Br. J. Pharmac. (1977), **61**, 615–626

PATHWAYS OF PROSTAGLANDIN $F_{2\alpha}$ METABOLISM IN MAMMALIAN KIDNEYS

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1 High-speed cytoplasmic supernatants of rat, rabbit, pig and guinea-pig kidneys were prepared and the metabolism of 10 μg/ml prostaglandin $F_{2\alpha}$ labelled with [$^3H_1$-9β]-prostaglandin $F_{2\alpha}$ studied by thin layer radiochromatography and bioassay.

2 The metabolism of prostaglandin $F_{2\alpha}$ measured by radiochromatography parallels biological inactivation in all species except the rabbit.

3 Kidneys metabolize prostaglandin $F_{2\alpha}$ by two divergent pathways, yielding a mixture of prostaglandin E and F metabolites.

4 15-Hydroxyprostaglandin dehydrogenase and prostaglandin Δ-13 reductase are present in all species in characteristic proportions. Thus prostaglandin $F_{2\alpha}$ is metabolized sequentially to 15-keto prostaglandin $F_{2\alpha}$ and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$. The rate and profile of formation of these metabolites is species-dependent.

5 13,14-Dihydro-15-keto prostaglandin $F_{2\alpha}$ is the principal prostaglandin F series metabolite in all species.

6 Pig and guinea-pig kidney contain an unidentified enzyme which converts 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ to 13,14-dihydro prostaglandin $F_{2\alpha}$.

7 Rat kidney contains a high concentration of a prostaglandin 9-hydroxy dehydrogenase which converts 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ to 13,14-dihydro-15-keto prostaglandin E_2.

8 Rabbit kidney contains a novel 9-hydroxydehydrogenase which oxidises prostaglandin $F_{2\alpha}$ directly to E_2, thus producing a compound with more potent renal actions. The possible implications of this enzyme for kidney homeostasis are discussed.

Introduction

The metabolism of prostaglandins is initiated by the sequential action of the enzymes 15-hydroxyprostaglandin dehydrogenase (PGDH) and prostaglandin Δ-13 reductase (Änggård, 1971; Samuelsson, 1972). Both enzymes appear to be distributed ubiquitously in mammalian tissues, with largest concentrations found in lung, kidney and placenta (Änggård, Larsson & Samuelsson, 1971; Marrazzi & Andersen, 1974). Since the enzymes are cytoplasmic, they may be separated easily from the membrane-bound prostaglandin synthetase complex by ultracentrifugation of tissue homogenates (Änggård & Samuelsson, 1967; Änggård *et al.*, 1971). The products of metabolism, 15-keto and 13,14-dihydro-15-keto prostaglandins, show greatly reduced biological potency (Änggård 1966; Crutchley & Piper, 1975, 1976). More recently, it has been found that certain primary prostaglandins may be interconverted in some tissues, resulting in compounds of different or even enhanced biological activity, rather than inactive metabolites Examples include a plasma isomerase which converts prostaglandin A_1 to C_1 (Jones, 1973), a prostaglandin E 9-ketoreductase which converts prostaglandin E_2 to $F_{2\alpha}$ and is found in several tissues (Hamberg & Israelsson, 1970; Leslie & Levine, 1973), and a prostaglandin 9-hydroxydehydrogenase converting prostaglandin $F_{2\alpha}$ metabolites to their corresponding E derivatives and found in rat kidney and rat lung (Pace-Asciak & Miller, 1974; Pace-Asciak, 1975a).

We describe here a study of prostaglandin $F_{2\alpha}$ metabolism in renal homogenates from four species. As yet there are no comparative surveys of species variations in the different pathways of prostaglandin metabolism and their time courses. The kidney was selected as the organ for study since it contains several enzymes implicated in prostaglandin metabolism, and there is evidence that prostaglandins modulate renal physiological processes (Lee, 1973; Terragno, Malik, Nasjletti, Terragno & McGiff, 1976). Interconversion of F to E prostaglandins (or *vice versa*) in the kidney would be of special interest, since prostaglandin E_2 has more

potent effects on renal functions and is the principal prostaglandin formed by the kidney.

The rate of breakdown of prostaglandin $F_{2\alpha}$ and identity of metabolites formed by high-speed cytoplasmic supernatants of renal homogenates were measured by thin layer radiochromatography (radio-t.l.c.) and the results compared by simultaneous measurement of biological activity on a smooth muscle bioassay.

Methods

Kidneys from male rats (Wistar, 300 g), guinea-pigs (Duncan-Hartley, 350 g) and rabbits (New Zealand White, 1.5 kg) were removed rapidly to ice after the animals had been killed; the kidneys were weighed and homogenized in 4 vol ice-cold pH 7.5 phosphate buffer (K_2HPO_4 40 mM, KH_2PO_4 10 mM, disodium edetate (EDTA) 1 mM, cysteine 1 mM) by forcing them past the blades of an Ultra-Turrax homogenizer type 18/2N in three or four strokes (about 5 seconds). The crude homogenate was centrifuged twice at 4°C: first at 3000 *g* for 10 min and again in an MSE Superspeed 50 at 100,000 *g* for 45 minutes. Pellets were usually discarded, and the high-speed supernatant stored overnight at −20°C. Pig kidneys (animal weight and sex not known) were obtained from a local slaughterhouse, and 100,000 *g* supernatants prepared as above.

For metabolism studies, incubations containing 10 μg prostaglandin $F_{2\alpha}$ (28.6 μM) labelled with 0.07–0.13 μCi [$^3H_1$-9_β] prostaglandin $F_{2\alpha}$, and 3.3 mg NAD^+ (5 mM) in each ml of thawed supernatant were started by transfer from ice to a 37°C water bath; 0.2 ml aliquots were withdrawn at timed intervals, added to an ice cold mixture of 0.2 ml ethanol and 0.2 ml 1.0 N formic acid (final pH 3.5) and extracted twice into 0.8 ml ethyl acetate. The organic phase was removed and evaporated in an air stream at 35°C. The residue was dried *in vacuo* and resuspended in 25 μl methanol for t.l.c. on plastic-backed sheets coated with silica gel (Kodak chromagram sheet 13181). The chromatograms were developed in one of the solvents listed in Table 1. Extraction efficiency of prostaglandin $F_{2\alpha}$ was 85.2 ± 1.2% (n=18). Formic acid was used in preference to hydrochloric or citric acids to acidify the samples for extraction because it could be removed completely by evaporation. All three acids were transferred in significant quantities into the ethyl acetate phase during the extraction.

In some experiments samples of incubations were reduced by sodium borohydride after extraction. The extract was dissolved in 0.5 ml methanol, transferred to ice and 10 mg $NaBH_4$ added slowly. After 10 min, the sample was removed from ice and the reaction continued at room temperature for a further 20 minutes. After dilution with 0.75 ml distilled water and acidification to pH 3.5 with 1.0 N formic acid, the samples were re-extracted twice into 1.5 ml ethyl acetate and prepared for t.l.c. as above.

After chromatography together with authentic standards, which were visualized in an iodine chamber (R_F values listed in Table 1), the chromatograms were cut into appropriate sections ('zones') and radioactivity in each zone counted in a Beckman LS-233 liquid scintillation counter using 7 ml scintillant (toluene containing 0.5% 2,5-diphenyloxazole w/v, 0.01% 1,4-di-2-(5-phenyloxazolyl)-benzene w/v and 30% v/v ethoxyethanol). The amount of each metabolite present was calculated after subtraction for background as the percentage of the total radioactivity recovered from each timed extract.

To determine the conversion of prostaglandin $F_{2\alpha}$ to non-radioactive prostaglandin E-series compounds, 0.05 ml samples from the incubation mixtures were

Table 1 Solvent systems for thin layer chromatography and R_F † values of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and metabolites

	*Solvent system**		
	F6	*P–A*	*A–IV*
$PGF_{2\alpha}$	0.20	0.21	0.14
13,14-Dihydro $PGF_{2\alpha}$	0.27	0.32	0.22
15-Keto $PGF_{2\alpha}$	0.34	0.36	0.23
13,14-Dihydro-15-keto $PGF_{2\alpha}$	0.41	0.41	0.35
PGE_2	0.38	0.36	0.29

* F6 = ethyl acetate : acetone : glacial acetic acid 90 : 10 : 1. 75 minutes. (Andersen, 1969.)
P–A = chloroform : methanol : glacial acetic acid : water 90 : 9 : 1 : 0.65. 60 minutes. (Pace-Asciak, Morawska & Wolfe, 1970.)
A–IV = (upper phase of) ethyl acetate : benzene : glacial acetic acid : 2,3,4-trimethylpentane : water 80 : 30 : 10 : 30 : 100. 90 minutes. (Gréen & Samuelsson, 1964.)

† R_F values are means of 6–12 observations. Standard errors are less than 5% of mean in all cases.

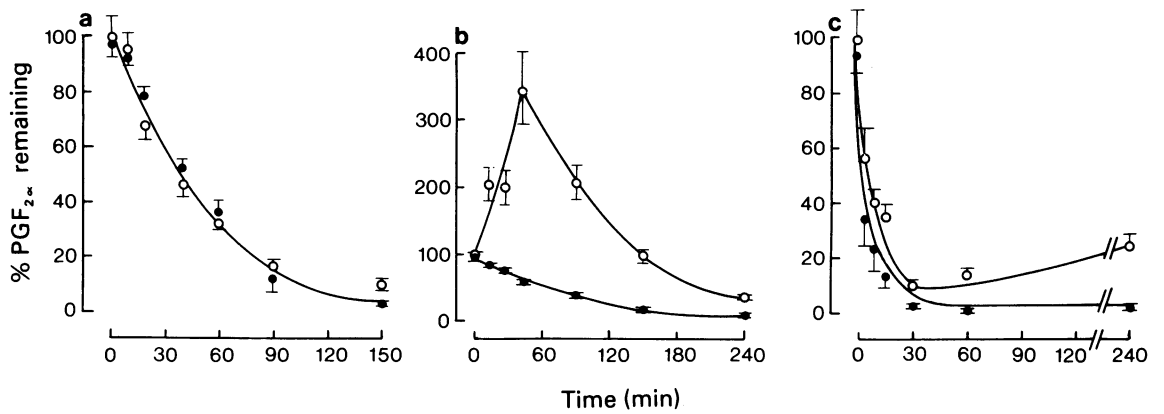

Figure 1 Metabolism of 10 μg/ml prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) labelled with [$^3H_1$-9β]-$PGF_{2\alpha}$ by high-speed supernatants of kidneys from (a) rats, (b) rabbits, and (c) guinea-pigs measured by biological assay (O) and by thin-layer radiochromatography (●). Metabolism is expressed as % $PGF_{2\alpha}$ remaining in the incubation, and results show mean from 6 experiments, using kidneys from 3–6 male animals, with 6 determinations for each radio-t.l.c. point and 6–21 for each bioassay point. Vertical lines show s.e. means.

removed, added to 0.1 ml ethanol to terminate enzyme activity and evaporated *in vacuo* to a residue to ensure removal of water. The sample was then dissolved in methanol, and an aliquot taken for scintillation counting. In other experiments, the total radioactivity of each sample appearing in all zones of the chromatogram after extraction and thin-layer chromatography was taken as a measure of F-series prostaglandins. For both methods, the conversion of prostaglandin $F_{2\alpha}$ to E-series prostaglandins was calculated by subtracting these values from the value obtained from a sample taken at time zero and which was carried through the same extraction procedure, and in which all the recovered label is present as unchanged prostaglandin $F_{2\alpha}$.

Samples of the incubation mixtures (0.5–5 μl) as appropriate) were withdrawn at the same timed intervals for immediate biological assay on the rat fundus strip preparation. Strips were mounted in 2.5 ml organ baths at 37°C and bathed in a Krebs solution (composition (mM): NaCl 65.3, KCl 4.75, KH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25, $CaCl_2$ 1.9 and glucose 11.1) gassed with 95% O_2 and 5% CO_2 and containing antagonists (propranolol 0.77 μM, atropine 0.72 μM, mepyramine 0.7 μM, methysergide 0.57 μM, and phenoxybenzamine 0.33 μM) to block the effects of other agonists and indomethacin (2.8 μM) to inhibit the generation of prostaglandins by the tissues themselves and to increase their sensitivity to exogenous postaglandins. Contractions were recorded isometrically with an applied tension of 2.0–4.0 g using Grass FT 10 isometric strain gauges coupled to a four channel Grass polygraph. The tissues were cut into shorter and broader strips than is usual with the rat stomach strip preparation because we found that this gave more reliable and sensitive preparations under isometric conditions. The tissues were generally sensitive to 0.2–0.4 ng/ml prostaglandin E_2 or to 2.0–4.0 ng/ml $F_{2\alpha}$. Relaxation after washout by overflow was rapid, and afforded a dose-cycle time of 3 to 4 minutes. Unknown samples from the renal homogenate incubations were assayed without extraction on three or four tissues simultaneously by bracketing between doses of standard.

Protein concentrations were measured using Folin's reagent (Lowry, Rosebrough, Farr & Randall, 1951) with bovine serum albumen as standard.

The following drugs were used: β-nicotinamide adenine dinucleotide (NAD^+) and indomethacin (Sigma), methysergide bimaleate (Sandoz), atropine sulphate (Wellcome), phenoxybenzamine hydrochloride (SK & F), mepyramine maleate (M & B), propranolol hydrochloride (ICI). Other laboratory chemicals were analytical grade where appropriate. Prostaglandins and prostaglandin metabolites were generous gifts from the Upjohn Company and from May & Baker Ltd, and [$^3H_1$-9β]-prostaglandin $F_{2\alpha}$ (specific activity 81.6 mCi/mmol) was provided by Dr A. Brash.

Results

Metabolism of prostaglandin $F_{2\alpha}$

Metabolism of prostaglandin $F_{2\alpha}$ by high-speed supernatants of rat, rabbit, and guinea-pig kidneys were measured by radio-t.l.c. and by bioassay. The radio-t.l.c. results show that more than 95% of 10 μg/ml prostaglandin $F_{2\alpha}$ was metabolized within the 150–240 min incubation period in all species

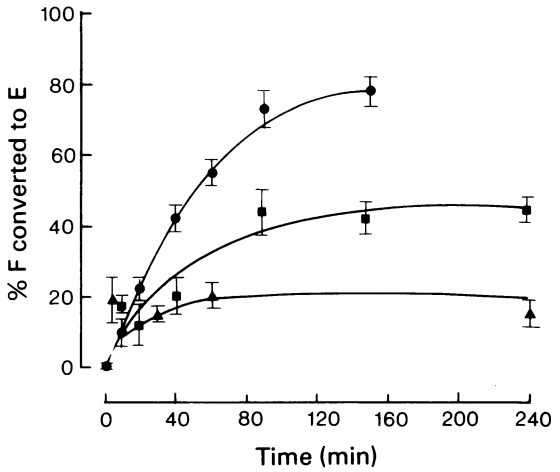

Figure 2 Conversion of 10 μg/ml prostaglandin $F_{2\alpha}$ (or $PGF_{2\alpha}$ metabolite) to E-series prostaglandins by oxidation at C-9 and removal of tritium label in high-speed renal supernatants of rat (●), rabbit (■) and guinea-pig (▲). Conversion to E-series prostaglandins is derived from the % loss of radioactivity of the extracted samples, as explained under methods. Results show mean for 6 experiments on kidneys from 3–6 male animals. Vertical lines show s.e. means.

(Figure 1). The rate of metabolism expressed in terms of soluble protein was much greater in the guinea-pig (112.8 ± 21.3 ng min^{-1} mg^{-1} protein, $n=6$) than in the rat (8.05 ± 0.66 ng min^{-1} mg^{-1} protein, $n=6$) and rabbit (7.03 ± 1.08 ng min^{-1} mg^{-1} protein, $n=6$), when calculated from the initial linear portion of the curves. Prostaglandin $F_{2\alpha}$ was not broken down when it was incubated with buffer alone or with boiled kidney high-speed supernatants of the three species. Metabolism of prostaglandin $F_{2\alpha}$ by kidney microsomal pellets of rat, rabbit and guinea-pig was less than 10% of that observed with the corresponding high-speed supernatants.

The results obtained by simultaneous bioassay showed good agreement with the radio-t.l.c. data for all points obtained for the rat kidney incubations (Figure 1a) and for all but the last two of the guinea-pig (Figure 1c). By contrast, there was no agreement in the case of the rabbit, as there was a large increase in the observed biological activity of the reaction mixture which increased to $346.7 \pm 59\%$ ($n=18$) of the initial level at 40 min and thereafter declined steeply over the next 200 min (Figure 1b).

Conversion of prostaglandin $F_{2\alpha}$ to prostaglandins of the E-series

Prostaglandin $F_{2\alpha}$ (or metabolites) may be converted to E-type prostaglandins by oxidation of the secondary alcohol group at C-9 to a ketone. In the case of the radiolabelled [^{3}H-9β]-prostaglandin $F_{2\alpha}$ used here, the tritium label at the 9-β position is removed, and the oxidation may be measured as a progressive decline in the total radioactivity in the incubation mixture after evaporating off the resulting tritiated water. Tritium loss, i.e. conversion of prostaglandin $F_{2\alpha}$ (or metabolite) to a non-radioactive prostaglandin of the E series, occurred in all three species examined (Figure 2), especially in the rat kidney. In this species, about 80% of the prostaglandin $F_{2\alpha}$ was metabolized by this pathway.

Identification of metabolites derived directly from prostaglandin $F_{2\alpha}$

Radioactive metabolites formed from prostaglandin $F_{2\alpha}$ were separated and identified by radio-t.l.c. in several solvent systems. In our hands, F6 was the best solvent system because it resolved satisfactorily the principal metabolites 15-keto prostaglandin $F_{2\alpha}$ and 13,14-dihydro-prostaglandin $F_{2\alpha}$ from the parent prostaglandin $F_{2\alpha}$ (Table 1).

Rat. In six experiments using rat kidney homogenates, there was a steady accumulation of

Table 2 Identification of F-series metabolites formed from prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in rat kidney homogenates, using three t.l.c. solvent systems

Incubation time (min)	*20*		*40*		*60*		*90*	
t.l.c. solvent system used†	*F6*	*P–A*	*F6*	*A–IV**	*F6*	*P–A*	*F6*	*A–IV**
$PGF_{2\alpha}$	79.2	69.5	58.6	66.4	36.3	38.3	15.3	15.7
13,14-Dihydro $PGF_{2\alpha}$	0.5	1.0	0	10.0	0.4	0.7	0	0.1
15-Keto $PGF_{2\alpha}$	5.1	3.0	12.3		8.9	4.4	6.2	
13,14-Dihydro-15-keto $PGF_{2\alpha}$	11.0	14.4	19.2	19.3	50.0	44.4	60.7	79.4

Amount of each metabolite expressed as % of total radioactivity recovered for that sample; values are for single experiments taken from those included in Figure 1 and Figure 3.
*This solvent system does not resolve 13,14-dihydro $PGF_{2\alpha}$ and 15-keto $PGF_{2\alpha}$. †For details of solvent systems and R_F values, see Table 1.

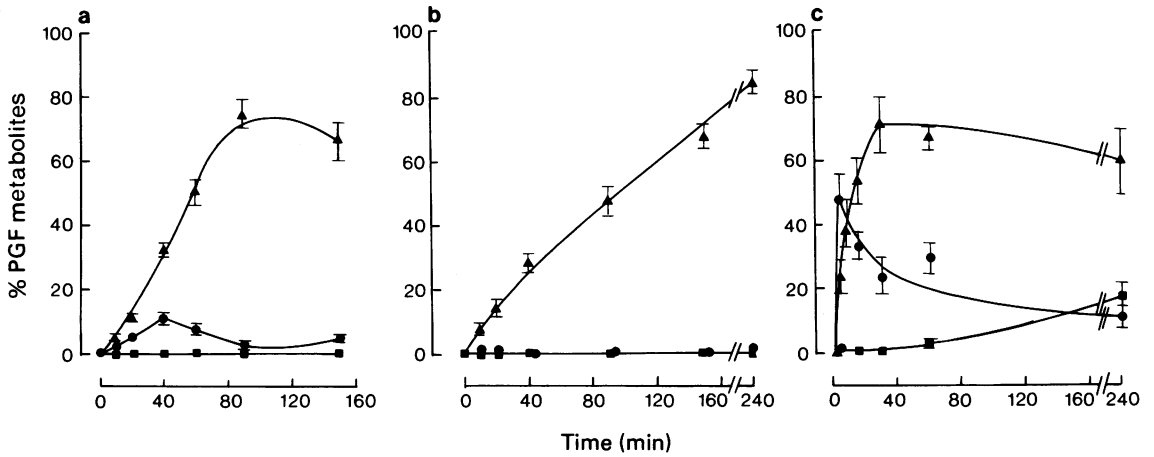

Figure 3 Radioactive F-series metabolites formed from 10 μg/ml [$^3H_1$-9β]-prostaglandin $F_{2\alpha}$ incubated at 37°C with renal high-speed supernatants of (a) rat, (b) rabbit and (c) guinea-pig. Metabolites are expressed as % of total radioactive material recovered after separation by thin-layer radiochromatography in solvent system F6: (●)=15-keto $PGF_{2\alpha}$; (■)=13,14-dihydro $PGF_{2\alpha}$; (▲)=13,14-dihydro-15-keto $PGF_{2\alpha}$. Results show mean of 6 experiments using kidneys from 3–6 male animals. Vertical lines shown s.e. means.

13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ up to 150 min (Figure 3a). Small amounts of 15-keto prostaglandin $F_{2\alpha}$ were also detected in the early stages of the incubation, and the remainder of the radioactivity on the plate was associated with the starting material, prostaglandin $F_{2\alpha}$. However, at the end of the incubation period, these radioactive metabolites accounted only for about a quarter of the total starting material since most of the prostaglandin $F_{2\alpha}$ was converted to compounds of the E series (Figure 2). The presence of these other metabolites towards the end of the incubation reduced the accuracy of the chromatography: thus, some 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ migrated further than the authentic standard, and the proportion of this metabolite at 90–150 min shown in Figure 3a is probably underestimated.

Further evidence for the identity and sequence of metabolites formed from prostaglandin $F_{2\alpha}$ by rat kidney was obtained by chromatography of selected samples in two other solvents in which the mobilities of the three metabolites differ relative to each other and to prostaglandin E_2 and $F_{2\alpha}$. These experiments confirmed that the principal metabolite co-chromatographed with 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$, and that small quantities of 15-keto prostaglandin $F_{2\alpha}$ appeared early in the incubation (Table 2).

Rabbit. The radioactive metabolites of prostaglandin $F_{2\alpha}$ accounted for just over half of the total formed during the incubation; the remainder was converted to metabolites of the E series (Figure 2). Of the radioactive prostaglandin F material, there was a

Table 3 Identification of F-series metabolites formed from prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in rabbit kidney homogenates, using three t.l.c. solvent systems

Incubation time (min)	*50*				*240*			
t.l.c. solvent system used†	*F6*		*P–A*	*A–IV**	*F6*		*P–A*	*A–IV**
$PGF_{2\alpha}$	68.3	(64.7)‡	63.7	68.6	2.9	(6.5)‡	2.5	2.7
13,14-Dihydro $PGF_{2\alpha}$	0	(29.5)	0.8	0.2	0	(71.2)	5.3	3.3
15-Keto $PGF_{2\alpha}$	1.8	(0.7)	3.5		8.7	(3.7)	5.7	
13,14-Dihydro-15-keto $PGF_{2\alpha}$	29.2	(1.4)	27.2	30.6	60.2	(10.1)	84.3	78.8

Amount of each metabolite expressed as % of total radioactivity recovered for that sample; values are for single experiments taken from those included in Figure 1 and Figure 3.

*This solvent does not resolve 13,14-dihydro $PGF_{2\alpha}$ and 15-keto $PGF_{2\alpha}$. † For details of solvent systems and R_F values, see Table 1. ‡ Numbers in brackets refer to % of total radioactivity in specified zone after borohydride reduction.

progressive accumulation with time of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (Figure 3b). This was the only radioactive metabolite detected in significant amounts, and further evidence for its identity was obtained by chromatography in two alternative solvents (Table 3). In addition, samples obtained at 50 and 240 min were treated after extraction with sodium borohydride which reduces the C-15 ketone group of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ to yield 13,14-dihydro prostaglandin $F_{2\alpha}$ (and its C-15 epimer) but does not affect prostaglandin $F_{2\alpha}$ itself. After borohydride reduction, radioactivity associated with the 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ zone was lost, and it reappeared in the 13,14-dihydro prostaglandin $F_{2\alpha}$ zone as predicted (Table 3).

Guinea-pig. The sequence of metabolites formed from prostaglandin $F_{2\alpha}$ by guinea-pig kidney homogenates was more complex than in the rat or rabbit, and showed more quantitative variation between animals (Figure 3c). After 5–15 min incubation, most radioactivity was found in the zone corresponding to 15-keto prostaglandin $F_{2\alpha}$. The amount of 15-keto prostaglandin $F_{2\alpha}$ detected was greatest in those preparations in which prostaglandin $F_{2\alpha}$ was metabolized most rapidly. Thus, in one animal, 95.8% prostaglandin $F_{2\alpha}$ was metabolized by 5.5 min, and 77.8% of the radioactivity appeared in the 15-keto prostaglandin $F_{2\alpha}$ zone with 12.6% as 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (mean of two experiments). By contrast, in another animal only 41.6% prostaglandin $F_{2\alpha}$ was metabolized at 5.5 min and, of this, the majority appeared as 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ rather than 15-keto prostaglandin $F_{2\alpha}$ (26.8% and 12.6% respectively, mean of two experiments).

As the incubation proceeded, radioactivity shifted from the 15-keto prostaglandin $F_{2\alpha}$ zone to that corresponding to 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$. Towards the end of the incubation, at 60–240 min, some radioactivity was detected in a third zone migrating just ahead of the prostaglandin $F_{2\alpha}$ starting material, and with an R_F value corresponding to 13,14-dihydro prostaglandin $F_{2\alpha}$ (Figure 3c). Chromatography of selected samples in two other solvents gave results consistent with the sequential formation of three different metabolites (Table 4).

We attempted to identify these three metabolites in two further experiments by treating half of each timed sample with sodium borohydride before chromatography. As expected, the radioactive material which behaved as 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ before reduction appeared in the 13,14-dihydro prostaglandin $F_{2\alpha}$ zone after borohydride treatment (Figure 4), and much of that corresponding to 15-keto prostaglandin $F_{2\alpha}$ produced in the early samples appeared after reduction to co-chromatograph with prostaglandin $F_{2\alpha}$ (Figure 4).

The third metabolite (provisionally identified by radio-t.l.c. as 13,14-dihydro prostaglandin $F_{2\alpha}$) appeared after 60–240 min incubation, at a time when the biological activity had increased significantly from the minimum value at 30 min of 1.06 ± 0.15 μg/ml prostaglandin $F_{2\alpha}$ equivalent ($n=8$) to 2.35 ± 0.33 μg/ml prostaglandin $F_{2\alpha}$ equivalent ($n=6$) at 240 min, $P<0.01$ (see also Figure 1c). In separate experiments, the biological activity of authentic 13,14-dihydro prostaglandin $F_{2\alpha}$ on the rat fundus assay tissue was shown to be considerably greater than that of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (relative activities of $21 \pm 1\%$ and $7 \pm 4\%$ respectively compared to prostaglandin $F_{2\alpha} = 100\%$, strips prepared from 5 animals), lending support to the proposed identity of the third metabolite as 13,14-dihydro prostaglandin $F_{2\alpha}$.

Direct conversion of prostaglandin $F_{2\alpha}$ to E_2 by the rabbit kidney

The breakdown of prostaglandin $F_{2\alpha}$ by the rabbit kidney was accompanied by a large increase in the biological activity of the incubation (Figure 1b). There

Table 4 Identification of F-series metabolites formed from prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in guinea-pig kidney homogenates, using three t.l.c. solvent systems

Incubation time (min	*5.5*			*240*		
t.l.c. solvent system used†	*F6*	*A–IV**		*F6*	*P–A*	*A–IV**
$PGF_{2\alpha}$	43.0	49.2		0	0	0
13,14-Dihydro $PGF_{2\alpha}$	2.0	40.3		7.4	12.1	10.3
15-Keto PGF	32.5			8.2	6.4	
13,14-Dihydro-15-keto $PGF_{2\alpha}$	15.0	5.5		72.1	67.6	74.8

Amount of each metabolite expressed as % of total radioactivity recovered for that sample; values are for single experiments taken from those included in Figure 1 and Figure 3.
* This solvent system does not resolv13,14-dihydro $PGF_{2\alpha}$and 15-keto $PGF_{2\alpha}$. † For details of solvents and R_F values, see Table 1.

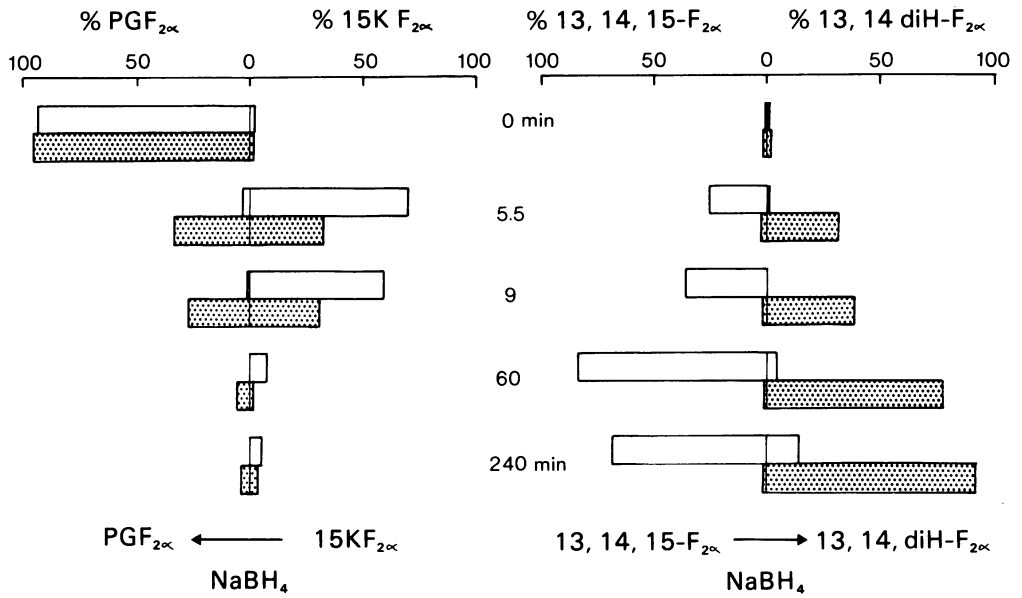

Figure 4 Sequential formation of three F-series metabolites formed from 10 μg/ml prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) labelled with [$^3H_1$-9β]-$PGF_{2\alpha}$ by high-speed supernatants of guinea-pig kidney homogenates. Samples were taken at the times indicated, extracted and chromatographed in solvent system F6 before (open columns) and after (shaded columns) reduction with sodium borohydride. Metabolites are expressed as % of total radioactive material recovered from each sample, and the results show the means of two experiments. The chemical transformations expected after borohydride reduction are indicated on the figure (15K-$F_{2\alpha}$ = 15-keto $PGF_{2\alpha}$; 13,14diH-$F_{2\alpha}$ = 13,14-dihydro $PGF_{2\alpha}$; 13,14,15-$F_{2\alpha}$ = 13,14-dihydro-15-keto $PGF_{2\alpha}$).

was also a significant loss of tritium from the 9-position of the labelled prostaglandin $F_{2\alpha}$ (Figure 2), suggesting conversion to a prostaglandin of the E series. Since the potency of prostaglandin E_2 on the rat fundus strip is greater than that of $F_{2\alpha}$ by a factor of 5.8 ± 0.5 (strips prepared from 11 animals), these results could be explained by a direct conversion of prostaglandin $F_{2\alpha}$ to E_2.

This hypothesis was tested in two further experiments by assaying the biological activity of material eluted from different regions of chromatograms prepared from samples taken at 50 min (at the height of the increased biological activity) and at 150 min, when most activity had disappeared. In the first experiment (Figure 5), when F6 was used as the chromatography solvent, 82.0% of the biological activity in the 50 min sample was found in the zone in which prostaglandin E_2 and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ co-chromatograph. When the A-IV system was used to resolve prostaglandin E_2 from 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (see Table 1), 83.5% of the activity was found in the zone corresponding to prostaglandin E_2, but only 3.8% continued to co-chromatograph with 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$. Similar results were obtained in the second experiment; in addition, after reduction of a portion of the extracted material with sodium borohydride, 88% of the biological activity was found in the prostaglandin $F_{2\alpha}$ and $F_{2\beta}$ zone, as expected if the biologically active material before reduction corresponded to prostaglandin E_2. In both experiments samples taken at 150 min showed only traces of biological activity in the prostaglandin $F_{2\alpha}$ and E_2 zones (<50 ng in each case).

Pig kidney homogenates

Radio-t.l.c. experiments were performed on 100,000 *g* supernatants prepared from the kidneys of 4 pigs. These animals could not be age or sex-matched. The homogenates rapidly metabolized prostaglandin $F_{2\alpha}$, with an initial rate calculated from 10 radio-t.l.c. experiments of 176.3 ± 23.3 ng min^{-1} mg^{-1} protein (i.e. the fastest rate of all four species investigated). Analysis by t.l.c. of the fate of the radiolabelled prostaglandin $F_{2\alpha}$ in samples withdrawn at various times up to 240 min showed that three different metabolites were formed in sequence. The sequence observed in four experiments was very

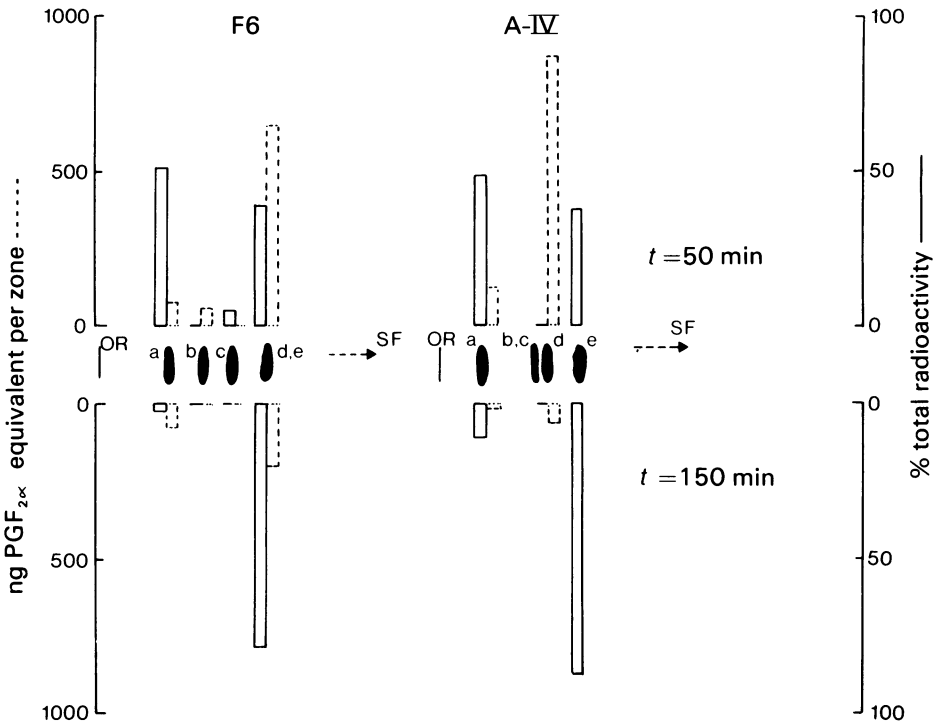

Figure 5 Identification of prostaglandin E_2 (PGE_2) formed directly from 10 μg/ml radiolabelled prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by rabbit renal homogenate. The incubation was extracted after 50 min or 150 min at 37°C (biological activity = 28.6 and 9.3 μg/ml $PGF_{2\alpha}$ equivalent, respectively) and radiolabelled PGF metabolites identified by t.l.c. in solvents systems F6 and A-IV (open columns). Part of each chromatogram was extracted then dissolved in Krebs solution, and assayed on three rat fundus strips (dashed columns) using PGE_2 and $PGF_{2\alpha}$ as standards. The figure shows one of two identical experiments, and the positions of the prostaglandin standards after t.l.c. are drawn approximately to scale (a = $PGF_{2\alpha}$; b = 13,14-dihydro $PGF_{2\alpha}$; c = 15-keto $PGF_{2\alpha}$; d = PGE_2; e = 13,14-dihydro-15-keto $PGF_{2\alpha}$; OR = origin; SF = solvent front).

similar to that of the guinea-pig illustrated in Figure 3c. In the early stages of the incubation, from 1 to 40 min, large amounts of 15-keto prostaglandin $F_{2\alpha}$ were detected (e.g. 54.1 ± 1.7% at 15 min), followed by the formation of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ which reached a maximum of 82.0 ± 0.9% at 120 minutes A third product, identified as 13,14-dihydro prostaglandin $F_{2\alpha}$, was formed in significant quantities (10–25%) during the last part of the incubation. In one experiment, the biological activity of the incubation was measured by simultaneous bioassay of unextracted samples on four rat fundus strips. Assay of six samples showed that the biological activity of this incubation declined rapidly from 11.1 ± 3.5 μg/ml prostaglandin $F_{2\alpha}$ equivalent to 0.53 ± 0.04 μg/ml in the first 10 min of incubation, but later increased significantly as the incubation proceeded (e.g. to 0.98 ± 0.09 μg/ml prostaglandin $F_{2\alpha}$ at 160 min, $P < 0.05$). This increase in biological activity is similar to that observed in the guinea-pig experiments (see Figure 1c), since it coincided with the formation of 13,14-dihydro prostaglandin $F_{2\alpha}$ in the latter part of the incubations.

Further analysis of prostaglandin $F_{2\alpha}$ metabolites extracted from a large scale incubation of pig renal homogenate sampled at 15 and 120 min was performed by radio-t.l.c. in several t.l.c. solvents, both before and after sodium borohydride reduction. These experiments confirmed that 15-keto prostaglandin $F_{2\alpha}$ was the predominant metabolite of prostaglandin $F_{2\alpha}$ at 15 min, whereas 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ was the major metabolite formed at 120 minutes. Conversion of prostaglandin $F_{2\alpha}$ to non-radioactive prostaglandins of the E series by pig renal homogenates did not appear to be important quantitatively, but this was not examined in detail.

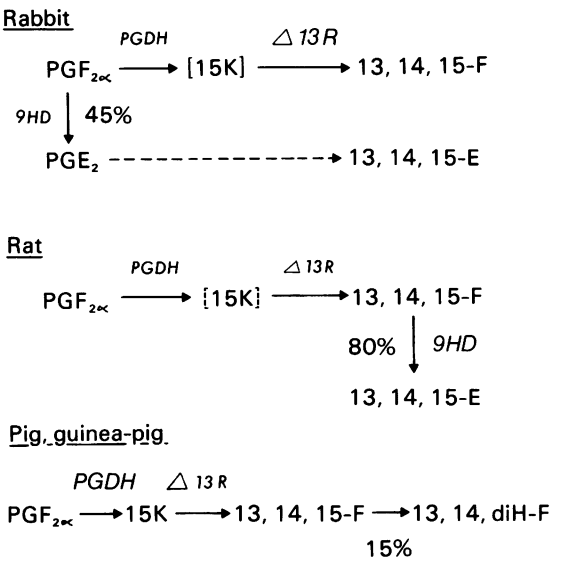

Figure 6 Pathways of kidney prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) metabolism *in vitro* in rat, rabbit, pig and guinea-pig. Solid lines indicate reactions which were identified in the present study; dashed lines represent those which are assumed to take place. Enzymes are indicated by italicized abbreviations; the size of the symbol indicates the relative abundance of the enzyme. Metabolites which were not detected are enclosed by square brackets; those which appeared fleetingly are enclosed by dashed square brackets. (15K = 15-keto $PGF_{2\alpha}$; 13,14 diH-F = 13,14-dihydro $PGF_{2\alpha}$; 13,14,15-F = 13,14 dihydro-15-keto $PGF_{2\alpha}$; 13,14,15-E = 13,14 dihydro-15-keto PGE_2; PGDH = prostaglandin 15-dehydrogenase; Δ13R = prostaglandin Δ-13 reductase; 9HD = prostaglandin 9-hydroxydehydrogenase).

Discussion

The present experiments were designed to compare renal prostaglandin $F_{2\alpha}$ metabolism in four mammalian species. The results show species variations both in the rate of prostaglandin $F_{2\alpha}$ breakdown and in the pathways of metabolism, and the principal findings are summarized in Figure 6.

We have also observed species differences in the rate of prostaglandin E_2 metabolism in rat, rabbit and guinea-pig kidney homogenates (Hoult & Moore, unpublished data). In all species, the initial rate of prostaglandin E_2 metabolism was faster than for $F_{2\alpha}$ and was in the ratio 50 : 5 : 1 (guinea-pig : rat : rabbit).

Figure 6 shows that there are two divergent pathways of prostaglandin $F_{2\alpha}$ metabolism in mammalian kidney *in vitro*, of which a two or three step conversion to metabolites of the F series is most usual. Alternatively, oxidation to a prostaglandin E compound may occur, and there is evidence that the cross-over may take place at more than one stage in the prostaglandin F metabolite sequence, so that the prostaglandin E compound may itself suffer further metabolic transformation (see below).

In the pig, guinea-pig and rabbit most prostaglandin $F_{2\alpha}$ is converted to F-series metabolites retaining the tritium label at the 9-position. In the rat, about 80% of the final products formed from prostaglandin $F_{2\alpha}$ are E series metabolites lacking the tritium label at C-9, and hence cannot be characterized further by radiochromatography. Nevertheless, the principal prostaglandin metabolite in the rat was found to be 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ as in the other three species, and further support for its identity in all species was provided by co-chromatography with authentic 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ in three t.l.c. solvents, by biological assay and by co-chromatography of the product after borohydride reduction with 13,14-dihydro prostaglandin $F_{2\alpha}$. Conclusive identification could be obtained from mass spectrometric analysis.

It is commonly accepted that 13,14-dihydro-15-keto metabolites of prostaglandins arise from the sequential action of PGDH, which converts the parent prostaglandin to the 15-keto derivative, and Δ-13 reductase which yields the 13,14-dihydro-15-keto compound. This forms an effective pathway for the biological inactivation of prostaglandins since both the 15-keto and 13,14-dihydro-15-keto derivatives possess only 1–10% or even less of the potency of the parent prostaglandins in most systems (Änggård, 1966; Crutchley & Piper, 1975; 1976). The experiments on pig and guinea-pig renal homogenates support this suggested metabolic pathway, since there was clear evidence of the sequential formation of 15-keto prostaglandin $F_{2\alpha}$ and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$, and this coincided with rapid biological inactivation. In the rat, a small peak of 15-keto prostaglandin $F_{2\alpha}$ appeared before the increase in 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ levels. We were unable to detect 15-keto prostaglandin $F_{2\alpha}$ in rabbit renal homogenates, and the sole radioactive metabolite identified was 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$.

These results emphasize that there are species differences not only in the absolute amounts of the two enzymes PGDH and Δ-13 reductase (accounting for variations in the rates of prostaglandin $F_{2\alpha}$ disappearance and in the rates of formation of the two metabolites) but also in their relative proportions, since it is the 'PGDH/Δ-13 reductase ratio' which determines the profile of metabolites formed. The PGDH/Δ-13 reductase ratio is high in guinea-pig and pig kidney, intermediate in the rat, and lowest in the rabbit. Similar conclusions were obtained for pig kidney (Änggård & Larsson, 1971; Änggård *et al.*, 1971) using different methods and prostaglandin E_1

as substrate, and for the rat kidney (Pace-Asciak, 1975b). Such findings also illustrate that misleading results may be obtained when measuring prostaglandin metabolism or PGDH activity in terms of the formation of 15-keto prostaglandins or NADH in crude cytoplasmic homogenates unless suitable precautions are taken to correct for product utilization by other enzymes. Other authors have recognized that the problem may be circumvented by measuring the enzymatic steps independently (e.g. Änggård *et al.*, 1971) or by attempting to prevent the subsequent conversions (e.g. Sun & Armour, 1974).

A third enzyme appears to be present in both pig and guinea-pig kidney, since a compound co-chromatographing with 13,14-dihydro prostaglandin $F_{2\alpha}$ in several solvent systems was detected in later samples taken from incubations of both species. The appearance of this metabolite coincided with an increase in biological activity of the incubation and it is known that 13,14-dihydro metabolites have more potent biological actions than 15-keto and 15-keto-13,14-dihydro metabolites (Änggård, 1966; Crutchley & Piper, 1975; 1976; this paper). The timing of the appearance of 13,14-dihydro prostaglandin $F_{2\alpha}$ in our experiments suggests that it is formed from 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ by reduction of the 15-keto group formed earlier in the metabolic sequence, rather than by direct reduction of the 13,14 double bond of prostaglandin $F_{2\alpha}$. It has been shown previously that reduction of the 13,14 double bond of the prostaglandin molecule in guinea-pig lung does not occur directly, but takes place after activation of the double bond by previous oxidation of the 15-hydroxyl group (Hamberg & Samuelsson, 1971).

Although either PGDH or prostaglandin E 9-ketoreductase might conceivably catalyse the conversion of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ to 13,14-dihydro prostaglandin $F_{2\alpha}$, the reaction probably represents the catalytic action of a hitherto unidentified enzyme. Partially purified PGDH has been shown to convert 15-keto prostaglandin E_1 back to prostaglandin E_1 (Marrazzi, Shaw, Tao & Matschinsky, 1972; Yamazaki & Sasaki, 1975) but the reaction proceeds better at low pH and is inhibited by NAD^+ which was present at 5 mM in our experiments. A prostaglandin E 9-ketoreductase has been found in kidneys from several species (Leslie & Levine, 1973; Lee, Pong, Katzen, Wu & Levine, 1975) with high concentrations in rabbit kidney (Stone & Hart, 1975). However, it does not seem to be a plausible candidate for catalysis of a reduction at C-15, especially since we did not observe 13,14-dihydro prostaglandin $F_{2\alpha}$ formation in rabbit kidney supernatants, and 9-ketoreductase activity is NADH or NADPH dependent.

Although the interconversion of E-type with F-type prostaglandins by prostaglandin E 9-ketoreductase occurs in many tissues and is now well documented (Lee & Levine, 1974; Hensby, 1974), there are few reports of the conversion of prostaglandin F compounds to E derivatives. Pace-Asciak & Miller (1974) have detected such a conversion in rat lung and rat kidney, and have shown that the substrate for the rat kidney enzyme (i.e. prostaglandin 9-hydroxydehydrogenase) is 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (Pace-Asciak, 1975a). Therefore, this pathway in the rat seems to have few implications physiologically, since both the 13,14-dihydro-15-keto metabolites of prostaglandin $F_{2\alpha}$ and E_2 are almost inactive on all systems so far tested. We have confirmed that rat kidney homogenates oxidize the 9-hydroxyl group of [$^3H_1$-9β] labelled prostaglandin $F_{2\alpha}$, resulting in non-radioactive products but we were unable to confirm that the reaction occurred subsequent to the formation of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$. However, the fact that the biological activity of the incubations declined rapidly and in step with the removal of prostaglandin $F_{2\alpha}$, as determined by radiochromatography, argues against the possibility of direct conversion of prostaglandin $F_{2\alpha}$ to E_2.

We have also detected 9-hydroxydehydrogenase activity in the rabbit kidney. However, there is clear evidence in this species of a direct conversion of prostaglandin $F_{2\alpha}$ to E_2, since incubation of the renal homogenates with prostaglandin $F_{2\alpha}$ was followed by a large increase in biological activity. A substance which had biological and chromatographic properties indistinguishable from authentic prostaglandin E_2 could be extracted from the incubations at the peak of their biological activity, but not from samples withdrawn at later times when the biological activity had declined. This suggests that prostaglandin E_2 is itself subsequently metabolized, and this has been confirmed in separate experiments.

These findings represent the first evidence for a direct and quantitatively important conversion of prostaglandin $F_{2\alpha}$ to E_2 and on this basis the rabbit enzyme may be distinguished from rat prostaglandin 9-hydroxydehydrogenase. Rabbit and other mammalian kidneys are capable of the synthesis and release of prostaglandin E_2 and $F_{2\alpha}$ *in vivo* (Davis & Horton, 1972) as well as *in vitro* (Hamberg, 1969). Prostaglandin E_2 is the principal product of arachidonate metabolism in rabbit renal medulla (Daniels, Hinman, Leach & Muirhead, 1967), possesses potent actions as a diuretic/natriuretic (Lee & Ferguson, 1969) and as a vasodilator (Nakano, 1968), and may exert a protective antihypertensive role (Lee, 1973). The second most abundant renal prostaglandin, prostaglandin $F_{2\alpha}$, lacks a readily demonstrable intrarenal action in that it neither amplifies neurogenic renal vasoconstriction nor affects renal vascular resistance at concentrations 400 times greater than the threshold dose of prostaglandin E_2 (McGiff, Crowshaw, Terragno, Malik & Lonigro, 1972). Thus the ratio of prostaglandin $F_{2\alpha}$ to E_2 might be critical for intrarenal homeostasis, and could be

effectively modulated by the actions of this novel rabbit renal prostaglandin 9-hydroxydehydrogenase.

P.K.M. is supported by a Science Research Council 'CASE' award held with the sponsorship of May & Baker Ltd. We thank Dr J.E. Pike (Upjohn Company, Kalamazoo, USA), Dr K. Crowshaw (May & Baker Ltd, Dagenham), and Dr A. Brash (Department of Clinical Pharmacology, Hammersmith Hospital) for generous gifts of prostaglandins.

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(Received April 5, 1977. Revised May 30, 1977)