An Electron-Capture Gas-Liquid-Chromatographic Method for the Determination of Prostaglandin F_{2α} in Biological Fluids

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A sensitive electron-capture gas-liquid-chromatographic method for the determination of sub-nanogram quantities of prostaglandin F_{2α} was developed. The method is based on the sub-microgram scale conversion of the prostaglandin into the electron-capturing pentafluorobenzyl ester, and analysis of the latter as the tris-trimethylsilyl ether. The lower limit of detection was 12.5 pg of the ester injected 'on-column' as the silylated product. The method was successfully applied to the determination of prostaglandin F_{2α} in monkey plasma. The specificity of the analytical procedure was increased by incorporating a thin-layer chromatographic fractionation before gas-liquid chromatography. The utility of the analytical methodology developed was demonstrated by its application to the determination of plasma concentrations of intact prostaglandin F_{2α} in a Rhesus monkey, after subcutaneous administration of a single dose of prostaglandin F_{2α}. The electron-capture gas-liquid-chromatographic assay is compared with the radioimmunoassay and the gas-liquid-chromatographic-mass-spectrometry assay for the determination of prostaglandin F_{2α}.

The biological significance of the prostaglandins and the current interest in them is well documented (Ramwell & Shaw, 1971; Karim, 1971; Hinman, 1970; Moghissi, 1972). Simplified analytical methods of high sensitivity and high specificity for the measurement of prostaglandins in biological fluids (e.g. plasma) are necessary to study their metabolic fate, physiological roles and mechanisms of action. Several methods have been considered for the determination of prostaglandins, among which are those based on biological responses (Horton, 1972; Horton & Jones, 1969; Vane, 1969), enzymic assay (Anggard *et al.*, 1969), u.v. and fluorescence spectroscopy (Gantt *et al.*, 1968; Shaw & Ramwell, 1969; Takeguchi *et al.*, 1971), radioimmunoassay (Caldwell *et al.*, 1971; Cornette *et al.*, 1972; Jaffe *et al.*, 1971; Levine & Van Vunakis, 1970; Cernosek *et al.*, 1972; Jubiz & Frailey, 1971), high-pressure liquid chromatography (Morozowich, 1972) and g.l.c. utilizing flame ionization detection (Albro & Fishbein, 1969; Ramwell *et al.*, 1968; Bygdeman & Samuelsson, 1964). Most of these methods lack adequate sensitivity and/or specificity. G.l.c. methods utilizing mass-spectrometric detection (Samuelsson *et al.*, 1970; Axen *et al.*, 1972; Baczynskyj *et al.*, 1973; Oates, 1972; Green *et al.*, 1973), as well as electron-capture detection (Jouvenaz *et al.*, 1970; Van Dorp, 1971; Levitt & Josimovich, 1971; Levitt *et al.*, 1972), which have been developed more recently, afford high sensitivity and also a high degree of specificity when combined with a preliminary t.l.c. separation. In terms of

general utility, the elegant g.l.c.-mass-spectrometric method has the disadvantage that it requires expensive and usually unavailable instrumentation, as well as the synthesis of the isotopically labelled (deuterated) prostaglandins for use as internal standards and carriers. Similarly, those reported electron-capture methods that are based on the inherent electron-capturing properties of the PG†B compounds (Jouvenaz *et al.*, 1970; Van Dorp, 1971; Sweetman *et al.*, 1973), are limited to the measurement of PGB-type compounds or to prostaglandins that can be readily converted into PGB types (e.g. the A and E prostaglandins).

Electron-capturing derivatives of the hydroxyl groups (Van Dorp, 1971; Levitt & Josimovich, 1971; Levitt *et al.*, 1972) of the prostaglandins reported thus far have found limited applicability. The heptafluorobutyrate derivatives in particular have been found to be unsuitable for g.l.c., because of their thermal instability (Levitt *et al.*, 1972). Multiple peak formation has been observed on g.l.c. at temperatures over 190°C, probably owing to loss of a heptafluorobutyrate group from the allylic C-15 position of the prostaglandin. Recently the F prostaglandins have been analysed by electron-capture g.l.c. of their bromo-silylated methyl ester derivatives (Jouvenaz *et al.*, 1973) (also see below). Since most prostaglandins and prostaglandin metabolites have at least one carboxylic function in the molecule, an electron-capturing derivative such as the pentafluorobenzyl ester appeared to merit consideration.

† Abbreviation: PG, prostaglandin.

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Pentafluorobenzyl esters of organic acids have been found to be ideal derivatives for electron-capture g.l.c. detection, on account of their excellent response (Kawahara, 1968). Further, additional specificity of detection is achieved with these derivatives since only organic acids in a biological extract would form the ester. The present paper describes the determination of sub-microgram amounts of $\text{PGF}_{2\alpha}$ present in biological specimens (i.e. plasma) by

electron-capture g.l.c. as the pentafluorobenzyl ester tris-trimethylsilyl ether.

Experimental

Tritium-labelled prostaglandin $F_{2\alpha}$

$[9\beta\text{-}^3\text{H}]\text{PGF}_{2\alpha}$ purchased from New England Nuclear Corp., Boston, Mass., U.S.A. (specific radioactivity 0.25 mCi/0.0062 mg), was mixed with

non-radioactive $\text{PGF}_{2\alpha}$, Tris salt (Ib). The specific radioactivity of the diluted [^3H] $\text{PGF}_{2\alpha}$ administered to the monkey was 9.55×10^6 d.p.m./mg of the free acid.

Biological methods

Animals. Female rhesus monkeys (*Macaca mulatta*; 4.3 kg body wt.) were used in the studies. The animals were housed in metabolism cages and did not have access to food and water during the dosing and blood collection. Food was withheld during the 16 h period preceding the dosing. Water was provided *ad lib*.

Dosing. Tritium-labelled $\text{PGF}_{2\alpha}$ (see above) was administered as a solution in saline (sterile 0.9% NaCl solution, supplied by Travenol Laboratories Inc., Deerfield, Ill., U.S.A.). A single dose of 3.5 mg of $\text{PGF}_{2\alpha}$ (free acid equivalents)/kg body weight (1 ml of the saline solution which contained 15.0 mg of $\text{PGF}_{2\alpha}$; 143.28×10^6 d.p.m.) was administered by subcutaneous injection into the right thoracic region.

Blood collection. Blood samples were drawn from the femoral vein of the monkey. Samples of blood (5 ml) were drawn at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0 and 10.0 h after administration of $\text{PGF}_{2\alpha}$. The blood samples were collected in heparinized tubes and centrifuged for 15 min at 3000 rev./min (in an International model UV centrifuge). The plasma was harvested and stored at -4°C .

Preparation and extraction of plasma standards and specimens from prostaglandin-treated monkeys

Non-radioactive $\text{PGF}_{2\alpha}$ standards. Two identical series of portions of a standard solution of the Tris salt of $\text{PGF}_{2\alpha}$ (Ib) in methanol containing 0.27–8.14 ng of $\text{PGF}_{2\alpha}$ salt (Ib) (corresponding to 0.202–6.059 ng of the $\text{PGF}_{2\alpha}$ free acid) were placed in glass-stoppered 15 ml centrifuge tubes. The samples were evaporated to dryness under a gentle stream of N_2 . A portion (1 ml) of control monkey plasma was added to each of the standards of one series and mixed well by using a Vortex mixer. A control plasma blank (1 ml; without the administration of $\text{PGF}_{2\alpha}$) was prepared in a 15 ml centrifuge tube and processed in the same manner as the other samples.

Radioactive $\text{PGF}_{2\alpha}$ standards. Portions of a standard solution of [$9\beta\text{-}^3\text{H}$] $\text{PGF}_{2\alpha}$ in methanol containing 0.21 ng (35961 d.p.m.)–5.33 ng (975610 d.p.m.) of the free acid were placed in glass-stoppered 15 ml centrifuge tubes, and evaporated to dryness under N_2 . A portion (1 ml) of the control monkey plasma was added to each of the radioactive standards.

Plasma specimens from the $\text{PGF}_{2\alpha}$ -treated monkeys. Portions (1 ml) of the plasma samples taken at each

time-interval were placed in 15 ml centrifuge tubes and processed along with the other standards.

Extraction. The non-radioactive plasma standards, the radioactive plasma standards and the plasma specimens from the $\text{PGF}_{2\alpha}$ -treated monkeys were extracted as follows. Each sample of plasma (1 ml) was mixed with 1 vol. of 0.9% NaCl solution (1 ml), 2 vol. of ethanol (2 ml), and extracted with light petroleum (b.p. $40\text{--}60^\circ\text{C}$; 2×8 ml) to remove neutral lipids and non-polar fatty acids. The aqueous phase was adjusted to pH 3.3 with formic acid (3%, v/v) and extracted with chloroform (Burdick and Jackson Labs. Inc., Muskegon, Mich., U.S.A.) (3×5 ml). The combined chloroform extracts were concentrated under a stream of N_2 . The concentrate was repeatedly diluted with chloroform and evaporated to aid the removal of formic acid. The extract was finally blown down to dryness under N_2 . The recoveries of $\text{PGF}_{2\alpha}$ extracted from monkey plasma (determined on the basis of the recovery of the added [$9\beta\text{-}^3\text{H}$]- $\text{PGF}_{2\alpha}$ radioactivity) are summarized in Table 1. The plasma extract residues were subjected to t.l.c. as described below.

T.l.c. of the monkey plasma extracts. T.l.c. was carried out on precoated silica gel F-254 plates of 250 μm thickness (supplied by Analtech Inc., Wilmington, Del., U.S.A. or by Merck, Brinkmann Instruments, Westbury, N.Y., U.S.A.), in a solvent system of chloroform–methanol–acetic acid (8:1:1, by vol.). Each t.l.c. plate was scored into lanes (each lane approx. 1.5 cm wide). Reference spots of authentic $\text{PGF}_{2\alpha}$ were used on marker lanes. The $\text{PGF}_{2\alpha}$ zones were located by exposure of the marker lanes to iodine vapour (by carefully touching the surfaces of the marker lanes with a few crystals of iodine wrapped in tissue paper). The $\text{PGF}_{2\alpha}$ zones were scraped out separately and the silica gel was extracted with methanol (3×1.5 ml). The extracts were evaporated to dryness under N_2 . Diethyl ether (Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A.) (1 ml) and 0.025 M-HCl (0.5 ml) were added to each residue and extracted. The aqueous phase was extracted twice more with ether (1 ml portions). To the combined ether extracts, a solution of NH_3 (58%, w/v; analytical reagent supplied by Mallinckrodt Chemical Works) (5 μl) was added, and mixed thoroughly by using a Vortex mixer. The extract was then blown down to dryness. Each residue was converted into the pentafluorobenzyl ester as described below.

Conversion of sub-microgram quantities of $\text{PGF}_{2\alpha}$ recovered into the pentafluorobenzyl ester (IIa)

The $\text{PGF}_{2\alpha}$ residue was treated with a solution of pentafluorobenzyl bromide (0.022 mmol) in acetonitrile (10 μl) and a solution of di-isopropylethylamine (0.007 mmol) in acetonitrile (10 μl). The reaction mixture was heated at 40°C for 5 min, and then

Table 1. Recovery of [9β - 3H]PGF_{2α} (a) from monkey plasma by solvent extraction and (b) from t.l.c. (silica gel), as measured by radioactivity analysis

	PGF _{2α} added		Recovery from monkey plasma (%)	Recovery from t.l.c. (%)
	Wt. (ng)*	Radioactivity (d.p.m.)		
Expt. 1.	0.28	2552	104.6	85.7
	0.57	5208	93.9	95.9
	1.14	10635	93.5	86.7
	2.27	21333	88.7	95.7
	3.41	32122	93.4	93.0
	5.68	53793	93.6	92.7
	10.22	96469	88.3	97.1
			Average = 93.7 ± 5.4	92.4 ± 4.5
Expt. 2	0.21	35961	89.0	83.2
	0.50	86475	98.7	82.4
	0.87	152888	102.3	79.6
	1.12	195577	96.0	82.4
	2.23	401214	90.3	86.6
	3.22	576374	100.8	72.5
	4.22	788414	90.1	77.3
	5.33	975610	96.8	76.3
			Average = 95.5 ± 5.1	80.04 ± 4.5
Expt. 3	0.50	77830	91.5	92.2
	1.12	176070	91.9	88.4
	2.23	369750	93.4	72.2
	3.22	519330	89.1	85.9
	4.22	668950	89.0	82.7
			Average = 91.0 ± 1.9	84.3 ± 7.6
Expt. 4	29.65	92255	90.4	
	29.65	92255	91.1	96.24
	29.65	92255	96.7	82.13
	29.65	92255	88.5	94.11
	29.65	92255	89.5	97.25
			Average = 91.2 ± 3.2	92.4 ± 6.9

* Weights in Expt. 4 are in μg .

evaporated to dryness under N₂. The residue was recycled with the reagents in a manner identical with that indicated above, and evaporated to dryness under N₂.

Tris-trimethylsilyl ether of PGF_{2α} pentafluorobenzyl ester (IIb)

The PGF_{2α} pentafluorobenzyl ester residues from the esterification were converted into the tris-trimethylsilyl ether (IIb) by treatment with *NO*-bis-(trimethylsilyl)acetamide (10 μl) at room temperature (approx. 23°C) for 5 min. Portions (1 μl) of the solution were directly analysed by electron-capture g.l.c.

Internal standard for g.l.c.

The tris-trimethylsilyl pentafluorobenzyl ester of 9-{3 α ,5 α -dihydroxy-2 β -[(3*S*)-3-hydroxy-*trans*-1-octenyl]-1 α -cyclopentyl}-*cis*-non-7-enoic acid (2*a*,2*b*-

dihomo-PGF_{2α}; III*a*) was used as an internal standard for g.l.c. The preparation and characterization of the ester (III*b*) was reported by Wickramasinghe *et al.* (1973). The pentafluorobenzyl ester product (III*b*) was converted into the tris-trimethylsilyl derivative (III*c*) by treatment with *NO*-bis-(trimethylsilyl)acetamide. A solution of the pentafluorobenzyl ester (III*b*) in *NO*-bis-(trimethylsilyl)-acetamide (66.174 $\mu\text{g}/\text{ml}$) was used as the internal standard for g.l.c. Portions (10 μl) of this solution were added to sub-microgram quantities of the PGF_{2α} pentafluorobenzyl ester (II*a*) and mixed well (by using a Vortex mixer). Samples (1 μl) of this solution were used for electron-capture g.l.c. analysis, as indicated below.

Electron-capture g.l.c.

Electron-capture g.l.c. was carried out on a Varian Aerograph, model 1740 gas chromatograph,

Fig. 1. Standard curves for the recovery of $PGF_{2\alpha}$ from monkey plasma

The electron-capture g.l.c. response of $PGF_{2\alpha}$ standards analysed directly after modification and the identical standards analysed after extraction from plasma, t.l.c. purification and modification. Further details are given in the text. Δ , $PGF_{2\alpha}$ standards; $\circ$, $PGF_{2\alpha}$ standards recovered from plasma.

equipped with a ^{63}Ni electron-capture detector, which was operated with a constant (d.c.) polarizing voltage. Spiral glass columns [0.61 m $\times$ 0.28 cm (internal diam.)] packed with 3% OV-1 on 80/100 mesh Gas-Chrom Q were used. High-purity N_2 was used as the carrier gas at a flow rate of 40 ml/min. The column oven was operated isothermally at 255°C; the flash heater at 275°C and the detector at 275°C. Samples of 1 μ l size were analysed. Under these conditions, the tris-trimethylsilyl ether of $PGF_{2\alpha}$ pentafluorobenzyl ester (IIb) and the internal standard (IIIc) had retention times of 8.9 min and 16 min respectively.

Calculations. Peak-height ratios were calculated by dividing the height of the peak of the tris-trimethylsilyl $PGF_{2\alpha}$ pentafluorobenzyl ester (IIb) by the height of the peak of the internal standard (IIIc). Standard curves were constructed by plotting the concentration of $PGF_{2\alpha}$ against the peak-height ratios. Typical standard curves for the recovery of $PGF_{2\alpha}$ from monkey plasma are presented (Fig. 1).

Analysis by measurement of radioactivity

The concentration of $PGF_{2\alpha}$ and/or related metabolites in the plasma samples from the [9β - 3H] $PGF_{2\alpha}$ -treated monkey was determined by radioactivity analysis. Portions (0.5 ml) of the plasma samples taken at various time-intervals were air-dried in cellophane sacs, and combusted by the Schöniger oxygen-flask technique. The resulting tritiated water was counted for radioactivity in Ditol scintillation

solvent [toluene-dioxan-methanol (5:5:3, by vol.) containing naphthalene (73 g), 2,5-diphenyloxazole (4.6 g) and 1,4-bis-(5-phenoxazol-2-yl)benzene (0.08 g) per litre] in a Packard model 314 EX Tri-Carb liquid-scintillation spectrometer. Efficiencies for 3H were determined by the internal-standard technique by using appropriately labelled toluene standards. Calculations of the absolute radioactivity for the 3H in each sample, as well as other routine calculations, were carried out with the aid of an IBM-1800 computer.

Results and Discussion

The electron-capture g.l.c. method developed for the determination of prostaglandins includes the following sequential steps: (a) isolation of the prostaglandins from the biological matrix (by solvent extraction), (b) purification and fractionation of the prostaglandins (by t.l.c.), (c) derivative formation to enhance volatility and sensitivity of detection (conversion into the tris-trimethylsilyl pentafluorobenzyl ester), and (d) measurement (by electron-capture detection).

Isolation of prostaglandin $F_{2\alpha}$

Several methods are currently available for the extraction of prostaglandins from blood and other tissues (Horton, 1972; Shaw & Ramwell, 1969). Many of these methods, however, have not been studied systematically with respect to reproducibility and recovery. Unger and co-workers (Unger *et al.*, 1971; Unger, 1972) have reported that the poor and inconsistent recoveries encountered in the extraction of prostaglandins from blood when protein-precipitating concentrations of ethanol or acetone (70–90%, v/v) were used resulted from binding of the prostaglandin to precipitated serum albumin. These authors also demonstrated that when the initial precipitation of albumin was avoided by the addition of non-precipitating amounts of ethanol (40–50%, v/v) to the plasma, quantitative extraction of the prostaglandin was possible. From this information, sub-microgram quantities of tritiated $PGF_{2\alpha}$ which were added to monkey plasma (in concentrations of 0.28–10.2 ng of [9β - 3H] $PGF_{2\alpha}$ /ml of plasma) were extracted with average recoveries of 93.4%. Similarly, microgram quantities of $PGF_{2\alpha}$ added to plasma (concentrations of 29.65 μ g/ml of plasma) were recovered in 91.2% average yield.

Purification of prostaglandin $F_{2\alpha}$

The crude extract containing the prostaglandin requires further purification and fractionation, before any quantitative g.l.c. determination can be made. Preliminary attempts to analyse the crude plasma

extract by electron-capture g.l.c. after pentafluorobenzyl esterification and silylation (see below for details), without a t.l.c. purification, proved to be unsatisfactory. Although there was no interfering peak corresponding to the retention time of the tris-trimethylsilyl PGF_{2α} pentafluorobenzyl ester (IIb) in the chromatogram, diffuse g.l.c. peaks were observed up to 100min after sample injection. Chromatography on silicic acid (Samuelsson, 1963), Sephadex LH-20 (Anggard, 1971), reverse-phase partition chromatography (Norman & Sjövall, 1958) and t.l.c. (Green & Samuelsson, 1964; Andersen, 1969) have all been used for the purification and separation of prostaglandins. The crude prostaglandin extract (described above) was purified and fractionated by t.l.c. on silica gel. Several solvent systems were examined for the t.l.c. purification and fractionation of the prostaglandins isolated by the extraction procedure (described above). Among them were: (1) ethyl acetate-acetic acid-2,2,4-trimethylpentane-water (9:2:5:10, by vol.) (organic phase), (2) ethyl acetate-methanol-water (8:2:5, by vol.) (organic phase), (3) ethyl acetate-acetic acid-methanol-2,2,4-trimethylpentane-water (11:3:3.5:1:10, by vol.) (organic phase) and (4) chloroform-methanol-acetic acid (8:1:1, by vol.). Solvent system (4) gave the maximum resolution of the PGF zone. Under the conditions used, PGF_{2α} was well resolved from the prostaglandins E, B and A, 15-oxo-PGF_{2α}, 13,14-dihydro-15-oxo-PGF_{2α} and the PGF_{2α}-nor metabolites. Resolution of PGF_{2α} from PGF_{1α} and PGF_{3α} was not achieved. PGF_{2α}, however, was resolved from PGF_{1α} under the conditions of g.l.c. (see below). The PGF_{2α} zones were located and extracted with methanol. The recoveries of [9β-³H]PGF_{2α} from the silica gel were satisfactory (Table 1). Adherence of nanogram quantities of prostaglandins to minute amounts of silica gel carried over in the methanol extract resulted in poor g.l.c. responses on subsequent derivative formation and analysis. The small amount of interfering silica gel was removed from the methanol-extract residue, by the addition of 0.025M-HCl and partition into ether. Traces of acid in the ether extracts were neutralized with an estimated quantity of NH₃. The ether and excess of NH₃ were removed by evaporation under N₂. Failure to neutralize traces of acid in the ether extract containing nanogram (or picogram) quantities of prostaglandin F_{2α} led to losses in g.l.c. response, probably owing to acid-dehydration on concentration. Similarly, attempts to remove the traces of acid from the ether extract by washing with water resulted in loss of the prostaglandin into the aqueous phase.

Derivative formation of prostaglandin F_{2α} and the internal standard (IIIa)

The success of an electron-capture g.l.c. method for the determination of sub-microgram quantities of

prostaglandins depends to a large extent on the ready conversion into a thermally stable derivative of high volatility and significant electron-capture properties. The sub-microgram scale reaction has to be quantitatively consistent and easy to carry out. A difficulty frequently encountered in such derivative formations is the incidence of high backgrounds on electron-capture analysis, which may usually be attributed to traces of excess of reagents responding to detection, by-products, or impurities included during the processing. Purification procedures that may be required to eliminate such traces of interfering contaminants are usually elaborate and time-consuming, thus rendering the method impractical for use for assay purposes.

Methods for the preparation of pentafluorobenzyl esters of carboxylic acids have been reported by Kawahara (1968) and Ehrsson (1971). These procedures, however, are either slow and hence impractical for use in assays, or they lack general applicability. The pentafluorobenzyl ester of PGF_{2α} was prepared by the reaction of PGF_{2α} with pentafluorobenzyl bromide and *NN*-di-isopropylethylamine {[$(\text{CH}_3)_2\text{CH}]_2\text{NC}_2\text{H}_5$] in acetonitrile, at room temperature. The synthetic methods, characterization of the ester and demonstration of its suitability for electron-capture g.l.c. were described by Wickramasinghe *et al.* (1973). A picogram or nanogram quantity of PGF_{2α} recovered from a plasma specimen was converted into the pentafluorobenzyl ester and the latter was converted into the tris-trimethylsilyl ether (IIb) (see the Experimental section for details). Hydrolysis of the trimethylsilyl groups was prevented, and maximum consistency of sample response was achieved, by injecting the analytical samples directly in the *NO*-bis-(trimethylsilyl)acetamide. Although conversion of the PGF_{2α} ester (IIa) into a bromomethyl-dimethylsilyl ether of enhanced electron-capture response is feasible, the excess of halogen-containing silylating reagent would have to be completely eliminated, since even traces may cause 'swamping' of the electron-capture detector (or high backgrounds). Attempts to remove rigorously all traces of the excess of halogen-containing reagent before dissolving in a dry solvent (e.g. ether) invariably results in partial hydrolysis of the trimethylsilyl groups of the sub-microgram sample, and a consequent loss in sample response. In an attempt to select a suitable internal standard for g.l.c. several prostaglandin analogues were converted into their pentafluorobenzyl esters on a micro-scale, and the g.l.c. properties of the tris-trimethylsilyl ethers of these esters were examined. Among those investigated the tris-trimethylsilyl pentafluorobenzyl ester of compound (IIIa) proved to be the most suitable for use as an internal standard. The synthesis of the pentafluorobenzyl ester (IIIb) and its characterization by g.l.c.-mass-spectrometry was described by

Table 2. Recovery of PGF_{2α} from monkey plasma as measured by electron-capture g.l.c. assay

PGF_{2α} was added to plasma as the Tris salt of PGF_{2α}. Recovery was measured after solvent extraction and t.l.c. purification.

PGF _{2α} (free acid equivalents) added (ng/ml of plasma)	PGF _{2α} found (ng/ml of plasma)	Recovery (%)
0.20	0.16	77.7
1.01	1.02	101.0
3.03	2.33	76.9
4.04	3.31	81.9
6.06	5.95	98.2
		Average = 87.1 ± 11.5

Wickramasinghe *et al.* (1973). Although in the present studies the internal standard (IIIa) was dissolved in the silylating agent, and added to PGF_{2α} pentafluorobenzyl ester before the g.l.c. analysis, it is feasible to add it as the free acid at an earlier stage (before t.l.c. or before extraction from plasma). The t.l.c. mobility of the internal standard (free acid) was very similar to that of PGF_{2α} itself ($R_F = 0.61$) in the solvent system chloroform-methanol-acetic acid (8:1:1, by vol.).

Under the g.l.c. conditions used the lower limit of detection was 12.5 pg of the PGF_{2α} pentafluorobenzyl ester, injected 'on-column'. The g.l.c. peak-height response caused by the tris-trimethylsilyl ether of PGF_{2α} pentafluorobenzyl ester (IIb) was linear over the concentration range 0.03–0.84 ng of the PGF_{2α} pentafluorobenzyl ester (IIa) injected 'on-column'.

Determination of prostaglandin F_{2α} in monkey plasma

The analytical methodology developed was applied to the determination of PGF_{2α} in monkey plasma. Monkey plasma samples containing added PGF_{2α} were analysed and a standard curve for the recovery of PGF_{2α} from monkey plasma was constructed (see Fig. 1). The recovery of PGF_{2α} was determined by comparison with a plot of the electron-capture g.l.c. response of identical standards that were not added to plasma, but were converted directly and analysed under the same conditions (see Fig. 1). The recovery of added PGF_{2α} in monkey plasma was satisfactory, as shown in Fig. 1 and Tables 1 and 2. The lower limit of detection sensitivity for added PGF_{2α} in monkey plasma was 0.2 ng/ml. This represents the smallest quantity of PGF_{2α} present in 1 ml of plasma which could be extracted, purified and analysed by electron-capture g.l.c.

The utility of the analytical method developed was demonstrated by application to the determination of

Fig. 2. Plasma concentrations of intact PGF_{2α} and its metabolites

Intact PGF_{2α} (measured by electron-capture g.l.c.) and PGF_{2α} including related metabolites (measured by analysis of radioactivity) were determined after single-dose subcutaneous administration of [³H]PGF_{2α} (15 mg, 143.28 × 10⁶ d.p.m.; 3.5 mg/kg) to a female Rhesus monkey. ○, Concn. of intact PGF_{2α}; △, concn. of PGF_{2α} and related metabolites; □, concn. of metabolites of PGF_{2α}.

Table 3. Plasma concentrations of intact PGF_{2α} in Rhesus monkey after single-dose (3.5 mg of PGF_{2α}/kg body wt.) subcutaneous administration of the prostaglandin, as measured by electron-capture g.l.c.

Time after PGF _{2α} administration (h)	Plasma concn. of intact PGF _{2α} (ng/ml)
0.25	736.03
0.50	200.86
0.75	184.31
1.00	66.03
1.50	12.69
2.00	2.51
2.50	1.96
4.00	<0.5 ng
5.00	0.85
10.00	1.49

PGF_{2α} concentrations in the plasma of a monkey treated with PGF_{2α}. A single dose of [9β-³H]PGF_{2α} (3.4904 mg of PGF_{2α}/kg body wt.) was administered subcutaneously to a female Rhesus monkey. Initial blood samples were collected at 15 min intervals after drug administration, and then at longer intervals. The plasma was harvested from these samples. The total exogenous PGF_{2α} and related metabolite content

Table 4. Analysis of monkey plasma (platelet-free) containing added PGF_{2α} by either (a) the electron-capture g.l.c. assay, (b) radioimmunoassay or (c) g.l.c.-mass spectrometric assay

The radioimmunoassay was that of Cornette *et al.* (1972) and the g.l.c.-mass spectrometry assay was done by the method of Axen *et al.* (1972).

PGF _{2α} added to plasma (ng/ml)	(a) Electron-capture g.l.c. assay		(b) Radioimmunoassay PGF _{2α} found (ng/ml of plasma)	(c) G.l.c.-mass spectro- metric assay PGF _{2α} found (ng/ml of plasma)
	PGF _{2α} found (ng/ml of plasma)	Exogenous PGF _{2α} (ng/ml of plasma)		
(1) 0.00	0.48	0	<0.5	<0.76
(2) 0.81	1.20	0.72	1.22	2.3
(3) 4.88	7.43	6.96	5.8	4.6
(4) 9.75	10.68	10.20	10.3	—
(5) 24.37	28.85	28.37	27.2	18.0
(6) 48.75	61.68	61.20	55.0	43.2

of the plasma samples was determined by measurement of radioactivity. The concentrations of intact PGF_{2α} in the plasma samples (excluding the related metabolites) were determined by electron-capture g.l.c. analysis, as indicated above.

Plasma concentrations of exogenously administered prostaglandin F_{2α} and/or related metabolites (as determined by measurement of radioactivity) are graphically represented in Fig. 2. A maximum plasma concentration of the exogenous PGF_{2α} and related metabolites (2.280 µg of PGF_{2α} equivalents/ml of plasma) was reached rapidly 15min after drug administration. The half-time disappearance of radioactivity in monkey plasma, estimated by semi-log plot, was 54min. The plasma concentrations of intact PGF_{2α} (as determined by the electron-capture g.l.c. assay) are tabulated in Table 3. A maximum plasma intact PGF_{2α} concentration of 0.736 µg/ml was reached at 15min after PGF_{2α} administration. The half-time of disappearance of intact PGF_{2α} in monkey plasma was graphically estimated to be 14min.

The differences between the total prostaglandin content in plasma (determined by measurement of radioactivity) and the concentrations of intact PGF_{2α} (determined by electron-capture g.l.c. assay) were attributed to the metabolites of PGF_{2α}. The peak plasma concentration of the metabolites of PGF_{2α}, which was 1.949 µg of PGF_{2α} equivalents/ml, was reached at 30min after PGF_{2α} administration (see Fig. 2).

Comparison of the electron-capture g.l.c. assay with the radioimmunoassay and the g.l.c.-mass spectrometric assay

It was considered desirable to compare the results of the electron-capture g.l.c. assay with those of the radioimmunoassay (Cornette *et al.*, 1972) and the g.l.c.-mass spectrometric assay (Axen *et al.*, 1972).

Differences in technique (i.e. methods of storage and extraction of the platelet-containing monkey plasma) are known to cause discrepancies in the actual amount of PGF_{2α} present in the samples, owing to biosynthesis and/or release of the prostaglandins from the platelets. Hence, for this experiment, monkey plasma was freed of platelets by centrifugation. To portions of the almost completely platelet-free monkey plasma known amounts of PGF_{2α} were added. Each portion was divided into three parts and analysed by the three methods. The results are summarized in Table 4.

The electron-capture g.l.c. method developed may be extended to the measurement of PGF_{2α} in other matrices or body fluids (e.g. urine), as well as to metabolites of PGF_{2α} (15-oxo-PGF_{2α} and 13,14-dihydro-15-oxo-PGF_{2α} etc.) and other prostaglandins.

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