

Spinal cats. Cats weighing 0·75–1·7 kg were anaesthetized with ethyl chloride and ether. A spinal section was made at C2 and the brain was destroyed. The cats were artificially ventilated with a Starling pump.

Arterial blood pressure was recorded from a carotid artery and prostaglandins were injected into an external jugular vein. Body temperature was maintained at 37° C by a rectal thermistor probe linked via a Sunvette temperature controller to the heating lamps of the operating table.

Cat cross-perfused hind limb

Donor cats weighing 3·8–4·2 kg and recipient cats weighing 2·7–3·0 kg were anaesthetized with pentobarbitone sodium, 40 mg/kg, injected intraperitoneally. The donor animal was placed approximately 10 cm lower than the recipient. Donor blood pressure was recorded from a carotid artery and recipient blood pressure from a brachial artery. The heart rate of the recipient was recorded with a Beckmann cardiotachometer coupler type 9857, triggered from the arterial pulse pressure. Blood was taken from a donor femoral artery by way of a Watson Marlow peristaltic pump and delivered into a recipient femoral artery. Blood was returned from the recipient femoral vein to a donor femoral vein under gravity. The donor cat was injected with heparin 1,000 u./kg. Hind limb perfusion pressure was recorded with a Statham pressure transducer P23Db.

In two experiments hind limb vessels proximal to the points of cannulation of the recipients' femoral artery and vein were ligated. In another experiment the skin and muscles of the upper thigh were sectioned with diathermy. A hole was drilled into the femur and the vascular connections within the bone destroyed with diathermy. During this procedure the exposed portions of the sciatic nerve and the sheath enclosing the femoral vessels and nerve were protected with cotton tapes soaked in 0·9% NaCl solution.

Prostaglandins were infused into an external jugular vein of the recipient cat using a Watson Marlow peristaltic pump.

Comparison of intra-aortic, intravenous and intraportal infusions of prostaglandins

Cats weighing 2·0–4·5 kg were anaesthetized with intraperitoneal pentobarbitone sodium, 40 mg/kg, and dogs weighing 6·0–9·4 kg with intravenous pentobarbitone sodium, 40 mg/kg.

The trachea was cannulated and a constant depth of anaesthesia was maintained by intravenous infusion of a 5 mg/ml. solution of pentobarbitone sodium in 0·9% NaCl solution using a Palmer slow injection apparatus. Blood pressure was recorded from a carotid artery.

The right external jugular vein was cannulated and the tip of the catheter was advanced to lie at the junction of the superior and inferior venae cavae. A femoral artery was cannulated retrogradely and the tip of the catheter was directed into the thoracic aorta. In some cats the thorax was opened along the line of the sternum, and a catheter inserted into the left atrium via a tributary of a pulmonary vein. Artificial ventilation was maintained throughout the experiment.

For intraportal infusions the abdomen was opened with a mid-line incision and a catheter inserted into a tributary of the splenic vein and advanced so that its tip lay in the portal vein.

The sciatic nerve was reached from the posterior surface of the thigh and sectioned. The femoral nerve and the sheath surrounding the femoral vessels were also sectioned. The hind limb vasculature was perfused at a constant flow rate using a Watson Marlow peristaltic pump and the perfusion pressure recorded. The cat was injected with heparin 500 u./kg. Prostaglandins were infused for 2 min by each of the three routes except in experiments 5 and 8, where the infusion time was 30 sec (see Table 4).

Source of prostaglandins

Prostaglandins E_1, A_1 and A_2 were kindly supplied by Dr. J. E. Pike, the Upjohn Company, Kalamazoo. 19-hydroxy prostaglandin A_1 containing about 30% 19-hydroxy prostaglandin B_1, and 19-hydroxy prostaglandin B_1 itself were prepared from human seminal plasma in this laboratory (see **Addendum**). Pure 19-hydroxy prostaglandin B_1 kindly supplied by Professor Bengt Samuelsson was used as a reference standard.

Stock solutions of prostaglandins containing 100 μg/ml. were made up in pH 6·5 0·9% NaCl solution and stored at $-15°$ C when not in use.

Results

Comparison of the prostaglandins A and prostaglandin E_1 on non-vascular smooth muscle

Table 1 gives the threshold doses of the prostaglandins A and their activities relative to prostaglandin E_1 on smooth muscle of the gastro-intestinal, respiratory, and male and female reproductive tracts. All non-vascular smooth muscle preparations investigated were less sensitive to prostaglandins A than prostaglandin E_1 and the relative activities ranged from 50 to 0·12%. The time courses of action of the prostaglandins A and prostaglandin E_1 were similar on all preparations, except for prostaglandin A_1 on the tracheal smooth muscle of the cat.

When dose-response curves to a prostaglandin A and prostaglandin E_1 on a particular preparation were obtained, they appeared to be parallel. Figure 1 shows the log dose-response curves to prostaglandins E_1, A_2 and A_1 on the rat fundal strip in the presence of bretylium tosylate 10 μg/ml. Prostaglandin E_1 was 10 times more potent than prostaglandin A_2 and 300 times more potent than prostaglandin A_1. The addition of bretylium tosylate, 10 μg/ml., to the bathing medium increased the sensitivity of the rat fundal strip to prostaglandins E_1, A_1 and A_2 by 6- to 10-fold.

Doses of prostaglandin A_1 and prostaglandin E_1 which were subthreshold for contraction of the isolated seminal vesicles of the guinea-pig potentiated the response of this preparation to both adrenaline and acetylcholine. Figure 2 shows that prostaglandin A_1 has approximately 1/300 of the activity of prostaglandin E_1.

TABLE 1. *Threshold doses and mean activities relative to prostaglandin E_1 of prostaglandins A_1, A_2, 19-OHA_1 and 19-OHB_1 on non-vascular smooth muscle preparations*

Preparation	Response	Mean activity relative to E_1=100	Threshold dose (ng/ml. and μg/kg)
Rat uterus	Contraction	4·1	600 (3)
Guinea-pig uterus	Contraction	11	20 (2)
Rabbit oviduct *in vivo*	Inhibition	4·0, < 5·0	25 (2)
Rat fundal strip A_1	Contraction	0·50	140 (4)
A_2		4·4	13 (3)
19-OH A_1		0·14	330 (2)
19-OH B_1		0·18	450 (2)
Guinea-pig ileum	Contraction	20	30 (6)
Rabbit jejunum A_1	Contraction	0·69	440 (4)
A_2		13	23 (3)
Chick crop strip	Contraction	1·4	150 (4)
Guinea-pig tracheal chain	Inhibition	< 0·15, < 0·50	>3,300 >6,500 (2)
Cat tracheal chain	Inhibition	3·4	1,000 (3)
Guinea-pig seminal vesicles	Potentiation	0·50	1,300 (4)

Values refer to prostaglandin A_1 unless otherwise stated. Figures in parentheses indicate number of estimates.

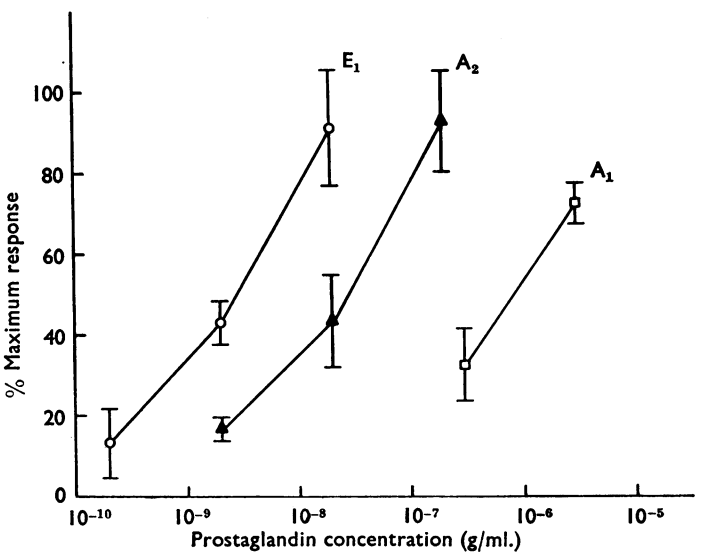

FIG. 1. Dose-response curves to prostaglandins E_1, A_2 and A_1 on the rat fundal strip. The Tyrode solution contained 10 μg/ml. bretylium tosylate. Each point represents the mean of three responses and the limits denote the limits of error at P=0·95.

Intravenous injection of 0·8–5 μg/kg prostaglandin E_1 into the anaesthetized rabbit reduced both intra-luminal pressure of the perfused oviduct and systemic arterial blood pressure. With prostaglandin A_1 the threshold dose for inhibition of the oviduct was 25 μg/kg, but this caused a substantial fall in the arterial blood pressure. In view of its potent depressor action higher doses of prostaglandin A_1 were not tested.

Both prostaglandin E_1 and A_1 inhibited the response to acetylcholine of isolated tracheal smooth muscle from the cat. The tracheal muscle was stimulated with acetylcholine at regular intervals and prostaglandins A_1 and E_1 were added to the organ bath 1 min before a dose of acetylcholine. The ensuing response to acetylcholine was inhibited by the prostaglandin and at the end of the acetylcholine contact time both prostaglandin and acetylcholine were washed out. Figure 3 shows the cumulative dose-response curves to acetylcholine on the cat tracheal chain in the presence of increasing concentrations of prostaglandin E_1 and prostaglandin A_1.

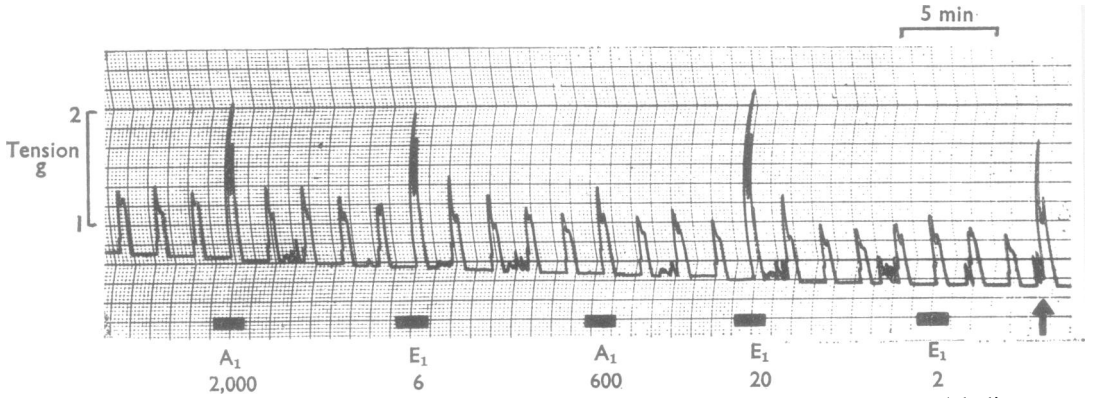

FIG. 2. Potentiation of the response of the guinea-pig isolated seminal vesicles to acetylcholine by prostaglandins E_1 and A_1. Acetylcholine chloride (200 ng) was added to the organ bath (5 ml.) for 30 sec every 5 min. Doses of prostaglandins (ng/ml.) were added to the organ bath 1 min before a dose of acetylcholine. At the arrow acetylcholine chloride (600 ng) was added to the bath.

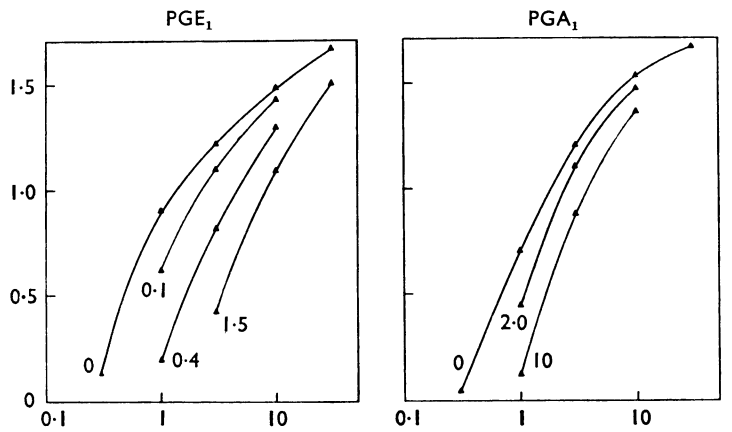

FIG. 3.—Cumulative dose-response curves to acetylcholine chloride on the cat tracheal chain preparation in the presence of prostaglandins E_1 and A_1. Figures adjacent to the curves refer to prostaglandin concentrations in μg/ml. Ordinate: tensions in g. Abscissa: acetylcholine concentration in μg/ml.

The antagonism by these prostaglandins resulted in a dose dependent shift to the right of the dose-response curves to acetylcholine. To produce a similar degree of inhibition the concentration of prostaglandin A_1 had to be some 30 times that of prostaglandin E_1.

The inhibition of the tracheal muscle was more prolonged with prostaglandin A_1 than with prostaglandin E_1, as shown in Fig. 4. The inhibition due to prostaglandin E_1 diminished to 50% of its initial magnitude $4{\cdot}2 \pm 0{\cdot}48$ min (nine determinations) after washout of the prostaglandin, compared with $13{\cdot}1 \pm 2{\cdot}2$ min (seven determinations) for prostaglandin A_1.

Cardiovascular preparations

Intravenous injection or infusion of the prostaglandins A into the pentobarbitone anaesthetized cat and dog and the urethane anaesthetized rabbit lowered the systemic arterial blood pressure. Table 2 shows the amounts of these prostaglandins required to produce a threshold effect.

In view of the high sensitivity of the cat to the depressor actions of prostaglandins A_1 and A_2, the cat blood pressure has been developed as an assay method. Pentobarbitone anaesthetized cats weighing about 0·8 kg and spinal cats weighing about 1·2 kg have been found satisfactory. Rapid intravenous injection of prostaglandins A_1 and A_2 into both preparations lowers the diastolic blood pressure to a greater extent than the systolic blood pressure. The fall in diastolic blood pressure in pentobarbitone anaesthetized cats is rapid both in onset and recovery and the fall in diastolic pressure in millimetres of mercury is a satisfactory measure of the response. Spinal cats, however, have a lower blood pressure than pentobarbitone anaesthetized cats and there is little curtailment of the depressor response by com-

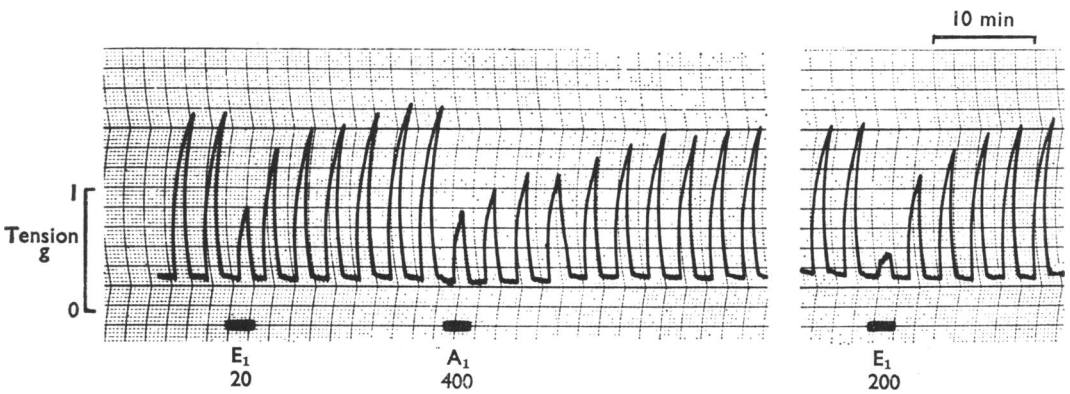

FIG. 4. Inhibition of the response of the cat isolated tracheal chain to acetylcholine chloride by prostaglandins E_1 and A_1. Acetylcholine chloride, 200 ng/ml., was added to the organ bath for 90 sec every 6 min to produce contractions of the tracheal muscle. Doses of prostaglandins E_1 and A_1 (ng/ml.) were added to the organ bath 1 min before a dose of acetylcholine.

TABLE 2. *Threshold depressor doses of prostaglandins E_1, A_1, A_2 and 19-OHA_1 on arterial blood pressure on intravenous infusion in the cat and dog (ng/kg per min) and intravenous injection in the rabbit (ng/kg)*

	E_1	A_1	A_2	19-OHA_1
Cat	180	45	8	250
Dog	440	370	110	—
Rabbit	1,000	6,300	—	—

pensatory mechanisms. Hence the area described by the fall in diastolic blood pressure yields a steeper dose-response curve in the spinal cat.

Both preparations gave measurable depressor responses to 5 to 10 ng of prostaglandin A_2 and 25 to 50 ng of prostaglandin A_1. The majority of cats showed a slight increase in sensitivity with time and were still suitable for assay of samples more than 12 hr after setting up.

Figure 5 shows the falls in blood pressure produced by intravenous injections of prostaglandins E_1, A_1, A_2, 19-hydroxy A_1 and 19-hydroxy B_1 in a spinal cat. Large secondary rises in blood pressure as illustrated in Fig. 5 were encountered in about one in three preparations, whether anaesthetized or spinal.

The dose-response curves to intravenous injections of prostaglandins E_1, A_1 and A_2 into a pentobarbitone anaesthetized cat are shown in Fig. 6. Doses of prostaglandin were given in random order at 10 min intervals. Each point represents the mean of three responses at a particular dose level. Analysis of variance reveals that the dose-response curves possess significant quadratic curvature at the 0·1% probability level and that the deviations from parallelism and the differences in quadratic curvature of the three curves are not significant at the 10% probability level.

On average, prostaglandin A_2 was about 20 times and prostaglandin A_1 about 5 times more potent than prostaglandin E_1. The 19-hydroxy A_1 which was extracted from human seminal plasma and which contained about 30% 19-hydroxy B_1 was slightly less active than prostaglandin E_1 in the pentobarbitone anaesthetized cat and slightly more active in the spinal cat. The depressor potency of the extract must be due to 19-hydroxy A_1 and not to the 19-hydroxy B_1, since 19-hydroxy B_1 itself has negligible depressor activity.

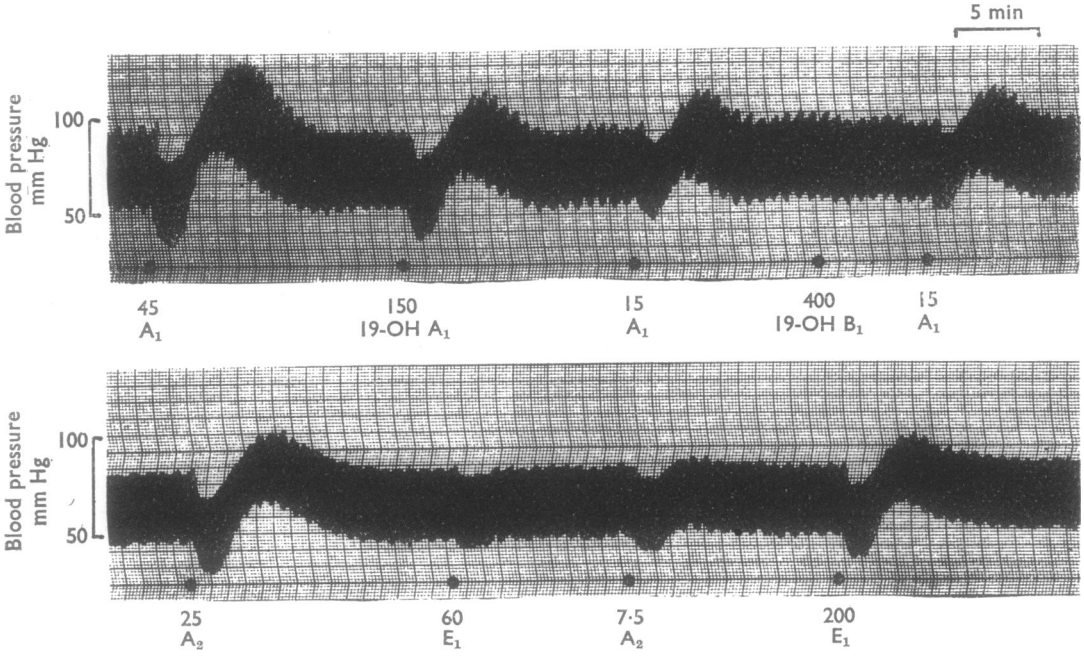

FIG. 5. Spinal cat, 0·83 kg. Responses to single intravenous injections of prostaglandins E_1, A_1, A_2, 19-OHA_1 and 19-OHB_1 on the carotid arterial blood pressure. Doses are in nanograms.

Mechanism of hind limb dilatation produced by intravenous and intra-arterial injections of prostaglandins

Intravenous infusions of prostaglandins E_1 and A_1 into the cat and dog caused a fall in both the systemic arterial blood pressure and the perfusion pressure of an auto-perfused hind limb. In the cat the mechanism of the hind limb vasodilatation was investigated using a cross-perfusion technique. The hind limb of a cat (recipient) was perfused at a constant flow rate with blood from a donor cat. As far as possible all nervous connections to the limb were left intact. Intravenous infusions of prostaglandins E_1 and A_1 into the recipient cat caused a fall in systemic arterial blood pressure and increase in heart rate of the recipient cat and a rise in hind limb perfusion pressure as shown in Fig. 7. However, close intra-arterial injections of prostaglandins A_1 and E_1 into the perfused hind limb resulted in a fall in perfusion pressure.

In one cat both cervical vagi were sectioned and the carotid bodies and sinuses were denervated by infusing 2% procaine hydrochloride solution around the

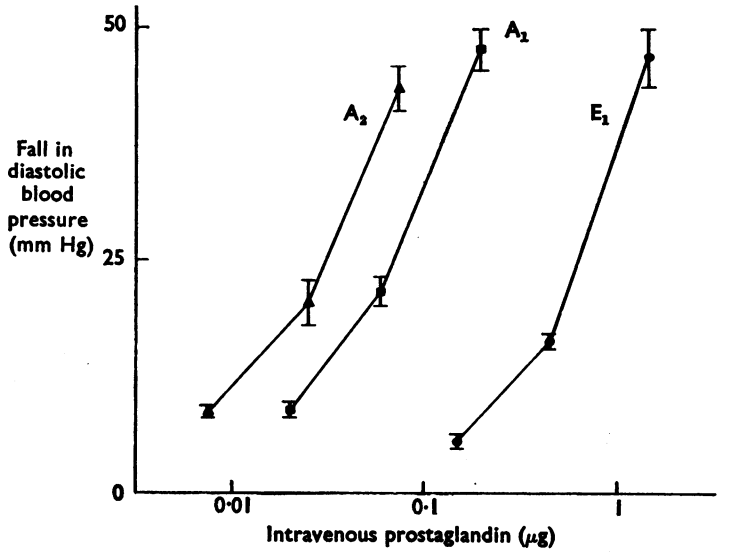

FIG. 6. Pentobarbitone-anaesthetized cat, 1·7 kg. Dose-response curves to prostaglandin A_2, A_1 and E_1 injected intravenously. Each point represents the mean of three responses and the limits denote the standard error of the mean. Ordinate: fall in diastolic pressure in mm Hg. Abscissa: dose of prostaglandin in μg.

TABLE 3. *Cat arterial blood pressure activities relative to E_1 (=1·0) and threshold doses of prostaglandins A_1, A_2, 19-OHA_1 and 19-OHB_1*

	Activity relative to E_1		Threshold dose (ng/kg)	
Prostaglandin	Spinal	Pentobarbitone	Spinal	Pentobarbitone
E_1	1·0	1·0	100 (6)	200 (5)
A_1	4·3 (2)	6·0 (6)	14 (2)	60 (5)
A_2	21·4 (6)	22·5 (4)	4·5 (6)	10 (4)
19-OHA_1	1·4 (2)	0·59 (2)	65 (2)	230 (2)
19-OHB_1	<0·06 (1)	0·004 (1)	>500 (1)	20,000 (1)

All compounds injected intravenously. Figures in parentheses indicate number of estimates.

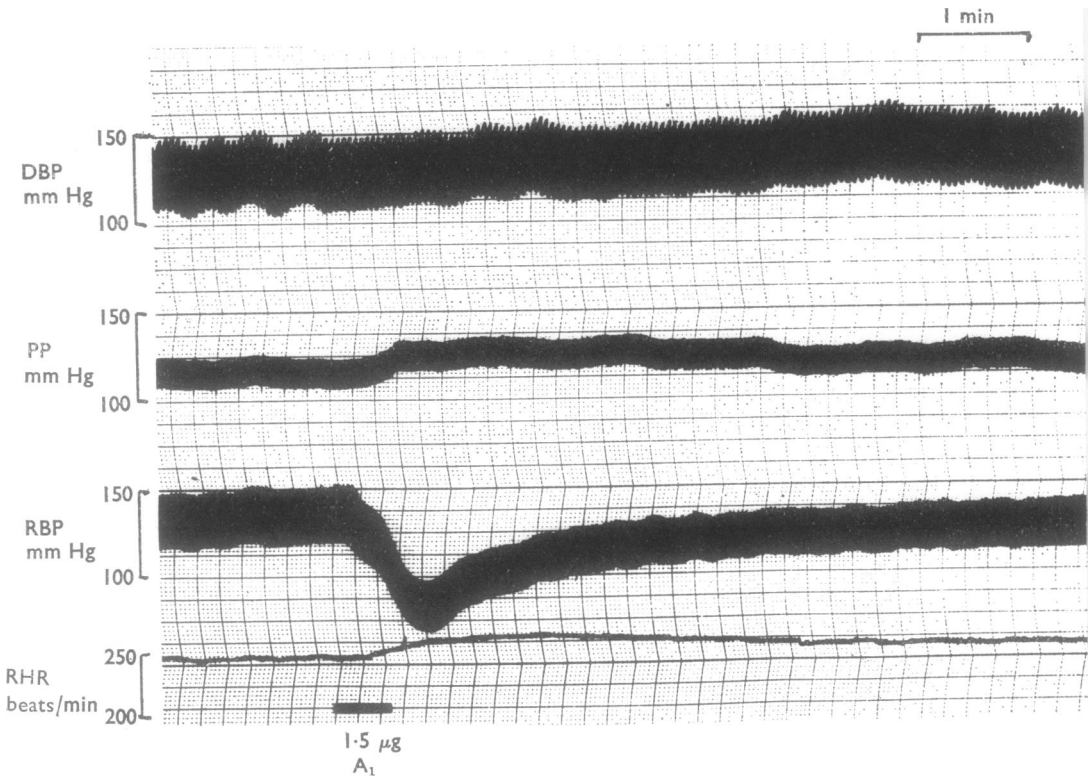

FIG. 7. Cat (2·7 kg) hind limb cross-perfusion. Traces from above downwards are donor arterial blood pressure (DBP); hind limb perfusion pressure (PP); recipient arterial blood pressure (RBP); and recipient heart rate (RHR). Prostaglandin A_1 (total dose 1·5 μg) was infused into a recipient external jugular vein.

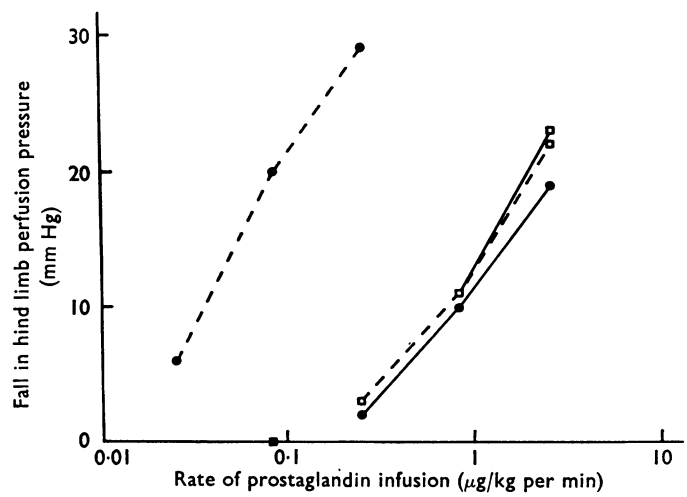

FIG. 8. Denervated auto-perfused hind limb of the dog. Comparison of intravenous (——) and intra-aortic (- - - -) infusions of prostaglandin E_1 (●) and prostaglandin A_1 (□).

carotid bifurcations. This treatment abolished the response to carotid occlusion. Intravenous infusion of prostaglandin still caused a fall in systemic arterial blood pressure but the rise in perfusion pressure was almost completely abolished and the increase in heart rate was diminished by 75%. It would therefore appear that the rises in perfusion pressure and heart rate are partly reflex responses to the fall in systemic arterial blood pressure.

We conclude that the vasodilatation of the hind limb blood vessels produced by intravenous prostaglandin is due to a direct action of the prostaglandin on the hind limb vessels and is not mediated via nerves supplying the hind limb vasculature.

TABLE 4. *Percentage loss of vasodilator activity on passage through the lungs and liver*

		E_1	A_1	A_2	19-OHA_1
Cat lung	Intravenous infusion rate (μg/kg per min)	0·1–1·1	0·03–0·4	0·006–0·06	0·8–2·5
	Expt. 1	99	15		
	Expt. 2	92	0	30	
	Expt. 3	81	10	10	
	Expt. 4	93 95	0 0		
	Expt. 5	99 98			0 0
	Expt. 6	97			0 0
	Expt. 7	95 95		0 0	
Cat liver	Intra-portal venous infusion rate	0·75–30	0·08–0·7	0·01–3·0	
	Expt. 1	55	73	70	
	Expt. 8	65		89	
Dog lung	Intravenous infusion rate	0·15–2·5	0·5–2·5	0·15–0·5	
	Expt. 9	73 75	10 13	0 12	
	Expt. 10	92 95	0 0		

Values in the first and second columns were calculated from arterial blood pressure and hind limb perfusion pressure respectively.

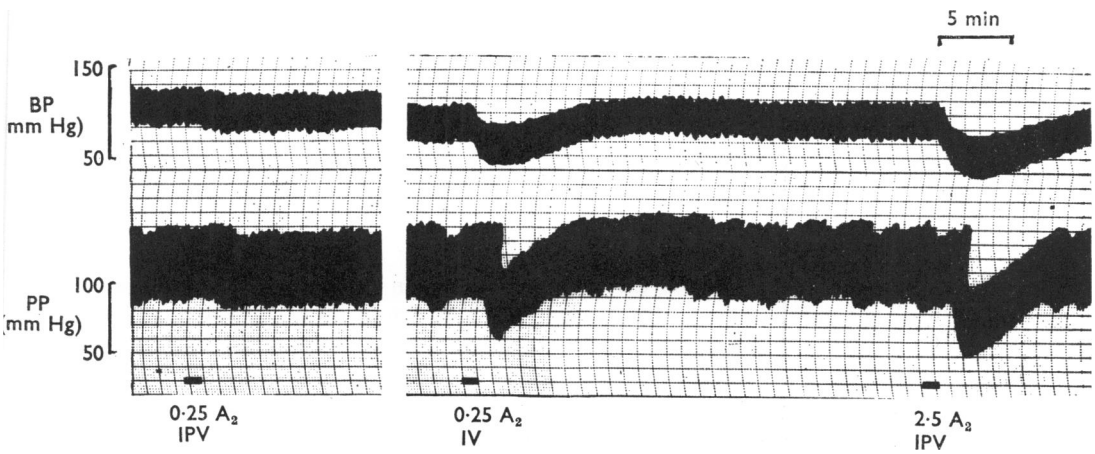

FIG. 9. Comparison of intravenous (IV) and intrahepatic portal venous (IPV) infusions of prostaglandin A_2 on the carotid arterial blood pressure (BP) and perfusion pressure (PP) of a denervated hind limb of a cat (3·2 kg). Doses are in μg.

Loss of vasodilator activity on passage through the liver and lungs of the cat and lungs of the dog

Intravenous infusions of prostaglandin E_1 and the prostaglandins A were compared with intra-aortic infusions on the arterial blood pressure and hind limb perfusion pressure of the cat and dog. To produce equivalent reductions of blood pressure and perfusion pressure the intravenous infusion rate of prostaglandin E_1 had to be 5 to 100 times the intra-aortic rate, whereas the intravenous infusion rate of a prostaglandin A had to be equal to or only slightly greater than the intra-aortic rate. Since we have shown that the hind limb vasodilatation is due to a direct action of the prostaglandin on the hind limb blood vessels, a single passage through the pulmonary circulation must result in a substantial loss of the vasodilator activity of prostaglandin E_1 but little if any loss of the vasodilator activity of a prostaglandin A.

The results of one experiment are shown in Fig. 8. To produce equivalent vasodilator responses of the perfused hind limb of the dog the intravenous infusion rate of prostaglandin E_1 must be about twenty times the intra-aortic rate, whereas the intravenous infusion rate of prostaglandin A_1 must be equal to the intra-aortic rate. This would indicate 95% loss of vasodilator activity of prostaglandin E_1 and no loss of vasodilator activity of prostaglandin A_1 during passage through the pulmonary circulation of the dog.

Table 4 shows that, irrespective of whether arterial blood pressure or hind perfusion pressure was used as the index of the arterial blood concentration of a prostaglandin, the losses of vasodilator activity of prostaglandin E_1 ranged from 73 to 99% and of the prostaglandins A from 0 to 30%, due to a single passage through the pulmonary circulations of the cat and dog.

Similarly intravenous and intraportal venous infusions of prostaglandins E_1, A_1 and A_2 have been compared on the arterial blood pressure and hind limb perfusion pressure of the cat. A single passage through the liver of the cat caused between 55 and 89% loss of vasodilator activity of prostaglandins E_1, A_1 and A_2. Fig. 9 shows part of an experiment to determine the loss of vasodilator activity of prostaglandin A_2 during a single passage through the hepatic portal circulation of the cat.

Discussion

Non-vascular smooth muscle

Dehydration of the β-ketol structure of prostaglandins E_1 and E_2 yields the corresponding prostaglandins A_1 and A_2. This change in structure results in decreased potency on isolated smooth muscle from the gastro-intestinal, respiratory and male and female reproductive tracts of a variety of species.

It has previously been reported that prostaglandin E_1 potentiates the response of the isolated guinea-pig seminal vesicles to adrenaline (Clegg, 1966) and acetylcholine and adrenaline (Eliasson & Risley, 1966). We have confirmed this action of prostaglandin E_1 and have further shown that high concentrations of prostaglandin A_1 potentiate the response to acetylcholine and adrenaline. Clegg stated that the potentiating effect of prostaglandin E_1 lasted up to 30 min, whereas Eliasson & Risley reported a rapid decline after washing. In our experiments the potentiations both to E_1 and A_1 were short lasting.

Prostaglandin A_1, like prostaglandins of the E and F series (Main, 1964 ; Horton & Main, 1965), has been shown to inhibit isolated tracheal smooth muscle of the cat. The mechanism of the longer lasting inhibition produced by prostaglandin A_1 has not been investigated.

Several groups of workers have determined the activity of prostaglandins A_1 and A_2 relative to prostaglandin E_1 on the isolated rabbit jejunum (Daniels *et al.*, 1965 ; Pike *et al.*, 1967 ; Bygdeman & Hamberg, 1967 ; Weeks *et al.*, 1969). The relative activities ranged from 0·02 to 8% for prostaglandin A_1 and 0·3 to 14% for prostaglandin A_2. In our experiments prostaglandin A_1 had 0·69% of the activity of E_1 and prostaglandin A_2, 13% of the activity of E_1 on this preparation.

The prostaglandins A are also less active than prostaglandin E_1 on non-vascular smooth muscle *in vivo*. Horton, Main & Thompson have shown that the tone of the rabbit oviduct is reduced by intravenous prostaglandins E (Horton, Main & Thompson, 1965 ; Horton & Main, 1963). They concluded that this effect was due to a direct action of the prostaglandins on the oviduct smooth muscle and not secondary to the accompanying fall in arterial blood pressure. This conclusion is confirmed by our finding that prostaglandin A_1 caused a substantial fall in arterial blood pressure but had little effect on the oviduct.

The smooth muscle stimulating activity of prostaglandin A_1 on the rat fundal strip was reduced by 3 to 4-fold by the substitution of a hydroxyl group at the 19 position in the molecule. This diminished potency may be applicable to non-vascular smooth muscle preparations in general, as Bygdeman & Hamberg (1967) have shown that the 19-hydroxy prostaglandins A and B are less active than the prostaglandins A and B on the isolated human uterus and isolated rabbit jejunum (Bygdeman, Hamberg & Samuelsson, 1966 ; Bygdeman & Hamberg, 1967).

Depressor actions of the prostaglandins A

Prostaglandin A_1 in the dog (Bergström *et al.*, 1967) and prostaglandins A_1 and A_2 in the rat and dog (Weeks *et al.*, 1969) have been shown to be potent blood pressure lowering agents. We have confirmed these results in the dog and also shown that the cat and rabbit are sensitive to the depressor actions of the prostaglandins A.

Intravenous injections of prostaglandins E_1 (Nakano & McCurdy, 1967) and E_1, A_1 and A_2 (Lee *et al.*, 1965 ; Weeks *et al.*, 1969) into the dog increase cardiac output and lower arterial blood pressure. Also close intra-arterial injections of prostaglandin E_1 have been shown to decrease the resistance of the vascular beds supplied by the carotid, femoral, brachial, coronary and renal arteries of the dog (Nakano & McCurdy, 1967) and the hind limb vasculature of the cat (Holmes, Horton & Main, 1963) and the rabbit (Beck, Pollard, Kayaalp & Weiner, 1966). These decreases in vascular resistance were not affected by atropine, tripelennamine or propranolol (Nakano & McCurdy, 1967 ; Smith, McMorrow, Covino & Lee, 1967). It can be concluded from the above results that prostaglandins E and A lower arterial blood pressure primarily by a direct vasodilator action on many peripheral vascular beds. The results of the hind limb perfusion experiments performed in this study support this conclusion. Furthermore, we have shown

that prostaglandins E_1 and A_1 do not cause a vasodilatation of the hind limb blood vessels of the cat by a change in nervous tone to these vessels.

Lung and liver inactivation of the prostaglandins A and E

Using the blood bathed organ technique, Ferreira & Vane (1967) have shown that a single passage through the pulmonary circulation results in the loss of more than 95% of the smooth muscle stimulating activity of prostaglandin E_2 in the cat and 95% of the smooth muscle stimulating activity of prostaglandin E_1 in the dog. In addition the liver of the cat was shown to cause the loss of about 80% of the smooth muscle stimulating activity of prostaglandin E_2.

In this study, substantial losses of peripheral vasodilator activity of prostaglandin E_1 on passage through the lung and liver circulation of the cat and dog have been found and the % losses agree well with those recorded by Ferreira & Vane for loss of smooth muscle stimulating activity. However, passage through the pulmonary circulation of the cat and dog caused little or no loss of the vasodilator activity of prostaglandins A_1, A_2 and 19-hydroxy A_1.

Prostaglandins of the E and F series have been found in many tissues and their release on nervous and chemical stimulation of a number of organs, including the dog spleen (Davies, Horton & Withrington, 1968 ; Ferreira & Vane, 1967), the rat stomach (Bennet, Friedmann & Vane, 1967 ; Coceani, Pace-Asciak, Volta & Wolfe, 1967) and cat and rat adrenals (Ramwell, Shaw, Douglas & Poisner, 1966 ; Shaw & Ramwell, 1967) has been demonstrated. In contrast, prostaglandins A_1 and A_2 and their 19-hydroxy derivatives have only been positively identified in human seminal plasma (Hamberg & Samuelsson, 1967). Prostaglandin A_2 was reported to be present in an extract of rabbit kidney medulla (Lee *et al.*, 1965 ; Lee, Gougoutas, Takman, Daniels, Grostic, Pike, Hinman & Muirhead, 1966), but was probably formed from prostaglandin E_2 during the extraction procedure (Strong, Boucher, Nowaczynski & Genest, 1966). It remains to be shown whether there are other tissue stores of the prostaglandins A and whether they are released on nervous and chemical stimulation. If a prostaglandin A were released into venous blood then this prostaglandin would lose little vasodilator activity on passage through the lungs and could have an effect on target organs and especially on the tone of the arterioles. The amounts of prostaglandin required to be released would be relatively small. For example, the intravenous infusion rate of prostaglandin A_2 for a threshold effect on the systemic arterial blood pressure of one cat was 4 ng/kg per min. If a cardiac output of 100 ml./kg per min is assumed then the venous blood concentration of prostaglandin A_2 must have been 0·04 ng/ml.

The nature of the loss of vasodilator or smooth muscle stimulating activity of the prostaglandins during passage through the pulmonary circulation is unknown, but enzymic conversion would appear to be the most likely mechanism. The presence in guinea-pig and swine lung homogenates of enzymes capable of metabolizing prostaglandins supports this suggestion (Änggård, Gréen & Samuelsson, 1965 ; Änggård & Samuelsson, 1965, 1966, 1967). The enzyme from swine lung has been isolated in pure form and has been shown to oxidize the 15-hydroxy of a prostaglandin to a keto group. It is interesting to note that this 15-hydroxy prostaglandin dehydrogenase oxidizes prostaglandins A_1, A_2 and 19-hydroxy A_1 at a slower rate than prostaglandins E_1 and E_2 (Änggård & Samuelsson, 1966, 1967).

Biological assay of prostaglandins A_1 and A_2

Column and thin-layer chromatography can be used to separate prostaglandins in tissue extracts. The least polar substances, prostaglandins A_1 and A_2, are eluted first, followed by prostaglandins of the E series followed by the F series. A biological assay system for the prostaglandins A_1 and A_2 must therefore detect small amounts of these prostaglandins and distinguish them from prostaglandins of the E series.

Three preparations are available for the biological assay of the prostaglandins A_1 and A_2, namely the cat and rat blood pressures and the rat fundal strip.

The cat blood pressure will detect 5–10 ng of prostaglandin A_2 and 25–50 ng of prostaglandin A_1 and is 20 and 5 times more sensitive to prostaglandins A_2 and A_1 than prostaglandin E_1 respectively. Coceani & Wolfe (1966) have shown that the rat fundal strip is sensitized to prostaglandin E_2 by the addition of bretylium to the bathing medium. We have shown that this potentiation extends to prostaglandins A_1 and A_2, and in these conditions the fundal strip will detect between 10 and 20 ng of prostaglandin A_2, between 100 and 200 ng of prostaglandin A_1 and less than 1 ng of prostaglandin E_1. The rat blood pressure has been used for the assay of renal depressor lipids (Hickler, Lauler, Saravis, Vagnucci, Steiner & Thorn, 1964 ; Lee *et al.*, 1965 ; Strong *et al.*, 1966). This preparation will detect about 250 ng of prostaglandins A_1 and A_2 and is approximately 3 times more sensitive to prostaglandin E_1 than prostaglandins A_1 or A_2.

Therefore, the cat blood pressure and rat fundal strip will detect small quantities of the prostaglandins A_1 and A_2 and by parallel biological assay on these preparations a prostaglandin A can be distinguished from a prostaglandin E, Gaddum's index of discrimination falling between 500 and 1,000 (Horton & Jones, 1969).

REFERENCES

ÄNGGÅRD, E., GRÉEN, K. & SAMUELSSON, B. (1964). Synthesis of tritium labelled prostaglandin E_2 and studies on its metabolism in guinea-pig lung. *J. biol. Chem.*, **240**, 1932–1940.

ÄNGGÅRD, E. & SAMUELSSON, B. (1965). Biosynthesis of prostaglandins from arachidonic acid in guinea-pig lung. *J. biol. Chem.*, **240**, 3518–3521.

ÄNGGÅRD, E. & SAMUELSSON, B. (1966). Purification and properties of a 15-hydroxyprostaglandin dehydrogenase from swine lung. *Arkiv Kemi*, **25**, 293–300.

ÄNGGÅRD, E. & SAMUELSSON, B. (1967). The metabolism of prostaglandins in lung tissue. In *Prostaglandins*, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. Bergström, S. & Samuelsson, B., pp. 97–106. Stockholm: Almqvist & Wiksell.

BECK, L., POLLARD, A. A., KAYAALP, S. O. & WEINER, L. M. (1966). Sustained dilatation elicited by sympathetic nerve stimulation. *Fedn Proc.*, **25**, 1596–1606.

BENNET, A., FRIEDMANN, C. A. & VANE, J. R. (1967). The release of prostaglandin E_1 from rat stomach. *Nature, Lond.*, **216**, 873–876.

BERGSTRÖM, S., CARLSON, L. A. & ORÖ, L. (1967). A note on the cardiovascular and metabolic effects of prostaglandin A_1. *Life Sci., Oxford*, **6**, 449–455.

BYGDEMAN, M. & HAMBERG, M. (1967). The effect of eight new prostaglandins on human myometrium. *Acta physiol. scand.*, **69**, 320–326.

BYGDEMAN, M., HAMBERG, M. & SAMUELSSON, B. (1966). The content of different prostaglandins in human seminal fluid and their threshold doses on the human myometrium. *Mem. Soc. Endocrinol.*, **14**, 49–64.

CLEGG, P. C. (1966). The effect of prostaglandins on the response of isolated smooth muscle preparations to sympathomimetic substances. *Mem. Soc. Endocrinol.*, **14**, 119–136.

COCEANI, F., PACE-ASCIAK, C., VOLTA, F. & WOLFE, L. S. (1967). Effect of nerve stimulation on prostaglandin formation and release from the rat stomach. *Am. J. Physiol.*, **213**, 1056–1064.

COCEANI, F. & WOLFE, L. S. (1966). On the action of prostaglandin E_1 and prostaglandins from brain on the isolated rat stomach. *Can. J. Physiol. Pharmac.*, **44**, 933–950.

DANIELS, E. G., HINMAN, B. A., JOHNSON, B. A., KUPIECKI, F. P., NELSON, J. W. & PIKE, J. E. (1965). The isolation of an additional prostaglandin derivative from the enzymatic cyclization of homo-γ-linolenic acid. *Biochem. biophys. res. Comm.*, **21**, 413–417.

DAVIES, B. N., HORTON, E. W. & WITHRINGTON, P. G. (1968). The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. *Br. J. Pharmac. Chemother.*, **32**, 127–135.

ELIASSON, R. & RISLEY, P. L. (1966). Potentiated response of isolated seminal vesicles to catecholamines and acetylcholine in the presence of PGE_1. *Acta physiol. scand.*, **67**, 253–254.

EVERETT, S. D. (1964). A sensitive preparation for the assay of 5-hydroxytryptamine. *J. Pharm. Pharmac.*, **16**, 767–768.

FERREIRA, S. H. & VANE, J. R. (1967). Prostaglandins: their disappearance from and release into the circulation. *Nature, Lond.*, **216**, 868–873.

HAMBERG, M. & SAMUELSSON, B. (1966). Prostaglandins in human seminal plasma. *J. biol. Chem.*, **241**, 257–263.

HAMBERG, M. & SAMUELSSON, B. (1967). New groups of naturally occurring prostaglandins. In *Prostaglandins*, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. Bergström, S. & Samuelsson, B., pp. 63–70. Stockholm: Almqvist & Wiksell.

HICKLER, R. B., LAULER, D. P., SARAVIS, C. A., VAGNUCCI, A. I., STEINER, G. & THORN, G. W. (1964). Vasodepressor lipid from the renal medulla. *Can. med. Ass. J.*, **90**, 280–287.

HOLMES, S. W., HORTON, E. W. & MAIN, I. H. M. (1963). The effect of prostaglandin E_1 on responses of smooth muscle to catecholamines, angiotensin and vasopressin. *Br. J. Pharmac. Chemother.*, **21**, 538–543.

HORTON, E. W. & JONES, R. L. (1969). The biological assay of prostaglandins A_1 and A_2. *J. Physiol., Lond.*, **200**, 56–57*P*.

HORTON, E. W. & MAIN, I. H. M. (1963). A comparison of the biological activities of four prostaglandins. *Br. J. Pharmac. Chemother.*, **21**, 182–189.

HORTON, E. W. & MAIN, I. H. M. (1965). A comparison of the actions of prostaglandins F_{2a} and E_1 on smooth muscle. *Br. J. Pharmac. Chemother.*, **24**, 470–476.

HORTON, E. W., MAIN, I. H. M. & THOMPSON, C. J. (1965). Effects of prostaglandins on the oviduct, studied in rabbits and ewes. *J. Physiol., Lond.*, **180**, 514–528.

KLOEZE, J. (1967). Influence of prostaglandins on platelet adhesiveness and platelet aggregation. In *Prostaglandins*, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. Bergström, S. & Samuelsson, B., pp. 241–252. Stockholm: Almqvist & Wiksell.

LEE, J. B., COVINO, B. G., TAKMAN, B. H. & SMITH, E. R. (1965). Renomedullary vasodepressor substance, medullin: Isolation, chemical characterisation and physiological properties. *Circulation Res.*, **17**, 57–77.

LEE, J. B., GOUGOUTAS, J. Z., TAKMAN, B. H., DANIELS, E. G., GROSTIC, M. F., PIKE, J. E., HINMAN, J. W. & MUIRHEAD, E. E. (1966). Vasodepressor and antihypertensive prostaglandins of the PGE type with emphasis on the identification of medullin as PGE_2–217. *J. clin. Invest.*, **45**, 1036.

MAIN, I. H. M. (1964). The inhibitory actions of prostaglandins on respiratory smooth muscle. *Br. J. Pharmac. Chemother.*, **22**, 511–519.

NAKANO, J. & MCCURDY, J. R. (1967). Cardiovascular effects of prostaglandin E_1. *J. Pharmac. exp. Ther.*, **156**, 538–547.

PIKE, J. E., KUPIECKI, F. P. & WEEKS, J. R. (1967). Biological activity of the prostaglandins and related analogs. In *Prostaglandins*, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. Bergström, S. & Samuelsson, B., pp. 161–172. Stockholm: Almqvist & Wiksell.

RAMWELL, P. W., SHAW, J. E., DOUGLAS, W. W. & POISNER, A. M. (1966). Efflux of prostaglandin from adrenal glands stimulated with acetylcholine. *Nature, Lond.*, **210**, 273–274.

ROBERT, A., NEZAMIS, J. E. & PHILLIPS, J. P. (1967). Inhibition of gastric secretion by prostaglandins. *Am. J. dig. Dis.*, **12**, 1073–1076.

SHAW, J. E. & RAMWELL, P. W. (1967). Prostaglandin release from the adrenal glands. In *Prostaglandins*, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. Bergström, S. & Samuelsson, B., pp. 293–299. Stockholm: Almqvist & Wiksell.

SMITH, E. R., MCMORROW, J. V., JR., COVINO, B. G. & LEE, J. B. (1967). Mechanism of the hypotensive and vasodilator action of prostaglandin E_1. *Clin. Res.*, **15**, 222.

STRONG, C. G., BOUCHER, R., NOWACZYNSKI, N. & GENEST, J. (1966). Renal vasodepressor lipid. *Proc. Staff Meeting Mayo Clin.*, **41**, 433–452.

WEEKS, J. R., CHANDRA SEKHAR, N. & DUCHARME, D. W. (1969). Relative activity of prostaglandins E_1, E_2, A_1 and A_2 on lipolysis, platelet aggregation, smooth muscle and the cardiovascular system. *J. Pharm. Pharmac.*, **21**, 103–108.

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Addendum overleaf

Addendum

The extraction of 19-hydroxy-prostaglandins from human seminal plasma

Human semen was obtained from the fertility clinic at the Chelsea Hospital for Women through the kind co-operation of Dr. I. F. Somerville. The method of extracting 19-hydroxy-prostaglandins was a modification of that described by Hamberg & Samuelsson (1967). The semen was diluted with an equal volume of water, adjusted to pH 3·0 by the addition of hydrochloric acid and partitioned twice with an equal volume of ethyl acetate.

The combined ethyl acetate phases were pooled and evaporated to smaller volume and partitioned twice with a pH 8·0 phosphate buffer. The aqueous phases were pooled, adjusted to pH 3·0 by the addition of hydrochloric acid and partitioned twice with an equal volume of ethyl acetate. The pooled ethyl acetate phases were evaporated to dryness. The residue was partitioned twice between equal volumes of 67% aqueous ethanol and heavy petroleum. The aqueous ethanol phase was evaporated to dryness.

The residue was taken up in about 5 ml. of 10% ethyl acetate in benzene and loaded on to a 10 g silicic acid column. The column was developed with increasing concentrations of ethyl acetate, fractions were collected, evaporated to dryness and their prostaglandin content estimated by biological assay on the rat fundus or by absorption at 217 mμ (prostaglandins A) and 278 mμ (prostaglandins B). The 19-hydroxy prostaglandins were eluted with 80% ethyl acetate in benzene a little ahead of but overlapping the prostaglandin F (Table 1).

Fraction 5 from this column was further purified by preparative thin-layer chromatography using the AI system of Gréen & Samuelsson (1964) to separate the 19-hydroxy prostaglandins from prostaglandin $F_{1\alpha}$ and $F_{2\alpha}$. The zone corresponding to the 19-hydroxy prostaglandins was separated and the eluted prostaglandins were chromatographed using the AII solvent system of Gréen & Samuelsson (1964), which separated the 19-hydroxy prostaglandins A_1 and B_1 from the 19-hydroxy prostaglandins A_2 and B_2.

Pure 19-hydroxy prostaglandins B_1 and B_2 were prepared by treating the mixture of 19-hydroxy prostaglandin A_1 and B_1 and the mixture of 19-hydroxy prostaglandin A_2 and B_2 separately with 0·5 N NaOH in 50% aqueous ethanol to convert the prostaglandin A into the corresponding B. These purified 19-hydroxy prostaglandins B_1 and B_2 were standardized spectrophotometrically against

TABLE 1. *Prostaglandin content of fractions from a silicic acid column loaded with extract derived from 374 ml. human semen, as described in the text*

No.	% Ethyl acetate in benzene	Fraction volume (ml.)	Biological activity (rat fundus)	Absorption at 217 mμ	Absorption at 278 mμ
1	30	100	≡0·04 mg E_1		
2	40	150	≡0·02 mg E_1		
3	40	450	≡4·0 mg E_1		
4	40	100	≡0·2 mg E_1		
5	80	100	≡0·6 mg $F_{2\alpha}$	≡3·25 mg A_1	≡2·75 mg B_1
6	80	450	≡2·0 mg $F_{2\alpha}$		≡0·12 mg B_1
7	80	100	≡0·4 mg $F_{2\alpha}$		≡0·06 mg B_1
8	100	100	≡0·08 mg E_1		
9	Methanol	100	≡0·8 mg E_1		

authentic samples of these compounds kindly supplied by Professor Bengt Samuelsson. All attempts to separate prostaglandin A_1 or its 19-hydroxy derivative from the corresponding prostaglandin B by thin-layer chromatography were unsuccessful. The following systems were investigated: Gréen & Samuelsson's solvent system I to X inclusive and the system of Horton & Thompson (1964) using silica gel plates with and without added silver nitrate. Furthermore, on reversed phase chromatography using Hyflo-Supercel no significant separation of prostaglandin A_1 from B_1 or of 19-hydroxy prostaglandin A_1 from 19-hydroxy prostaglandin B_1 could be achieved.

The 19-hydroxy prostaglandin B_1, 19-hydroxy prostaglandin B_2 and 19-hydroxy prostaglandin A_1 (containing 19-hydroxy prostaglandin B_1) separated by the method described above were used for investigations of their biological activities.

REFERENCES

GRÉEN, K. & SAMUELSSON, B. (1964). Prostaglandins and related factors: XIX. Thin-layer chromatography of prostaglandins. *J. lipid Res.*, **5**, 117–120.

HAMBERG, M. & SAMUELSSON, B. (1967). New groups of naturally occurring prostaglandins. In *Prostaglandins*, Proc. 2nd Nobel Symp., Stockholm, June 1966, ed. Bergström, S. & Samuelsson, B., pp. 63–70. Stockholm: Almqvist & Wiksell.

HORTON, E. W. & THOMPSON, C. J. (1964). Thin-layer chromatography and bioassay of prostaglandins in extracts of semen and tissues of the male reproductive tract. *Br. J. Pharmac. Chemother.*, **22**, 183–188.

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