Output of prostaglandins from the rabbit kidney, its increase on renal nerve stimulation and its inhibition by indomethacin

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Summary

1. In each of six experiments, prostaglandins were identified in the renal venous blood of the rabbit. The concentrations in renal venous blood were up to 45 times higher than in aortic blood, suggesting that most of the prostaglandins originate from the kidney.

2. Prostaglandins E_2, $F_{2\alpha}$ and a prostaglandin of the A, C or B series were estimated by biological assay after solvent partition and column or thin-layer chromatography.

3. Prostaglandins E_2 and $F_{2\alpha}$ were identified conclusively by combined gas chromatography-mass spectrometry.

4. Electrical stimulation of the renal nerve increased the output of prostaglandins.

5. Indomethacin (10 mg/kg) injected intravenously, reduced the output of prostaglandins into renal venous blood and prevented the increase in output on renal nerve stimulation.

Introduction

Prostaglandins A_2, E_2 and $F_{2\alpha}$ have been identified in high concentrations in the rabbit renal medulla (Lee, Crowshaw, Takman, Attrep & Gougoutas, 1967; Daniels, Hinman, Leach & Muirhead, 1967). We now report the mass spectrometric identification of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in renal venous blood of the rabbit. The output of these prostaglandins and of a prostaglandin A, C or B-like substance was increased by renal nerve stimulation and reduced by indomethacin.

Methods

Surgical procedure

Six experiments were performed using male rabbits weighing 1·5–3 kg. In each experiment, 3 rabbits, all litter-mates, were used. Anaesthesia was induced with 25% urethane (1·75 g/kg) injected into the marginal ear vein.

Two donor rabbits provided blood for the third rabbit. Heparin (1 i.u./g) was administered intravenously, then blood was collected from the abdominal aorta in polyethylene tubes.

In the third rabbit, the trachea, right external jugular vein and right femoral vein were cannulated. Blood pressure (1 mmHg≡1·333 mbar) was monitored from the

left carotid artery using a Statham pressure transducer (P.23 Dc) connected to a Grass polygraph. The abdomen was opened along the midline and the left renal vein and nerve were cleared. Heparin (1 i.u./g) was then injected intravenously into the rabbit and the left renal vein cannulated. This cannula was connected to a Y-piece, one arm of which was attached to the cannula in the right femoral vein and the other arm was connected to polyethylene tubing which allowed for the collection of renal venous blood samples. After cannulation of the renal vein, the 'collection side arm' was clamped and blood was directed from the kidney into the femoral vein. Heparinized blood (10 ml) was then slowly infused by a Watson–Marlow pump into the right external jugular vein of the rabbit, in order to compensate for the blood in the renal-femoral vein circuit. The renal nerve was cut 2 cm from the kidney and wound around platinum electrodes which were connected to a Grass stimulator (SD5). The nerve and kidney were bathed in a pool of liquid paraffin. The rabbit was maintained at a temperature of 37° C.

All cannulae inserted into blood vessels in these experiments were filled with a solution containing heparin (50 i.u./ml), except for the carotid artery and abdominal aorta cannulae, which were filled with a more concentrated solution (500 i.u./ml).

After surgery, a period of 30 min was allowed before collecting blood samples.

Collection of blood samples

The time periods for renal venous blood sample collections and the volumes of the samples are given in Table 2. During the collection of all blood samples, the right femoral venous cannula was clamped and blood was directed from the kidney into an ice-cold measuring cylinder.

A control sample of renal venous blood was initially collected. This was followed by a 5 min period during which blood was directed from the renal vein into the femoral vein.

In experiments 1 and 2 (Table 2), a test sample was then collected. During this period, the renal nerve was stimulated at 10 V, frequency 10 Hz and duration 0·5 ms. In experiment 2, the renal nerve was stimulated for a further 20 min and a second test sample (Test 2) was collected in the last 5 min of this 20 min stimulation period. A 20 min period was then allowed during which renal venous blood was directed into the femoral vein. This period allowed time for the kidney to revert to 'pre-test' conditions before a post-test sample was collected. In experiments 3, 4, 5 and 6, the renal nerve was stimulated (as above) for 10 min before a test sample was collected. A 15 min period was then allowed during which renal venous blood was directed into the femoral vein.

After the collection of the test sample in experiment 3, 2 ml of polyethylene glycol 200 (Analar) and 1 ml of 0·9% saline were mixed and slowly injected into the right external jugular vein. A 30 min period was then allowed before collecting a control polyethylene glycol sample (Control Glycol). After the collection of this sample, the renal nerve was stimulated (as above) for 10 min, before collecting a test polyethylene glycol sample (Test Glycol). A 15 min period was then allowed during which renal venous blood was directed into the femoral vein.

In experiment 3, after collection of the test glycol sample, and in experiments 4, 5 and 6, after collection of the test sample, indomethacin (10 mg/kg) dissolved in 2 ml of polyethylene glycol 200 and 1 ml of 0·9% saline was slowly injected into the

right external jugular vein. In experiment 4, a 10 min period, but in experiments 3, 5 and 6, a 30 min period was allowed before collecting a control indomethacin sample (Control Ind.). After collection of this sample, the renal nerve was stimulated (as above) for 10 min before collecting a test indomethacin sample (Test Ind.).

In experiment 6 (Table 2), a 20 ml blood sample was collected from the abdominal aorta, before collection of the initial renal venous control blood sample.

During the collection of all blood samples, heparinized blood at $37°$ C, from the donor animals, was infused into the right external jugular vein. Blood was infused at a slightly faster rate than it was being removed. The blood pressure, therefore, remained fairly constant.

After collection, $0·1$ μCi each of (5,6-^{3}H$_2$) PGA$_1$, (5,6-^{3}H$_2$) PGE$_1$ and (9-^{3}H$_2$) PGF$_{2\alpha}$ were added to each blood sample. This radioactivity ($0·1$ μCi) represented 100 ng PGA$_1$, 150 ng PGE$_1$ and $3·3$ ng PGF$_{2\alpha}$. Two volumes of $0·9\%$ saline at $0°$ C were then added to the blood samples. The samples were kept at $0°$ C until extraction.

Prostaglandin solvent extraction procedure

The diluted blood samples were centrifuged for 10 min at 1,000 g and $4°$ C. The plasma/saline was separated from the cells and stored at $0°$ C, while the cells were washed twice with an equal volume of $0·9\%$ saline at $0°$ C. The saline was centrifuged from the cells and bulked with the plasma.

The plasma/saline samples were adjusted to pH $4·5$ with glacial acetic acid, then extracted by the methods illustrated in Figure 1.
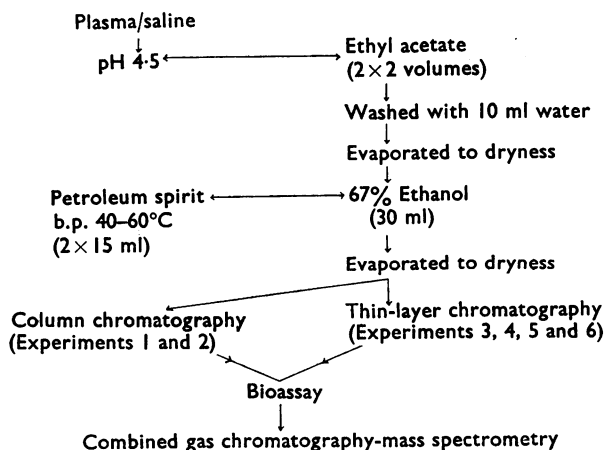

FIG. 1. Outline of extraction procedure.

Silicic acid column chromatography

Silic acid, $4·5$ g (Sigma, Sil-R, 100 mesh) was used for 1×10 cm glass columns. It was activated by heating at $110°$ C for 1 hour. After cooling, it was suspended in 10% ethyl acetate in toluene and poured into the column. The dried residue from the solvent extraction procedure was dissolved in 1 ml of 10% ethyl acetate in toluene and applied to the column with a Pasteur pipette. The residue flask was then washed with five 2 ml portions of 10% ethyl acetate in toluene and these separate washings were applied dropwise to the column. Thus, 10 ml of the first

80 ml of 10% ethyl acetate in toluene was used for transferring activity from the residue flask on to the column.

The columns were eluted under reduced pressure at a rate of approximately 1 ml/min, with increasing concentrations of ethyl acetate in toluene, namely, 10% (80 ml); 20% (80 ml); 40% (80 ml); 65% (150 ml); 80% (150 ml); 100% ethyl acetate (80 ml); methanol (80 ml).

Radioactive tracer prostaglandins were eluted from the columns with the following concentrations of ethyl acetate in toluene; PGA_1 with 40%; PGE_1 with 65%; $PGF_{2\alpha}$ with 80%. The 40% eluates are also likely to contain any PGC (Jones, 1972a, b) or PGB in the extract.

All eluates from the columns were evaporated to dryness and redissolved in a known volume of methanol for liquid scintillation counting. After this procedure the samples were again evaporated to dryness. The 10% and 20% eluates were dissolved in 1 ml of 0·9% saline, and two-fifths of the 40% eluate were dissolved in 0·4 ml of 0·9% saline. These samples were assayed on the kitten blood pressure preparation. The remainder of the 40% eluate was used for combined gas chromatography-mass spectrometry (GC/MS). The other eluates were dissolved in 1 ml of water ready for bioassay on the rat fundal strip.

These assay procedures were used because the rat fundus is very sensitive to PGE and PGF, but insensitive to PGA and PGB, whereas the kitten blood pressure is very sensitive to PGA (Horton & Jones, 1969) and PGC (Jones, 1972a), but insensitive to intravenous injections of PGB, PGE and PGF. Since both assay preparations were insensitive to PGB, these prostaglandins could only be detected using combined GC/MS.

All eluates from column chromatography were stored at −15° C. Redistilled solvents were used for chromatography and all flasks for the collection of eluates were rinsed with methanol in order to remove traces of detergent (' Teepol ') which had been found to interfere with further prostaglandin identification using GC/MS.

Thin-layer radiochromatography

Glass grooved plates (Chromalay, May & Baker) of dimensions 20 × 5 cm were used. The plates were coated with a 0·75 mm layer of silica gel G (Merck). The silica gel, 30 g, was suspended in 57 ml of redistilled water for spreading the plates. The plates were allowed to dry overnight at room temperature and they were then used without further activation.

Each extract, containing tracer PGA_1, PGE_1 and $PGF_{2\alpha}$, was dissolved in 0·5 ml methanol and applied, as a band, using an Agla micrometer syringe, to the groove of a plate. The extract flask was washed with 2 × 0·2 ml methanol and these aliquots were also applied to the groove of the plate.

The thin-layer plates were developed in the following solvent system; redistilled toluene:dioxane:acetic acid (50:30:1) and a 16 cm run was used. Using this solvent system, PGA_1 has an R_F value of approximately 0·5 and prostaglandins of the E and F series have lower R_F values. This solvent system allows the plate to be developed twice without prostaglandins of the A series running too far up the plate, but it does not separate prostaglandins of the A, C or B series.

During the initial 16 cm run, the prostaglandins were eluted from the groove but pigments and polar lipids remained in the groove or had a low R_F value. The exact location of the tracer prostaglandins on the thin-layer plate was determined using a

Panax thin-layer scanner RTLS-1A. The silica gel in the groove and approximately 1 cm further up the plate was then scraped off, leaving a 1 cm length of silica gel intact below the PGF zone. The plate was then redeveloped in the same solvent system, up to the 16 cm line, and the positions of the tracer PGA, PGE and PGF zones were determined by radioactive scanning. These zones were scraped off and transferred to test-tubes which had been previously rinsed with methanol. The prostaglandins in the silica gel were eluted with 2 × 5 ml of methanol. The methanol was evaporated to dryness, then the samples were redissolved in a known volume of methanol ready for liquid scintillation counting. All other silica gel zones on each plate were also extracted and their radioactivity determined by liquid scintillation counting, to ascertain that no prostaglandin had been left on the plates.

After scintillation counting, the PGE, PGF and PGA (C or B) eluates were evaporated to dryness. The PGE and PGF eluates were dissolved in 1 ml of water ready for bioassay on the rat fundus, whereas 40% of the PGA (C or B) eluate was dissolved in 0·4 ml of 0·9% saline, ready for bioassay on the kitten blood pressure preparation ; the other 60% was retained for combined GC/MS.

Liquid scintillation counting

Radioactivity was measured in a Nuclear Chicago, Mark II, liquid scintillation system, using the channels radio method.

Samples for liquid scintillation counting were dissolved in a known volume of methanol or ethyl acetate. Aliquots (50 or 100 μl) were pipetted into vials and 10 ml of scintillation solution was added to the counting vial. A scintillation solution with the following composition was used ; toluene, 2·5 l. ; 2,5, diphenyloxazole (PPO), 10·65 g ; 1,4-di-(2-(4-methyl-5-phenyloxazolyl)-benzene), 0·275 g. Counting efficiencies for the samples, in organic solvents, averaged between 40 and 50%.

Further purification of prostaglandin F fractions

After thin-layer chromatography and biological assay, samples which contained a sufficiently high concentration of PGF for identification using combined GC/MS, were purified further. To each sample, 15 ml of pH 8 phosphate buffer was added, and this solution was extracted as in Figure 2. The pH 8 buffer was prepared by adding 189·4 ml of 0·5 M disodium phosphate to 5·3 ml of 1 M sodium dihydrogen phosphate and making up to 1 litre with distilled water.

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pH 8 phosphate buffer  ←————————→ Ethyl acetate
       (15 ml)                      (2 × 15 ml)
          ↓
  Adjusted to pH 4
  with acetic acid
          ↓
        pH 4  ←————————————→ Ethyl acetate
                               (2 × 30 ml)
                          Washed with 2 × 5 ml water
                                    ↓
                          Evaporated to dryness
                                    ↓
                          Dissolved in methanol for
                          scintillation counting
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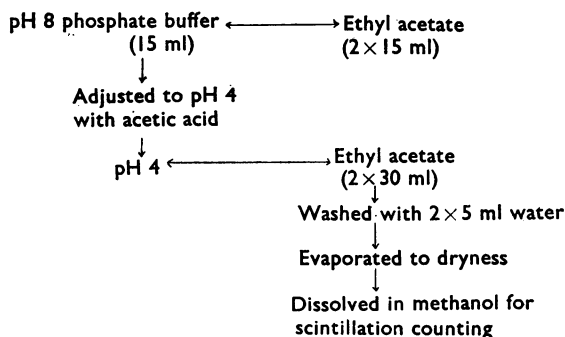
FIG. 2. Outline of additional purification procedure for PGF fractions after thin-layer chromatography.

Conversion of prostaglandins of the A, C or E series to PGB

After biological assay, prostaglandins of the A, C and E series, detected in extracts, were converted to prostaglandins of the B series, before final identification using combined GC/MS. The extracts were evaporated to dryness and 1 ml of 0·1 N KOH in methanol was added to them. After 1 h at approximately 18° C, water (20 ml) was added to each sample. This solution was adjusted to pH 4 with glacial acetic acid and partitioned twice against an equal volume of redistilled ethyl acetate. The ethyl acetate was retained and washed with 2×5 ml of redistilled water before being evaporated to dryness. Each sample was dissolved in a known volume of methanol, ready for liquid scintillation counting.

PGE was converted to PGB, as the methoxime derivatives of PGE require a minimum of 5 μg of PGE for their preparation. Low concentrations (300 ng) of PGE can be detected, however, by converting the PGE to PGB. The methyl ester/trimethylsilyl ether derivative of PGB is identifiable in low concentrations (100–200 ng) using GC/MS. Similarly, prostaglandins of the A and C series were converted to PGB, as part of any released PGA was possibly converted to PGC or PGB during the extraction procedure (see *Estimates of recovery*). Any PGB already in an extract would not be affected by this conversion procedure.

Gas chromatography-mass spectrometry

The methyl ester/trimethylsilyl ether (Me/TMS), the methyl ester/trifluoroacetate (Me/TFA) and the trimethylsilyl ester/trimethylsilyl ether (TMS/TMS) derivatives were prepared for combined GC/MS.

The derivatives were prepared on a microscale using 0·5 ml stoppered tubes and the methyl esters were initially formed using the methods of Thompson, Los & Horton (1970).

For blood extracts, trimethylsilyl ether derivatives (Me/TMS and TMS/TMS) were formed by the addition of 13 μl of bis (trimethylsilyl)- trifluoroacetamide (BSTFA) to the methyl ester (Me/TMS), or dry extract (TMS/TMS). PGB derivatives required 15 min at 60° C and PGF derivatives required 3 h at room temperature for conversion to the trimethylsilyl ether derivatives. Without removal of the BSTFA, approximately 12 μl was injected on to the gas chromatographic column. Authentic PGB_1 and PGB_2 (1 μg) were used for the preparation of Me/TMS and TMS/TMS derivatives and authentic $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (1 μg) were used for the preparation of Me/TMS derivatives. BSTFA (25 μl) was added to these authentic prostaglandins and 10 μl fractions (400 ng) were injected on to the column.

Trifluoroacetates (Me/TFA) were prepared by reacting the methyl esters for 2 h with 200 μl trifluoroacetic anhydride, which was then removed in a vacuum desiccator. The extract residues were dissolved in 13 μl hexane and approximately 12 μl was injected on to the column. The Me/TFA derivative of authentic $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (1 μg) was dissolved in 25 μl hexane and 10 μl fractions (400 ng) were injected on to the column.

Analyses were performed on an LKB 9000 gas chromatograph-mass spectrometer as described previously (Davis, Horton, Jones & Quilliam, 1971). Authentic PGB and PGF derivatives were injected on to the gas chromatograph column, their retention times were noted and a mass spectrum was taken at these times. The extract derivatives were then injected on to the column and mass spectra were taken at the retention times of authentic prostaglandin derivatives.

Biological assays

The rat fundus, rabbit jejunum and kitten blood pressure preparations, used for the biological assay of prostaglandins, have been described in a previous paper (Davis *et al.*, 1971).

Estimates of recovery

Tracer amounts (0·1 μCi) of PGA_1, PGE_1 and $PGF_{2\alpha}$ were each added to all the blood samples collected in the six experiments. The samples were extracted, as previously described, and after column or thin-layer chromatography the amount of tracer PGA_1, PGE_1 and $PGF_{2\alpha}$ was determined by scintillation counting.

The following recoveries were obtained ; PGA_1, 55–89% ; PGE_1, 42–70% ; $PGF_{2\alpha}$, 60–90%. The recovery results for tracer PGE_1 and $PGF_{2\alpha}$ agreed well with recovery results, obtained after chromatography, by biological assay of non-labelled prostaglandins (Davis, 1971). However, previous recovery results obtained using non-labelled PGA_1 added to rabbit blood, suggested that after chromatography, very low concentrations of PGA_1 were recovered, as assayed on the kitten blood pressure (Davis, 1971). The recovery of PGA_1 from rabbit blood was, therefore, investigated further.

PGA_1 was added to four 40 ml rabbit aortic blood samples at 37° C, containing heparin (20 i.u./ml), to give a final concentration of 100 ng/ml in two samples and 200 ng/ml in the other two samples. These concentrations of PGA_1 included 0·2 μCi ($\equiv$0·2 μg) of tracer PGA_1. The blood samples were extracted, as previously described. After thin-layer chromatography, the material with the chromatographic behaviour of tracer PGA_1 was eluted and the percentage recovery estimated by scintillation counting and assay on the kitten blood pressure. The results presented in Table 1, suggest that PGA_1 is at least partially converted to biologically inactive material.

TABLE 1

Sample number	Added PGA_1 concentration (ng/ml)	% Recovery by biological assay	% Recovery by scintillation counting
1	100	17	75
2	100	19	63
3	200	19	64
4	200	20	53

After biological assay, the Me/TMS derivatives of the remaining prostaglandin-like material in the four extracts were prepared for combined GC/MS. Mass spectra taken at the retention times of authentic PGA_1 (7·9 min) and PGB_1 (11·9 min) confirmed that all the extracts contained PGB_1 but only extract number 1 (Table 1) contained any PGA_1. Since the extracts contained many other substances quantitation by GC/MS was not possible and the percentage of PGA_1 converted to PGB_1 could not be calculated.

PGA_1 was, therefore, converted partly to biologically inactive PGB_1 after addition to rabbit blood and subsequent extraction. Since PGA_1 is very rapidly converted to PGC_1 by the enzyme prostaglandin isomerase in rabbit blood (Jones, 1970) and PGC_1 is then isomerized more slowly to PGB_1 (Jones, 1972a ; Fig. 3), it is probable that PGC_1 was also present in the blood extracts. Since PGC_1

is more active than PGA_1 in lowering kitten blood pressure (Jones, 1972a) and since PGA_1 could not be detected by GC/MS, PGC_1 may have been partly responsible for the fall in kitten blood pressure produced by these extracts. The behaviour of PGC_1 on GC/MS has not yet been fully investigated.

Even though the blood samples were cooled to 0° C after addition of PGA_1, it is now known that PGC_1 is formed rapidly under these conditions (Jones, personal communication). PGC_1 is unstable and rapidly converts to PGB_1 during purification procedures, particularly thin-layer chromatography (Jones, 1972b).

Results
Output of prostaglandins into renal venous blood

The release of prostaglandins from the rabbit kidney into renal venous blood was demonstrated in each of the six experiments. This output was increased by electrical stimulation of the renal nerve.

In four experiments, indomethacin was injected intravenously. This reduced the output of prostaglandins from the rabbit kidney and prevented the increase in output on renal nerve stimulation. These effects could not be attributed to the solvents, polyethylene glycol 200 and 0·9% saline, in which the indomethacin was dissolved for injection. The results are summarized in Table 2 and presented in detail below.

TABLE 2. *Amounts of prostaglandin (ng PG equivalent released/min) detected after column or thin-layer chromatography in renal venous blood samples*

Experiment number	Samples collected	Collection time (min)	Volume of blood sample (ml)	PGA	PGE_2	$PGF_{2\alpha}$
1	Control	5	39	<26	237	133
	Test	5	30	54	610	281
2	Control	5	46	<7	303	216
	Test 1	5	49·5	17	461	327
	Test 2	5	48	52	678	327
	Post-test	5	46	<11	356	238
3	Control	4	55	99	903	475
	Test	4	46	129	1700	949
	Control Glycol	4	42	39	168	240
	Test Glycol	4	36	49	322	272
	Control Ind.	4	40	<11	15	23
	Test Ind.	4	25	<10	14	21
4	Control	7	41	12	424	170
	Test	7	36·5	33	787	430
	Control Ind.	7	26	33	121	160
	Test Ind.	7	23	16	41	81
5	Control	7	33	<12	30	72
	Test	7	36	<12	54	180
	Control Ind.	7	41	<12	14	10
	Test Ind.	7	30	<12	15	9
6	Control	5	50	38	541	357
	Test	5	40	46	618	431
	Control Ind.	5	22	<16	<1	<23
	Test Ind.	5	16	<16	3	23

Samples collected before (Control) and during (Test) renal nerve stimulation; after polyethylene glycol 200 and 0·9% saline, samples collected before (Control Glycol) and during (Test Glycol) nerve stimulation; after indomethacin, samples collected before (Control Ind.) and during (Test Ind.) nerve stimulation. PGA (C or B)-like eluates assayed on kitten blood pressure preparation in terms of PGA_1. PGE and PGF-like eluates assayed on rat fundus in terms of PGE_2 and $PGF_{2\alpha}$, respectively. Amounts (ng) of tracer PGE_1 and $PGF_{2\alpha}$ recovered in each sample were deducted from total amounts of PG detected. PGE and PGF concentrations were then corrected for recovery. The estimates of PGA were not corrected for recovery.
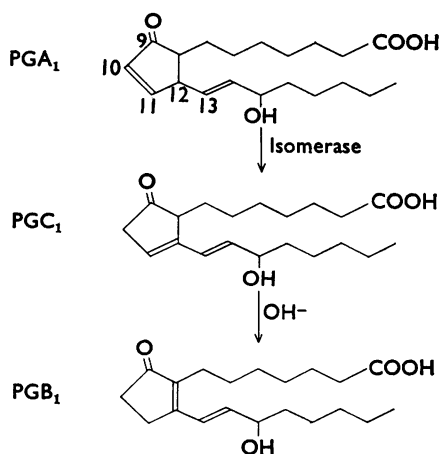
FIG. 3. Structural formulae of prostaglandins A_1, C_1 and B_1.
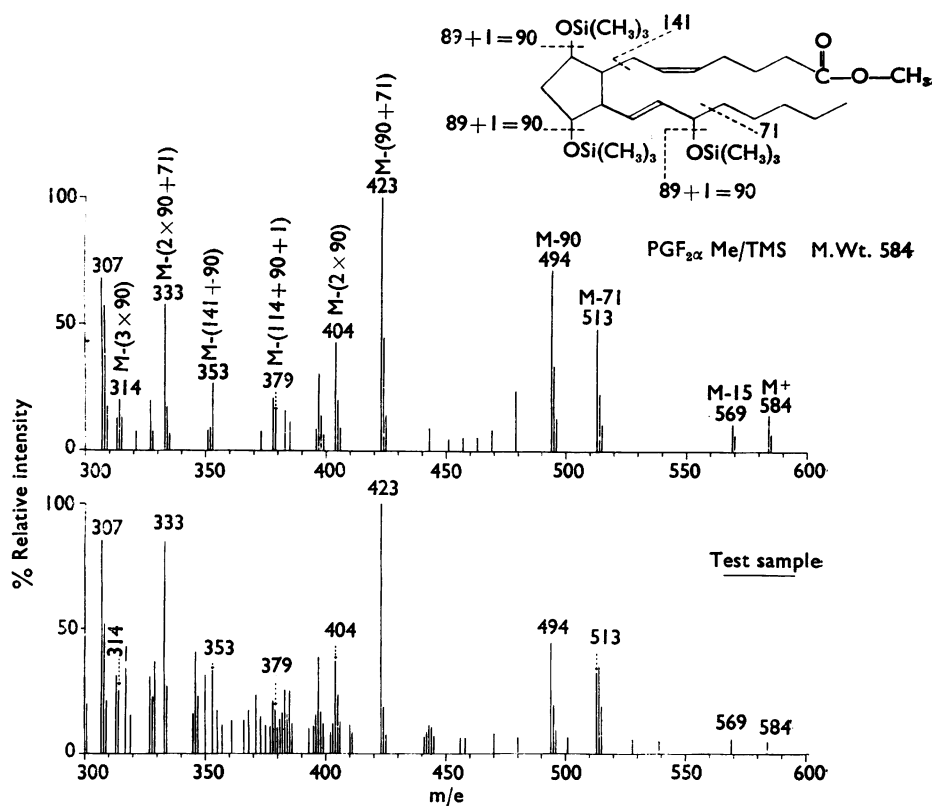
FIG. 4. Mass spectra of the methyl ester/trimethylsilyl ether derivatives of authentic prosta-glandin $F_{2\alpha}$ (upper) and half the prostaglandin F fraction from the test sample in experiment 3.

Identification of prostaglandins in renal venous blood collected before and during renal nerve stimulation

The prostaglandins were identified by column or thin-layer chromatography, biological assay and combined GC/MS ; this is illustrated by detailed reference to experiment 3 (Table 2). Similar evidence was obtained in the other five experiments.

In the renal venous blood samples collected before (control) and during (test) renal nerve stimulation in experiment 3, biological activity was detected in the zones of the thin-layer chromatogram corresponding to the chromatographic behaviour of added tracer amounts of $PGF_{2\alpha}$, PGE_1 and PGA_1.

Identification of prostaglandins of the F series

In experiment 3 (Table 2) the material in the control and test samples with the chromatographic behaviour of a prostaglandin of the F series was assayed on the rat fundus in terms of $PGF_{2\alpha}$. After bioassay, the PGF-like material was purified further using a phosphate buffer partition. Each sample was then divided into two parts for the preparation of the Me/TMS and Me/TFA derivatives respectively. Combined GC/MS was performed on these derivatives.

Mass spectra taken at the retention times of authentic Me/TMS and Me/TFA derivatives of $PGF_{2\alpha}$ and $PGF_{1\alpha}$ confirmed that the control and test samples contained $PGF_{2\alpha}$ but no detectable $PGF_{1\alpha}$. At a column temperature of 206° C, the gas chromatographic retention times for the Me/TMS derivatives of $PGF_{2\alpha}$ and $PGF_{1\alpha}$ were 9·9 and 10·4 min respectively. At a column temperature of 190° C, the retention times for the Me/TFA derivatives of $PGF_{2\alpha}$ and $PGF_{1\alpha}$ were 5·5 and 6·0 min respectively. Line diagrams of the mass spectra for the Me/TMS and Me/TFA derivatives of the test sample in experiment 3, and of authentic $PGF_{2\alpha}$, are presented in Figures 4 and 5. The amount of tracer $PGF_{2\alpha}$ (3·3 ng) initially added to each blood sample would not have been sufficient for identification using GC/MS.

In the mass spectra for the Me/TMS derivatives, only peaks with m/e values above 300 were considered significant, since characteristic ' prostaglandin peaks ' below this value were obscured by the background ' noise '. The Me/TMS derivative of authentic $PGF_{2\alpha}$ has 11 prominent m/e peaks above 300, at 584 [M+], 569 [M−15], 513 [M−71], 494 [M – 90], 423 [M−(90+71)], 404 [M−(2×90)], 379 [M−(114+90+1)], 353 [M−(141+90)], 333 [M−(2×90+71)], 314 [M−(3×90)] and 307. The presence of these 11 peaks, in approximately the same ratios, in the mass spectra of the control and test PGF-like material (Me/TMS) confirmed that this material was $PGF_{2\alpha}$ (Fig. 4).

The mass spectrum for the Me/TFA derivative of authentic $PGF_{2\alpha}$ showed five prominent m/e peaks above 300, at 542 [M−114], 511 [M−(31+114)], 441 [M−(101+114)], 428 [M−(2×114)] and 314 [M−(3×114)]. The presence of these peaks, in similar ratios, in the mass spectra of the control and test PGF-like material (Me/TFA) again confirmed the presence of $PGF_{2\alpha}$ in these samples (Fig. 5).

In the five other experiments (Table 2), PGF-like material was estimated in the control and test samples by biological assay after chromatography. Combined GC/MS was performed on the Me/TMS and Me/TFA derivatives. $PGF_{2\alpha}$ was identified in every sample. No $PGF_{1\alpha}$ was detected.
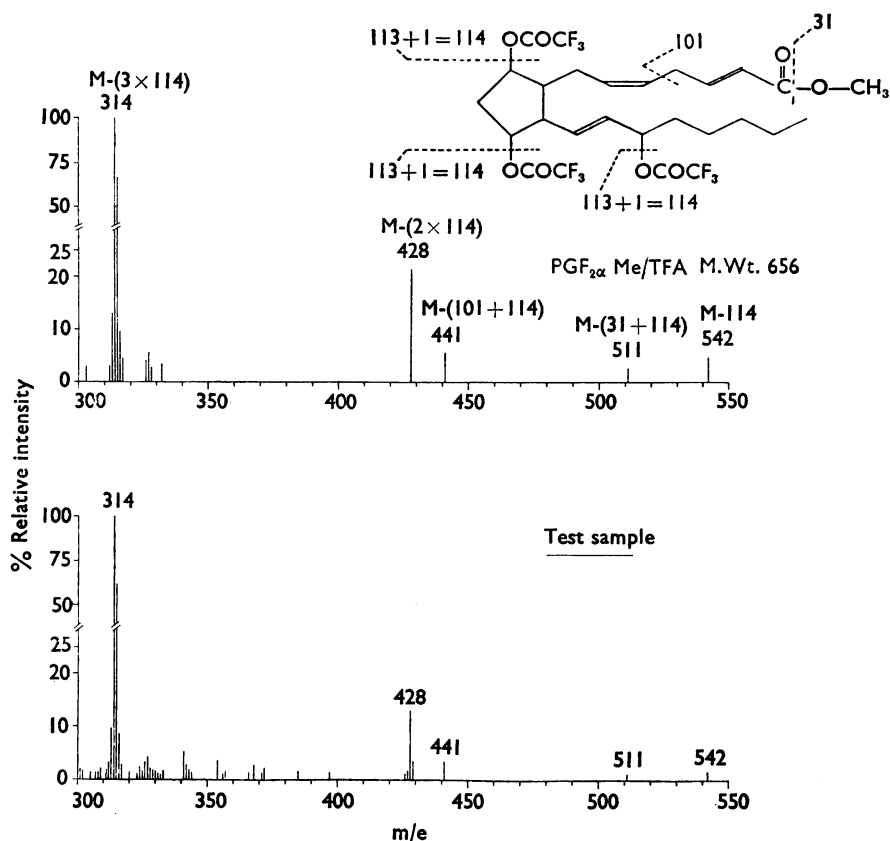
FIG. 5. Mass spectra of the methyl ester/trifluoroacetate derivatives of authentic prostaglandin F$_{2\alpha}$ (upper) and half the prostaglandin F fraction from the test sample in experiment 3.

Identification of prostaglandins of the E series

In experiment 3 (Table 2), the material in the control and test samples with the chromatographic behaviour of a prostaglandin of the E series was assayed on the rat fundus in terms of PGE$_2$. After bioassay, the PGE-like material was converted to PGB and extracted.

Combined GC/MS was then performed on the Me/TMS derivatives of the PGB-like material in the control and test samples. Mass spectra taken at the retention time (8·9 min) of the Me/TMS derivative of authentic PGB$_2$ (PGB$_1$, Me/TMS, had the same retention time on this column ; column temperature 206° C) confirmed that the control and test samples contained PGB$_2$ but no detectable PGB$_1$. The mass spectrum for PGB$_1$ is distinguishable from that of PGB$_2$ as it shows many characteristic peaks at two mass units higher. Line diagrams of the mass spectra for the test sample (Me/TMS) and authentic PGB$_2$ (Me/TMS) are presented in Figure 6. In each mass spectrum, only peaks with m/e values of 199 or above were of significance since characteristic ' prostaglandin peaks ' below this were obscured by the background ' noise '. The Me/TMS derivative of authentic PGB$_2$ has 10 prominent peaks above 199, at 420 [M$^+$], 405 [M−15], 389 [M−31], 349 [M − 71], 330 [M−90], 321 [M − (71+28)], 279 [M−141], 247 [M−173], 221 [M−199], 199.
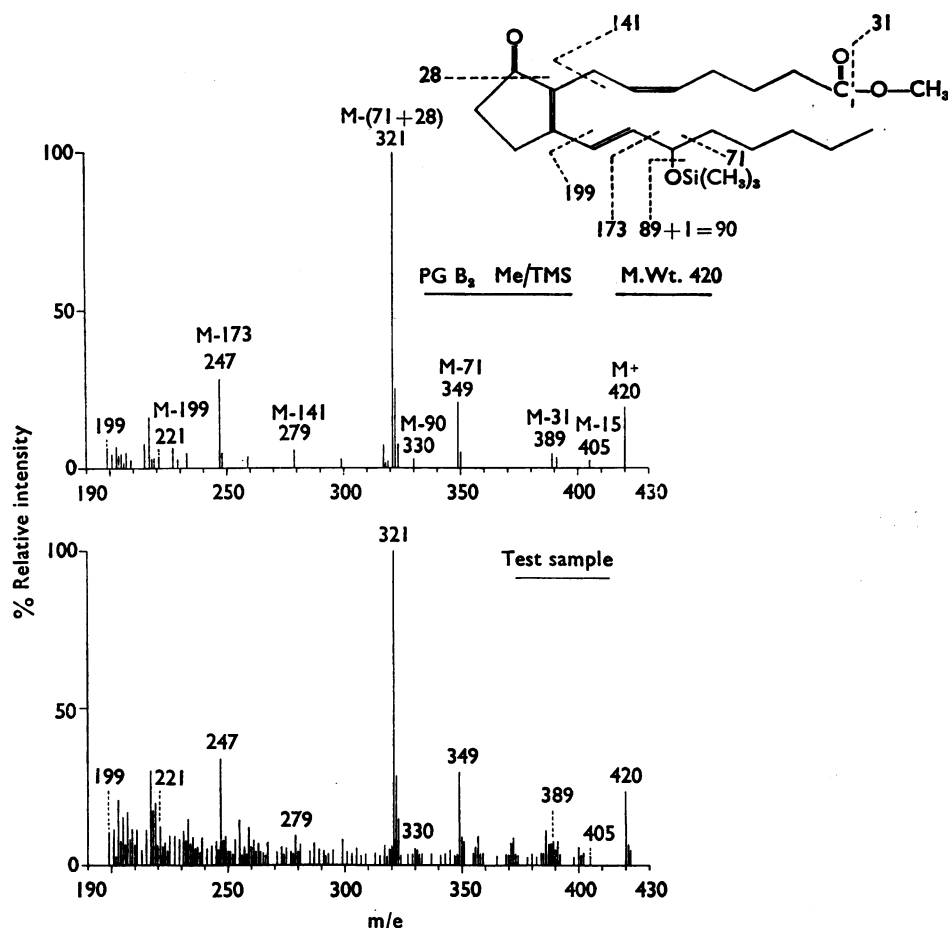
FIG. 6. Mass spectra of the methyl ester/trimethylsilyl ether derivatives of authentic prosta-glandin B_2 (upper) and the prostaglandin E fraction (converted to prostaglandin B) from the test sample collected in experiment 3.

The presence of these 10 peaks, in approximately the same ratios, in the mass spectra of the control and test PGB-like material (Me/TMS) confirmed that this material was PGB_2 (Fig. 6). Since the PGB had been derived from PGE, mass spectrometry confirmed that PGE_2, and no detectable PGE_1, was present in the renal venous blood samples in experiment 3.

In the five other experiments (Table 2), PGE-like material was estimated in the control and test samples by biological assay after chromatography.

In experiments 4 and 6 (Table 2), the PGE-like material in the control and test samples was identified as PGE_2 using combined GC/MS. As in experiment 3, the PGE_2 was identified as the Me/TMS derivative of PGB_2. No PGE_1 was detected in the samples.

In experiments 1 and 2 (Table 2), the PGE-like material was converted to PGB. In experiment 1, this material was lost during a further purification procedure. In experiment 2, the control and post-test samples were pooled and the two test samples were also pooled. The TMS/TMS derivative of the PGB-like material was pre-pared for combined GC/MS. Mass spectra for the two extracts, taken at the

retention time (18·6 min ; column temperature 200° C) of authentic PGB$_2$ and PGB$_1$ (TMS/TMS) showed peaks with m/e values identical to those of authentic PGB$_2$ (TMS/TMS). No PGB$_1$ was detectable. There were many other larger peaks attributable to interfering substances present in the extracts, thus, the identification of PGE$_2$ in this experiment must be regarded as tentative. In experiment 5 (Table 2), there was insufficient PGE-like material released to attempt identification by combined GC/MS.

Identification of prostaglandins of the A, C or B series

In experiment 3 (Table 2), the material with the chromatographic behaviour of a prostaglandin of the A, C or B (Jones, 1972a, b) series (Fig. 3) was assayed on the kitten blood pressure in terms of PGA$_1$. This material lowered the kitten blood pressure when injected intravenously, a characteristic of a prostaglandin of the A series (Horton & Jones, 1969), C series (Jones, 1972a) or high concentrations of a prostaglandin of the B series (Horton & Jones, 1969).

Since PGA$_2$ is 4 times and PGC$_1$ is 1·5 times (Jones, personal communication) more active than PGA$_1$ in producing a fall in kitten blood pressure, when injected intravenously, an even lower concentration of PGA$_2$ or PGC$_1$ could have been present in the extracts. PGB$_1$ is 40 times and PGB$_2$ is 12 times less active than PGA$_1$ on the kitten blood pressure preparation ; thus much higher concentrations of PGB could have been present in the extracts.

After biological assay, the remaining 60% of the control and test PGA, C or B-like material was converted to PGB and extracted. Combined GC/MS was performed on the Me/TMS derivatives of the PGB-like material in the control and test samples. Mass spectra taken at the retention time (10·0 min ; column temperature 205° C) of the Me/TMS derivatives of authentic PGB$_2$ and PGB$_1$, indicated that the control and test samples did not contain detectable amounts of PGB$_2$ or PGB$_1$.

If the fall in kitten blood pressure had been attributable to a prostaglandin of the B series there would have been microgram quantities which would have been easily detectable by combined GC/MS (Table 3). If the fall in kitten blood pressure pro-

TABLE 3. *Amounts (ng) of prostaglandin A$_1$ and equivalent amounts (ng) of prostaglandins A$_2$, C$_1$, B$_1$ and B$_2$ remaining after biological assay in the control and test samples collected in experiment 3 (Table 2)*

Sample	Prostaglandin				
	A$_1$	A$_2$	C$_1$	B$_1$	B$_2$
Control	238	59	159	9,528	2,858
Test	309	77	206	12,360	3,708

duced by the extracts was attributable to prostaglandin A$_1$, A$_2$ or C$_1$, there would have been insufficient remaining after biological assay (Table 3) for identification by GC/MS, especially in the presence of large amounts of interfering substances in the extracts. Furthermore, approximately 60% of these remaining prostaglandins were lost on conversion to the PGB compound. It was, therefore, very likely that the fall in kitten blood pressure produced by the extracts was attributable to a low concentration of a prostaglandin of either the A or the C series (see *Estimates of recovery*). In addition, low concentrations of PGB could have been present.

In four of the five other experiments (Table 2), PGA, C or B-like material was estimated after chromatography by assay on the kitten blood pressure (Fig. 7). As in experiment 3, neither PGB_1 nor PGB_2 was identified by GC/MS. These results also suggest that a low concentration of a prostaglandin of the A or C series was present in the extracts.
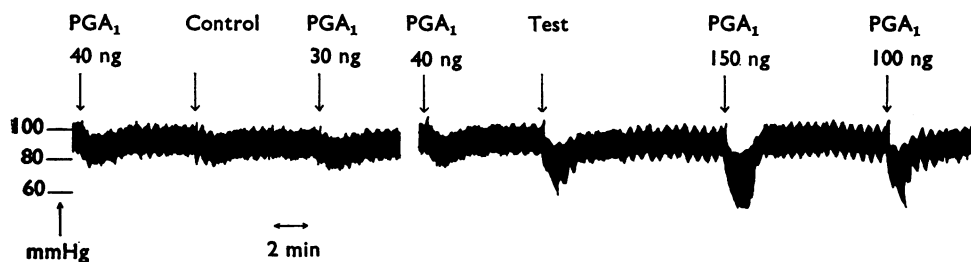

FIG. 7. Assay on the kitten blood pressure preparation. Kitten (1·4 kg), anaesthetized with Nembutal (40 mg/kg). Responses to single intravenous injections of PGA_1 on the carotid arterial blood pressure are compared with injections of 40% of the PGA (C or B)-like eluates from chromatography of the control and test samples collected in experiment 4. These extract samples were dissolved in 0·4 ml of 0·9% saline and 0·35 ml was injected intravenously.

The effect of polyethylene glycol 200 on prostaglandin concentrations in the renal venous blood before and during renal nerve stimulation

In experiment 3, a mixture of 2 ml of polyethylene glycol 200 and 1 ml of 0·9% saline was injected intravenously. Following the injection, systolic blood pressure rose by 22 mmHg and diastolic blood pressure rose by 12 mmHg. This effect lasted for 10 minutes.

Renal venous blood samples were collected before (Control Glycol) and during (Test Glycol) renal nerve stimulation. There was a fall in output of prostaglandins of the A (C or B), E and F series in the control glycol and test glycol samples compared with the output in the control and test samples in experiment 3 (Table 2). Polyethylene glycol 200 did not, however, abolish the output of prostaglandins from the kidney, neither did it prevent the increased release of prostaglandins in response to renal nerve stimulation (Table 2).

Identification of prostaglandin F after polyethylene glycol 200

After biological assay, the remaining PGF-like material present in the control and test polyethylene glycol samples was identified as $PGF_{2\alpha}$ by combined GC/MS. Two derivatives, the Me/TMS and Me/TFA, were prepared and identified for each sample.

Identification of prostaglandin E after polyethylene glycol 200

After bioassay, the remaining PGE-like material present in the control and test polyethylene glycol samples was identified as PGE_2 by combined GC/MS. As previously described, the PGE_2 was identified as the Me/TMS derivative of PGB_2.

Attempted identification of prostaglandin A, C or B after polyethylene glycol 200

After bioassay, the remaining PGA, C or B-like material in the two samples was converted to PGB. An attempt was made to identify the Me/TMS derivative of the PGB-like material by combined GC/MS. Neither PGB_2 nor PGB_1 could be detected.

The effect of indomethacin on prostaglandin concentrations in the renal venous blood before and during renal nerve stimulation

In four experiments (nos. 3, 4, 5 and 6), indomethacin (10 mg/kg), dissolved in polyethylene glycol 200 and 0·9% saline, was injected intravenously. Following the injection, systolic blood pressure rose by 10 to 40 mmHg and diastolic blood pressure rose by 16 to 28 mmHg. This effect lasted for more than 1 h in all experiments. In experiments 4 and 6 (Table 2), total renal blood flow decreased.

Renal venous blood samples were collected before (Control Ind.) and during (Test Ind.) renal nerve stimulation. Indomethacin reduced the output (Control Ind.) into renal venous blood of prostaglandins of the E and F series in all four experiments, and of prostaglandins of the A (C or B) series in two experiments (nos. 3 and 6, Table 2). In all experiments, indomethacin prevented the increased release of prostaglandins of the A (C or B), E and F series in response to renal nerve stimulation (Table 2).

In experiment 4, only 10 min was allowed after the injection of indomethacin before the collection of the initial Control Ind. sample. In the other experiments, 30 min was allowed. After 30 min, but not after 10 min, the concentration of prostaglandins of the A (C or B), E and F series in the renal venous blood was very low (Table 2).

Identification of prostaglandin F after indomethacin

After bioassay, the remaining PGE-like material present in the control and test indomethacin samples in experiment 4 (Table 2) was identified as PGE_2, by combined GC/MS. Two derivatives, the Me/TMS and Me/TFA, were prepared for each sample. In each of the experiments, 3, 5 and 6, the PGF-like material in the control and test indomethacin samples was pooled and identified as $PGF_{2\alpha}$, using the Me/TMS derivatives only, on GC/MS.

Identification of prostaglandin E after indomethacin

After bioassay, the remaining PGE-like material present in the control and test indomethacin samples in experiment 4 (Table 2) was identified as PGE_2, by combined GC/MS. As previously described, the PGE_2 was identified as the Me/TMS derivative of PGB_2. In experiments 3, 5 and 6, after indomethacin, insufficient PGE-like material was released for identification by combined GC/MS.

Attempted identification of prostaglandin A, C or B after indomethacin

After bioassay, the remaining PGA, C or B-like material in experiments 3, 4, 5 and 6 (Table 2) was converted to PGB. An attempt was made to identify the Me/TMS derivative of the PGB-like material, by combined GC/MS. Neither PGB_2 nor PGB_1 could be detected.

Concentrations of prostaglandins in abdominal aortic blood

In experiment 6 (Table 2), a 20 ml blood sample was collected from the abdominal aorta, before the collection of the control renal venous blood sample. The aortic blood, to which tracer amounts of PGA_1, PGE_1 and $PGF_{2\alpha}$ were added, was extracted, purified by thin-layer chromatography and eluates were bioassayed by identical methods to those described for the renal venous blood samples. The concentrations of detectable PGE_2 and $PGF_{2\alpha}$ were corrected for recovery. The PGA was not corrected for recovery.

No detectable levels of PGA (<2 ng/ml PGA_1 equivalent) or PGF ($<3·5$ ng/ml $PGF_{2\alpha}$ equivalent) were found but a concentration of 1·5 ng/ml of PGE_2 equivalent was estimated. Since the total quantity of PGE-like material available for assay was very low, the error in this estimation may be considerable. There was also insufficient PGE-like material to attempt further identification by combined GC/MS.

Discussion

In all six experiments, PGE_2, $PGF_{2\alpha}$ and PGA, C or B-like material were detected in rabbit renal venous blood, collected before and during renal nerve stimulation. Renal nerve stimulation increased the output of these prostaglandins. PGE_2 and $PGF_{2\alpha}$ were conclusively identified by combined GC/MS. Since the concentrations of prostaglandins in renal venous blood were up to 45 times higher than in aortic blood, they must have originated from the kidney.

Piper & Vane (1971) have demonstrated that handling of organs may cause prostaglandin release. In the present experiments, however, the kidney was not handled and 30 min was allowed after the surgical procedure before collecting blood samples. It is, therefore, unlikely that the presence of prostaglandins in the control renal venous blood samples could be attributed to this cause.

It has previously been suggested that PGA_2 isolated from the rabbit kidney was derived, at least in part, from PGE_2 during the extraction procedure (Lee *et al.*, 1967). It is not known whether the PGA, C or B-like material in our experiments was similarly derived, but an organic acid was employed in the extraction procedure and this has been reported to reduce the dehydration of PGE considerably (Andersen, 1969).

In four experiments, an intravenous injection of indomethacin decreased the output of PGE_2, $PGF_{2\alpha}$ and the PGA, C or B-like material in renal venous blood and prevented the increased output of these prostaglandins in response to renal nerve stimulation. These effects could not be entirely attributed to the solvents, polyethylene glycol 200 and 0·9% saline, in which the indomethacin was dissolved for injection. These results confirm previous work in which indomethacin has been shown to inhibit prostaglandin release (Vane, 1971 ; Ferreira, Moncada & Vane, 1971 ; Aiken & Vane, 1971 ; Smith & Willis, 1971 ; Smith & Lands, 1971 ; Poyser, 1972).

After the injection of indomethacin, a prolonged rise in systemic arterial blood pressure resulted in all four experiments and total renal blood flow was reduced in two. These results suggest that, in the absence of indomethacin, prostaglandins are continually released in the rabbit kidney, to exert a regulatory antihypertensive rôle. Such a rôle has previously been proposed (Lee, Covino, Takman & Smith, 1965 ; Lee, 1967; Lee, Kannegiesser, O'Toole & Westura, 1971; Muirhead, Brooks, Pitcock & Stephenson, 1972). The renal prostaglandins may exert such a rôle by intrarenal

vasodilatation or they may circulate to produce arteriolar dilatation in other vascular beds. In particular, prostaglandins of the A or C series, which are not metabolized by the lungs, may act as circulating antihypertensive hormones (Horton & Jones, 1969 ; McGiff, Terragno, Strand, Lee, Lonigro & Ng, 1969 ; Jones, 1972a).

Prostaglandins may also be associated with autoregulation in the kidney. When the sympathetic nerves to the cat kidney are stimulated at physiological frequencies, renal blood flow initially decreases markedly, it then increases over the next 3 to 5 min, to a value slightly below the control level (Johansson, Sparks & Biber, 1970). It has been suggested that this ' escape ' of the renal blood flow may be the result of a decrease in the amount of transmitter released per impulse (Johansson *et al.*, 1970). This suggestion was proposed because on prolonged sympathetic stimulation the outflow of noradrenaline from perfused skeletal muscle or spleen decreases with time (Stjärne, Hedqvist & Bygdeman, 1969). Since prostaglandins of the E series reduce the release of noradrenaline from adrenergic nerve terminals (Hedqvist, 1971), the increased output of PGE_2 on renal nerve stimulation in our experiments may be exerting a modulator function by reducing transmitter output and thus allowing the ' escape ' of renal blood flow. Since Hedqvist (1971) did not investigate the effects of prostaglandins of the A, B or F series on noradrenaline release from adrenergic nerve terminals, no similar function for the increased output of $PGF_{2\alpha}$ and PGA (C or B) on renal nerve stimulation observed in our experiments can yet be postulated.

Johansson *et al.* (1970) also suggested that the ' escape ' of renal blood flow during renal nerve stimulation in cats may be attributable to the redistribution of renal blood flow. This was suggested as stimulation of sympathetic nerves to the canine kidney results in decreased cortical but increased outer medullary blood flow, while total renal blood flow remains normal (Pomeranz, Birch & Barger, 1968). Since prostaglandins of the A and E series are potent vasodilators in the rabbit kidney (Lee, 1967) and since they were released in our experiments, they may be responsible for the redistribution of renal blood flow during nerve stimulation. The last hypothesis is not, however, entirely compatible with the observations that both PGA_1 and PGE_1 increase total renal blood flow in dogs by producing vasodilatation in the renal cortex not the medulla (Carrière, Friborg & Guay, 1971).

There is also some evidence from experiments on dogs that renal prostaglandins are concerned with autoregulation (McGiff, Crowshaw, Terragno & Lonigro, 1970 ; McGiff, Crowshaw, Terragno, Malik & Lonigro, 1972 ; Dunham & Zimmerman, 1970). McGiff *et al.* (1970 ; 1972) showed that during an infusion of noradrenaline or angiotensin II, prostaglandins were released from the dog kidney concomitantly with the return of renal blood flow and urine flow to control values. However, in contrast to our results in the rabbit, McGiff *et al.* (1972) found no increase in prostaglandin output on renal nerve stimulation in the dog. Previously, Dunham & Zimmerman (1970) had reported an increased output in the dog, but the evidence of identification was only sufficient to justify the term ' prostaglandin E-like '. In the present paper, the identification of PGE_2 and $PGF_{2\alpha}$ has been established unequivocally by mass spectrometric analysis.

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