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METABOLISM OF PROSTAGLANDIN E_2 IN THE ISOLATED PERFUSED KIDNEY OF THE RABBIT

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Abstract—In the Krebs-perfused rabbit isolated kidney, [^{3}H]PGE_2 (5 μCi, 165 Ci/mmole) was infused intra-arterially for 5 min; venous and urinary effluents were collected every 2 min for 20 min. Efflux of radioactive material peaked at 8 min and declined thereafter. The kidney retained 35% of the infused ^{3}H. Samples were extracted for acidic lipids; PGE_2, $PGF_{2\alpha}$ and metabolites were separated by TLC and quantified by a radiometric method. Efflux of [^{3}H]$PGF_{2\alpha}$ into urinary and venous outflows increased progressively over the first 12 min and then plateaued for the remaining 4 min. By 12 min, conversion of [^{3}H]PGE_2 to [^{3}H]$PGF_{2\alpha}$ was 70 and 80% as determined by radiolabeled products recovered in the urinary and venous effluents respectively. Estimates of total conversion of [^{3}H]PGE_2 to [^{3}H]$PGF_{2\alpha}$ were 62 and 52% of the radiolabeled material exiting in the urinary and venous effluents respectively. The 15-keto and 13,14-dihydro-15-keto metabolites of [^{3}H]$PGF_{2\alpha}$ appeared in the urine but were not found in the venous outflow. We conclude that PGE-9-ketoreductase (PGE-9KRD) activity is high in the rabbit isolated perfused kidney. Further, the extent of conversion of PGE_2 to $PGF_{2\alpha}$ and metabolism of newly formed $PGF_{2\alpha}$ may differ within the vascular and tubular compartments of the kidney. PGE-9KRD activity may be important in the regulation of renal vascular tone, compliance of veins, and salt and water balance.

Prostaglandin E-9-ketoreductase (PGE-9KRD) activity may be important to the catabolism of PGE_2, particularly in the rabbit [1, 2]. The action of PGE-9KRD is distinct from other metabolic pathways responsible for terminating the effects of PGE_2, as it forms another biologically active prostaglandin, namely prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and not an inactive metabolite. While $PGF_{2\alpha}$, unlike PGE_2, has negligible direct effects on renal blood flow and tubular function, it may have important effects on venous tone and adrenergic neurotransmission [3].

Renal PGE-9KRD activity may affect renal function and cardiovascular homeostasis by determining the ratio of PGE_2:$PGF_{2\alpha}$. As PGE_2 is a vasodilator and promotes salt and water excretion, and $PGF_{2\alpha}$ is a vasoconstrictor without effect on salt and water excretion, regulation of the relative levels of PGE_2 and $PGF_{2\alpha}$ through the activity of PGE-9KRD may be of importance in circulatory control. Further, its activity is thought to be inhibited by "loop" diuretics [2, 4] and modified by salt balance [5].

Estimations of PGE-9KRD activity have generally been limited to subcellular fractions [1–9], and for this reason we have examined its activity in an intact organ, the rabbit isolated Krebs-perfused kidney.

METHODS

Isolated perfused kidney. Male New Zealand rabbits were anesthetized with 25 mg/kg of sodium pentobarbitone given intravenously and the left kidney was cleared from the surrounding perirenal adipose tissue. The ureter was freed for a length of 2 cm and cannulated *in situ*. The renal artery was cannulated, and the kidney was flushed with 50 ml of warm, oxygenated modified Krebs–Henseleit solution (pH = 7.4) to remove blood elements. Within 1 min the kidney was removed, weighed, and transferred to a warming jacket for perfusion at a flow rate of 2 ml per g per min (25–40 ml/min) with Krebs–Henseleit solution. Stability of the preparation was determined by (1) a stable perfusion pressure in the physiological range of 60–90 mm Hg; (2) vascular responsiveness to norepinephrine (100–500 ng); and (3) a stable diuresis greater than 5% of the perfusion flow rate. The preparation was determined to be viable by these criteria; two of seven experiments were terminated because of failure to fulfil one or more of these requirements. For convenience, the perfusing solution recovered from the ureter will be termed urine, and that obtained from the renal vein, venous.

Biochemical analysis. Multi-labeled [^{3}H]PGE_2 was infused into the renal artery over a 5-min period at a rate of 0.5 ml/min in a vehicle of Krebs–Henseleit. A total of 5.0 μCi of radiolabeled PGE_2 (sp. act. = 165 Ci/mmole) was administered; the venous and ureteral effluents were collected every 2 min for 20 min from the start of the infusion. A small aliquot of each sample was taken to determine the time-course of radiolabeled material efflux. The remainder was acidified to pH 3.0 with 1 M citric acid, and the prostaglandins were extracted three times in an excess of ethyl acetate; the efficiency of extraction was 99 and 85% for urinary and venous prostaglandins respectively.

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These extracts were dried in a rotary evaporator, re-extracted with ethyl acetate (8 × 2 ml), and transferred to a scintillation vial. After drying under a stream of nitrogen, the residue was stored at −25°. Non-lipid contaminants were removed from the venous extracts on G-25 Sephadex columns, according to the method of Wuthier [10]. On the day of analysis, samples were reconstituted with 1 ml of dry acetone and applied to thin-layer chromatography (TLC) plates (Brinkmann). Individual prostaglandins were separated with the solvent system: ethyl acetate–iso-octane–water–acetic acid (66:30:60:12, by vol.). After radiochromatograph scanning, the zones co-migrating with authentic $PGF_{2\alpha}$, PGE_2, 15-keto-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, 15-keto-PGE_2 and 13,14-dihydro-15-keto-PGE_2 standards were scraped and suspended in 10 ml of scintillation fluid and counted for ^3H.

Prostaglandins. Standard prostaglandins were a gift of Dr. John Pike of the Upjohn Co. (Kalamazoo, MI). Tritiated PGE_2 was labeled in positions 5, 6, 8, 11, 12, 14 and 15 (New England Nuclear Corp., Boston, MA).

Analysis of results. The time-course of efflux of [^3H]PGE_2, [^3H]$PGF_{2\alpha}$ and their metabolites was established in each experiment. The activity of PGE-9KRD was quantified by the percentage conversion of [^3H]PGE_2 to [^3H]$PGF_{2\alpha}$, calculated from the following formula: cpm [^3H]$PGF_{2\alpha}$ recovered/cpm [^3H]PGE_2 and [^3H]$PGF_{2\alpha}$ recovered. Two estimations of conversion were made: (1) only unmetabolized [^3H]PGE_2 and [^3H]$PGF_{2\alpha}$ were incorporated into the formula; and (2) both metabolized and unmetabolized [^3H]PGE_2 and [^3H]$PGF_{2\alpha}$ were considered.

Statistical treatment of the results was performed with Student's *t*-test for either paired or unpaired samples, as appropriate. A P value of less than 0.05 was considered to be statistically significant. Results are expressed as their mean ± S.D.

RESULTS

Substantially more ^3H was recovered in the urine than in the venous effluent as reflected by the ratio ^3H urine/^3H perfusate = 1.9 ± 0.9, despite the intra-arterial route of administration of [^3H]PGE_2 and a 9-fold difference in venous and urinary flow rates. Venous flow rate was 30.0 ± 4.7 ml/min, whereas urine flow rate was 3.4 ± 3.0 ml/min (N = 5). The time-course of ^3H-recovery was, however, similar in both the urinary and venous outflows (Fig. 1). Recovery of ^3H peaked at 8 min and declined progressively thereafter. Approximately 35% of the infused ^3H remained in the renal mass after the conclusion of the experiment, 15 min after terminating the [^3H]PGE_2 infusion. Mean perfusion pressure, which ranged between 60 and 90 mm Hg, was unaltered by the [^3H]PGE_2 infusion; urine flow was similarly unaffected.

Radiolabeled material recovered in the venous effluent. The radioactive products recovered in the venous effluent for each 2-min collection are shown in Fig. 2, in which ^3H-content in TLC zones corresponding to authentic PGE_2 and $PGF_{2\alpha}$ and their metabolites is expressed as a percentage of the total

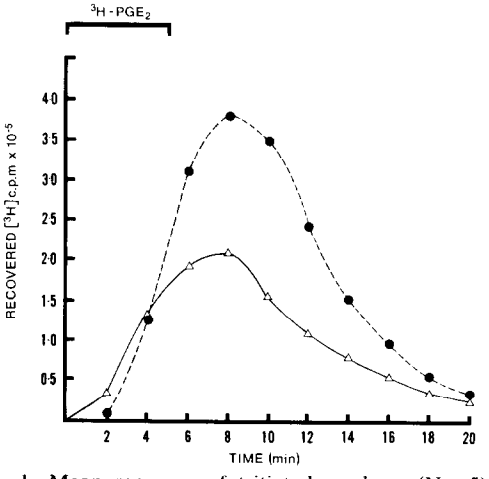

Fig. 1. Mean recovery of tritiated products (N = 5) in ureteral (●----●) and renal venous (△—△) effluents at 2-min intervals following a 5-min renal artery infusion of [^3H]PGE_2.

radioactivity on the TLC plate. In successive samples, there was a decline in PGE_2 levels with a concurrent increase in radioactivity in the $PGF_{2\alpha}$ zone. This time-dependent increase in $PGF_{2\alpha}$ was so marked that this area alone accounted for 45–50% of the total radioactivity in samples indicated at 12–16 min in Fig. 2, which were obtained 7–11 min after the completion of the [^3H]PGE_2 infusion. The corresponding levels of PGE_2 were, on the other hand, only 7–10% of the total radioactivity.

There was a time-dependent increase in ^3H-recovery in the zone of the thin-layer plate from the origin to $PGF_{2\alpha}$, which probably represents the products of β- or ω-oxidation as these are more polar than $PGF_{2\alpha}$ (see Fig. 3). The 15-keto and 13,14-dihydro-15-keto metabolites of [^3H]PGE_2 showed a time-dependent decline paralleling the changes in venous efflux of [^3H]PGE_2. However, the corresponding metabolites of $PGF_{2\alpha}$ in the venous effluent could not be detected above background levels of radioactivity.

Radiolabeled material recovered in the urine. While the urinary efflux of PGE_2 decreased and that of $PGF_{2\alpha}$ increased with time, these changes were less marked than those seen in the venous effluent (Fig. 2). Post-infusion [^3H]$PGF_{2\alpha}$ urinary levels represented only 24–28% of total radioactivity, an amount which was approximately half that found in the corresponding venous samples. The difference in $PGF_{2\alpha}$ efflux between venous and urine samples was significant ($P < 0.02$) for the 10-, 12- and 14-min samples of Fig. 2.

The time-dependent changes in percentage radioactivity in the origin zone of the thin-layer plates were essentially similar to those described for the venous effluent. The levels of 15-keto and 13,14-dihydro-15-keto urinary metabolites of $PGF_{2\alpha}$ were relatively constant. The 15-keto and 13,14-dihydro-15-keto-PGE_2 urinary metabolites, however, declined over the entire collection period in association with the decline of their precursor, [^3H]PGE_2, a pattern which was also evident for the

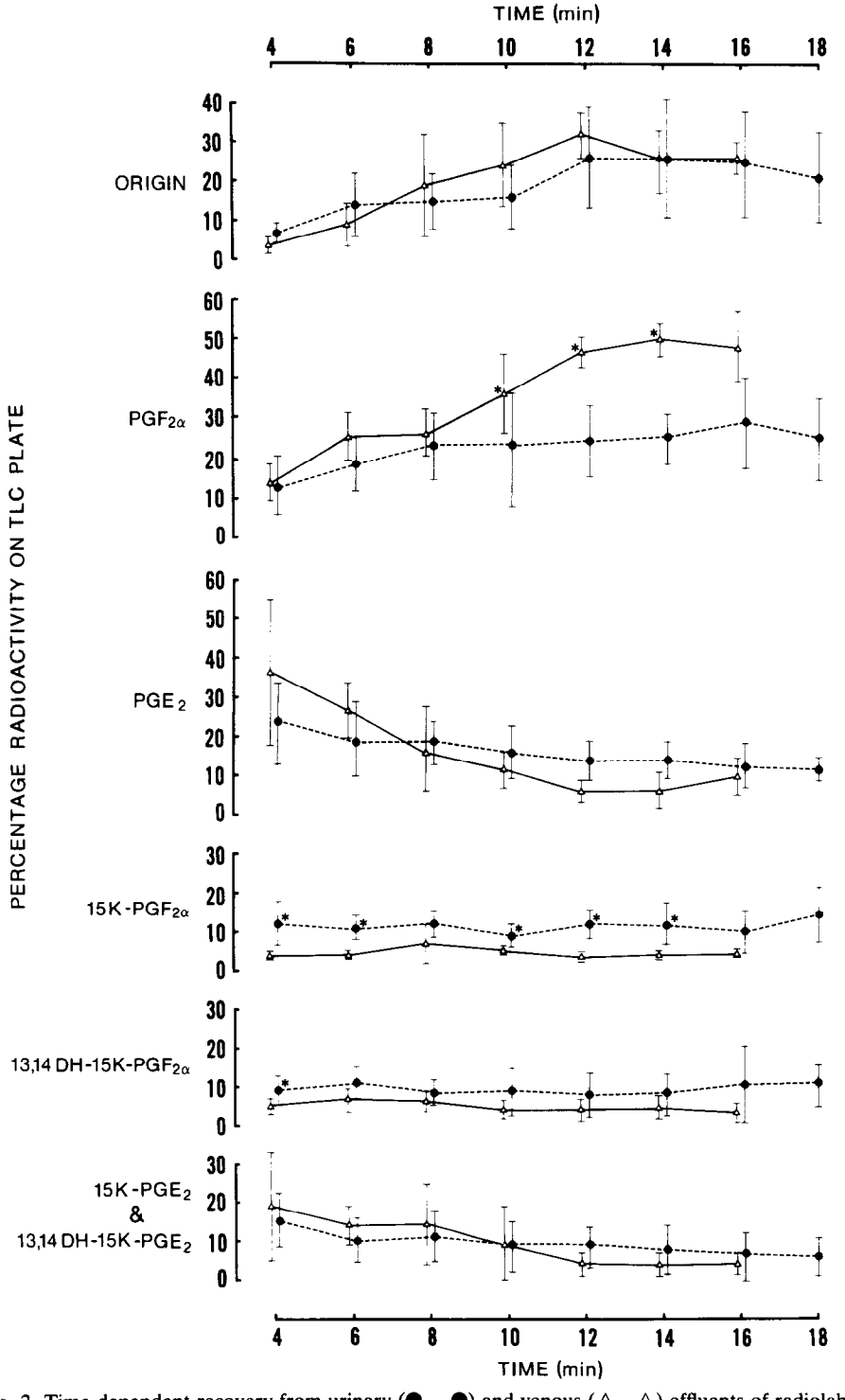

Fig. 2. Time-dependent recovery from urinary (●---●) and venous (△—△) effluents of radiolabeled products which have been separated on TLC plates and identified by co-migration of authentic prostaglandin standards. 15-Keto-PGE_2 and 13,14-dihydro-15-keto-PGE_2 have similar mobilities with this solvent system. Therefore, radioactivity co-migrating in this region has been expressed as if it were a single zone. Significant differences ($P < 0.05$) between simultaneous ureteral and venous samples are denoted by an asterisk (*).

efflux of these PGE_2 metabolites into the venous effluent.

Radiochromatograph scans from an experiment in which 22 μCi of [^{3}H]PGE_2 was administered are found in Fig. 3. Three urine samples were collected: during the [^{3}H]PGE_2 infusion (top panel), 1–5 min post-infusion (middle), and 6–13 min post-infusion (bottom). A time-related increase in the appearance of [^{3}H]$PGF_{2\alpha}$ in the urine occurred in association with a decline in the excretion of [^{3}H]PGE_2. A zone of radioactivity which lies before the $PGF_{2\alpha}$ peak, becoming apparent in the period 6–13 min after the [^{3}H]PGE_2 infusion, probably represents the products of β-oxidation.

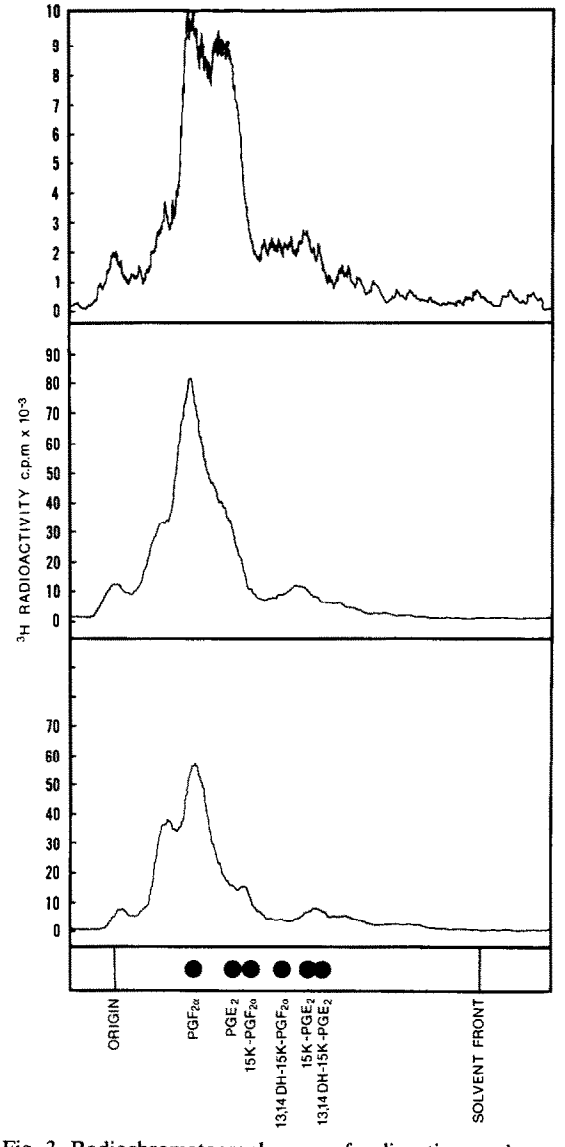

Fig. 3. Radiochromatograph scans of radioactive products extracted from ureteral effluent during and after a 5-min infusion of [^{3}H]PGE_2. The top panel is a scan of radiolabeled products obtained from urine collected during the [^{3}H]PGE_2 infusion; the middle and lower panels show radiolabeled products obtained from urine collected 1–5 min and 6–13 min, respectively, after completion of the infusion.

Conversion of PGE_2 to $PGF_{2\alpha}$. PGE-9-ketoreductase activity was determined by calculating the percentage conversion of [^{3}H]PGE_2 to [^{3}H]$PGF_{2\alpha}$. The possible influence of 15-hydroxyprostaglandin dehydrogenase (15-OH PGDH) or $\Delta^{13,14}$ reductase metabolic pathways was also considered. The estimates of conversion of PGE_2 to $PGF_{2\alpha}$ for each 2-min sample, and the mean total conversion as reflected in efflux of radiolabeled products in urine (min 2–18) and venous outflows (min 2–16), are found in Fig. 4. Estimates of conversion increased with time in both urinary and venous effluents, although this effect was substantially greater in the venous effluent. The calculated conversion for the 4- and 6-min samples was significantly lower in the venous effluent ($P < 0.05$) when compared to the corresponding urine samples (Fig. 4). On the other hand, the percentage conversion in the post-infusion samples was greater in the venous effluent than in the urine. This difference was significant for samples collected at 10-, 12- and 14-min, when only unmetabolized [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$ were considered ($P < 0.02$), and at 12 min for both metabolized and unmetabolized prostaglandins ($P < 0.05$). Thus, during the [^{3}H]PGE_2 infusion, estimations of conversion of PGE_2 to $PGF_{2\alpha}$ were lower for the venous effluent than for the urine, while the reverse was true for the greater part of the post-infusion sampling periods (Fig. 4). Indeed, in these later venous samples, nearly 85% of the [^{3}H]PGE_2 had been converted to [^{3}H]$PGF_{2\alpha}$.

There was another important difference between the efflux of radiolabeled products into urinary and venous effluents which became apparent when the

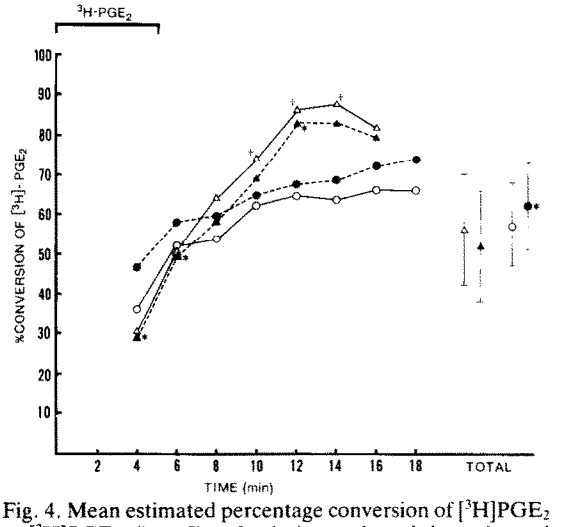

Fig. 4. Mean estimated percentage conversion of [^{3}H]PGE_2 to [^{3}H]$PGF_{2\alpha}$ (N = 5) at 2-min intervals and the estimated total conversion, in both ureteral and venous effluents. Estimates were made for both unmetabolized PGE_2 and $PGF_{2\alpha}$ alone [urine (○), venous (△)] and unmetabolized PGE_2 and $PGF_{2\alpha}$ together with their respective 15-keto and 13,14-dihydro-15-keto metabolites [urine (●), venous (▲)]. The asterisk (*) denotes a significant difference ($P < 0.05$) between urinary and venous estimates of PGE_2 conversion when both metabolized and unmetabolized PGE_2 and $PGF_{2\alpha}$ are considered together, and the single dagger (†) denotes a significant difference ($P < 0.05$) for only unmetabolized PGE_2 and $PGF_{2\alpha}$.

total conversion of [^{3}H]PGE_2 to [^{3}H]$PGF_{2\alpha}$ was determined not only from the unmetabolized species, but also from the 15-keto and 13,14-dihydro-15-keto metabolites of each prostaglandin. When only metabolized [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$ were used in the calculation, no difference was found between urine and venous effluents: 57 ± 10 and 56 ± 14% respectively. However, conversion of [^{3}H]PGE_2 to [^{3}H]$PGF_{2\alpha}$ accounted for 62 ± 11% of radiolabeled products in the urine and 52 ± 14% of those in the venous outflow ($P < 0.01$), when the 15-keto and 13,14-dihydro-15-keto metabolites of [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$ were used to calculate total conversion.

DISCUSSION

This study demonstrated PGE-9-ketoreductase activity in the perfused isolated kidney of the rabbit as indicated by substantial recovery of [^{3}H]$PGF_{2\alpha}$ in both urinary and venous effluents after having infused [^{3}H]PGE_2 into the renal artery. The extent and predominance of PGE-9KRD activity are remarkable as the concentration of [^{3}H]PGE_2 delivered intra-arterially was 2×10^{-10} M, whereas the K_m for partially purified PGE-9KRD has been reported to be $1–3 \times 10^{-4}$ M [8, 9, 11]. This apparently high K_m, in addition to the non-specific nature of this enzyme, was interpreted to indicate that prostaglandins are not endogenous substrates for PGE-9KRD [9, 11]. Our study re-emphasizes the need for extreme caution when interpreting the significance of enzymes based on apparent K_m values, particularly enzymes which are only partially purified.

Of the recovered tritium, more was found in the urine than in the venous effluent despite a 9-fold greater venous flow than urine flow and the intra-arterial route of administration. Bito and co-workers [12, 13] have made similar findings in the isolated rabbit kidney with bolus injections of tritiated $PGF_{2\alpha}$ or PGE_1, as had Rennick [14], with infused tritiated PGE_2, $PGF_{2\alpha}$ or 13,14-dihydro-15-keto-$PGF_{2\alpha}$ in an *in vivo* chicken preparation. Tritiated prostaglandins recovered in the urine may arise from either glomerular filtration or tubular secretion. We have found that probenecid treatment, which blocks tubular secretion, markedly reduced the efflux of radiolabeled compounds into the urine (unpublished observations), suggesting that radiolabeled urinary prostaglandins, under the conditions of our study, were transported from the vascular compartment across the tubular epithelium. Estimates of PGE-9KRD activity in the urinary effluent may, therefore, represent a composite of both vascular and tubular events, whereas radiolabeled $PGF_{2\alpha}$ exiting via the renal vein probably reflects vascular PGE-9KRD activity. As the estimates of conversion were similar in venous and urinary effluents, renal PGE-9KRD may be predominantly localized to the vasculature.*

As PGE-9KRD is predominantly a cytosolic enzyme, the recovery of [^{3}H]$PGF_{2\alpha}$ in either effluent must be preceded by cellular incorporation of [^{3}H]PGE_2, conversion to [^{3}H]$PGF_{2\alpha}$ by PGE-9KRD, and the release of the newly formed [^{3}H]$PGF_{2\alpha}$ into the extracellular fluid. It was not surprising, therefore, to observe a time-related activity of PGE-9KRD, as measured by a greater recovery of $PGF_{2\alpha}$ in the post-infusion samples. Recovery of [^{3}H]$PGF_{2\alpha}$ in the post-infusion venous samples was greater than in the corresponding urinary samples (Fig. 2). This may be attributable to a greater activity of PGE-9KRD in the vasculature, as well as to failure to metabolize either PGE_2 or $PGF_{2\alpha}$ to the 15-keto and 13,14-dihydro-15-keto species in the vascular compartment. Although these metabolites of $PGF_{2\alpha}$ were present in the effluent from the urinary compartment, only unmetabolized [^{3}H]$PGF_{2\alpha}$ was recovered from the venous effluent.

The presence of PGE_2 and $PGF_{2\alpha}$ metabolites in the urine effluent is presumed to represent the products of metabolism primarily by the tubular epithelium. McLain and Irish [15], however, found substantial conversion of tritiated PGE_2 to $PGF_{2\alpha}$ in isolated rabbit renal proximal tubules without significant 15-OH PGDH or 9-OH PGDH activity. It should be noted that the non-physiological intra-arterial route of administration of [^{3}H]PGE_2 in the present study may influence the distribution of recovered [^{3}H]prostanoids, and, consequently, their metabolism. Therefore, our findings may not accurately represent intrarenal events *in vivo*.

The appearance of [^{3}H]$PGF_{2\alpha}$ metabolites in urine presumably arises from catabolism of [^{3}H]$PGF_{2\alpha}$, as 15-keto-PGE_2 and 13,14-dihydro-15-keto-PGE_2 are poor substrates for PGE-9KRD [16] and, therefore, are unlikely to be converted into their respective $PGF_{2\alpha}$ metabolites. Our findings are consistent with a tubular compartmentalization of 15-OH PGDH, and are supported by the histochemical studies [17, 18] localizing Type I 15-OH PGDH [NAD^+-dependent] to the proximal and distal tubules and to the thick ascending loop of Henle. This enzyme, which irreversibly converts the hydroxyl group at C15 to a ketone, is the most important step in prostaglandin degradation. Sun *et al.* [19] concluded from experiments also conducted in the rabbit isolated perfused kidney that 15-OH PGDH was of minor importance in the vascular compartment. However, they could not determine the importance of tubular 15-OH PGDH as they did not cannulate the ureter.

Hassid and Levine [20] suggested that $NADP^+$-dependent 15-OH PGDH and PGE-9KRD activities belong to a single protein species classified as Type II prostaglandin dehydrogenase. This relatively non-specific enzyme may act as a dehydrogenase at C15 and a reductase at C9; both reactions are reversible and competitive. The enzyme(s) also reduces steroids, as well as sugars and quinones [9, 11, 21, 22] in the presence of NADPH, and may be similar to a non-specific carbonylreductase isolated from human brain [23]. The redox state of the pyrimidine nucleotides of the tissue is an important determinant of the direction of these reversible reactions and, clearly, in the rabbit isolated kidney, the reduction of the C-9 keto group of PGE_2 is favored.

In addition to PGE-9KRD/Type II 15-OH PGDH

* We did not determine intrarenal distribution of the retained tritium; however, Wong and Sun have found that in the rabbit isolated kidney most of the tritium was retained in the renal cortex (personal communication).

being a reversible reaction, $PGF_{2\alpha}$ may be converted to PGE_2 by the action of NAD^+-dependent 9-hydroxyprostaglandin dehydrogenase [23, 24]. Thus, PGE_2 may be converted to $PGF_{2\alpha}$ and then back to PGE_2. However, under the present conditions, the formation of $PGF_{2\alpha}$ by PGE-9KRD predominates, as renal artery infusions of $[^3H]PGF_{2\alpha}$ have failed to demonstrate any conversion to $[^3H]PGE_2$ in this preparation (unpublished observations).

Sun *et al.* [19] also identified [^{3}H]tetranor-$PGF_{1\alpha}$ in the venous effluent from rabbit kidneys infused with $[^3H]PGE_2$. The presence of this prostanoid, formed from PGE_2 by the combined effects of PGE-9KRD and β-oxidation, is consistent with our finding of increased radioactivity with successive samples on the thin-layer plate zone extending from the origin to $PGF_{2\alpha}$ (Fig. 3). This may well represent the effects of β-oxidation, particularly as its appearance is strongly associated with the appearance of $[^3H]PGF_{2\alpha}$. If this radioactivity is the result of either dinor-$PGF_{2\alpha}$ or tetranor-$PGF_{1\alpha}$ formation, then the conversion of PGE_2 to $PGF_{2\alpha}$ by PGE-9KRD approaches 100% during the post-infusion period in the venous effluent. The predominance of β-oxidation as a metabolic pathway in the study of Sun *et al.* [19] may be due to the low perfusion rate of 10 ml/min, whereas the rate was 30 ml/min in the present study.

The substantial conversion of PGE_2 to $PGF_{2\alpha}$ in the rabbit kidney raises a number of important considerations. For example, $PGF_{2\alpha}$, unlike PGE_2, facilitates adrenergic neurotransmission [25, 26]. Therefore, PGE-9KRD activity could be a major determinant of prostaglandin-dependent mechanisms which have been suggested to contribute to the regulation of the activity of the autonomic nervous system. In addition, $PGF_{2\alpha}$ has important effects on the vasculature in its own right, particularly on venous tone. The release of unmetabolized $PGF_{2\alpha}$ from vascular elements intrarenally may reduce the compliance of renal veins as well as of the vena cava, thereby increasing ventricular-filling pressure and cardiac output. One or more renal humoral factors have been suggested to affect cardiac performance and to link changes in renal function to changes in cardiac function [27].

As $PGF_{2\alpha}$ has limited, if any, effects on tubular function [28], PGE-9KRD activity in the tubular compartment may be one of a number of metabolic pathways available for terminating the effects of PGE_2 on salt and water transport.

In conclusion, PGE-9KRD activity has been demonstrated in the rabbit isolated perfused kidney. Changes in the activity of PGE-9KRD may be important in the regulation of renal vascular tone and venous compliance.

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