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Metabolism of prostaglandin E_1 in dog kidneys

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Summary

1. The biotransformation of prostaglandin E_1 (PGE_1) was studied in the isolated, perfused dog kidneys.

2. An average 43% of PGE_1 was converted into the less polar metabolite I by a single passage through the kidney. As the re-circulation of the perfusate continued, PGE_1 was converted not only into metabolite I but also the least polar metabolite II. The velocity of the conversion of PGE_1 into metabolite I was significantly greater than that into metabolite II. Usually, six passages elapsed before maximum degradation of PGE_1 occurred.

3. Further separation with silicic acid column chromatography and gas-liquid chromatography showed that metabolite II consists of two individual metabolites, metabolite IIa and metabolite IIb.

4. The present study indicates that the kidney biotransforms PGE_1 rather rapidly into three metabolites which are less polar than PGE_1.

Introduction

Änggård & Samuelsson (1964) found that, when incubated with guinea-pig lung homogenates, PGE_1, PGE_2 or PGE_3 were converted into two less polar metabolites, dihydro- and dihydro-15-keto-PGE compounds. On the other hand, each PGE compound was converted into one metabolite, a respective 15-keto-PGE compound, by swine lung homogenate (Änggård & Samuelsson, 1964). In the latter biotransformation was catalysed by a NAD^+-dependent enzyme, prostaglandin dehydrogenase (Änggård & Samuelsson, 1966; Nakano, Änggård & Samuelsson, 1969). Recently, using a histochemical technique, Nissen & Andersen (1968) showed that the similar prostaglandin dehydrogenase exists in the thick, ascending limb of the loop of Henle and in the distal tubules of rat kidneys. It is of interest that not only the kidney contains PGE_2 and $PGF_{2\alpha}$ in the medulla (Daniels, Hinman, Leach & Muirhead, 1967; Lee, Covino, Takman & Smith, 1965), but also prostaglandin-like substance was increased in the renal venous blood or medulla in hypertensive animals (Edwards, Strong & Hunt, 1969).

The present study was undertaken to examine (*a*) whether PGE_1 is metabolized in the isolated dog kidney by a similar mechanism, and (*b*), if so, to study the magnitude and speed of the metabolism of PGE_1 in dog kidney.

Methods

Synthesis and purification of 15-keto-prostaglandin E_1 (15-keto-PGE_1) and ^{3}H-prostaglandin E_1 (PGE_1)

Crystalline powders of PGE_1 and PGE_2 were obtained from Dr. J. E. Pike, Chemistry Department, Upjohn Co., Kalamazoo, Michigan. 11-α-Hydroxy-9,15-diketo-13-prostaenoic acid (15-keto-PGE_1) was synthesized by MnO_2 oxidation of PGE_1 in this laboratory according to the method described by Attenburrow, Cameron, Chapman, Evans, Hems, Jansen & Walker (1952). ^{3}H-PGE_1 was synthesized by reducing $\Delta 5$ *cis* double bond of PGE_2 with tritium gas according to the Samuelsson (1963) method. Tritiation was made by the Amersham/Searle Corporation, Des Plains, Illinois. ^{3}H-PGE_1 was purified from the tritiated product of PGE_2 by silicic acid column chromatography as described later and was ascertained by thin-layer chromatography using Silica Gel G with the following solvent system: ethylacetate-water-isooctane-acetic acid (110 : 100 : 20 : 10, v/v). The spots were identified by heating at 110° C after spraying 10% phosphomolybdic acid solution in 96% ethanol (Geyer, Matthews & Stare, 1949). The purified ^{3}H-PGE_1 had a specific activity of 41 mCi/mmol. After an aliquot of ^{3}H-PGE_1 (100 μCi) was evaporated, 10 μl of 96% ethanol was added and diluted further with Tyrode solution (pH 7·4) before perfusion.

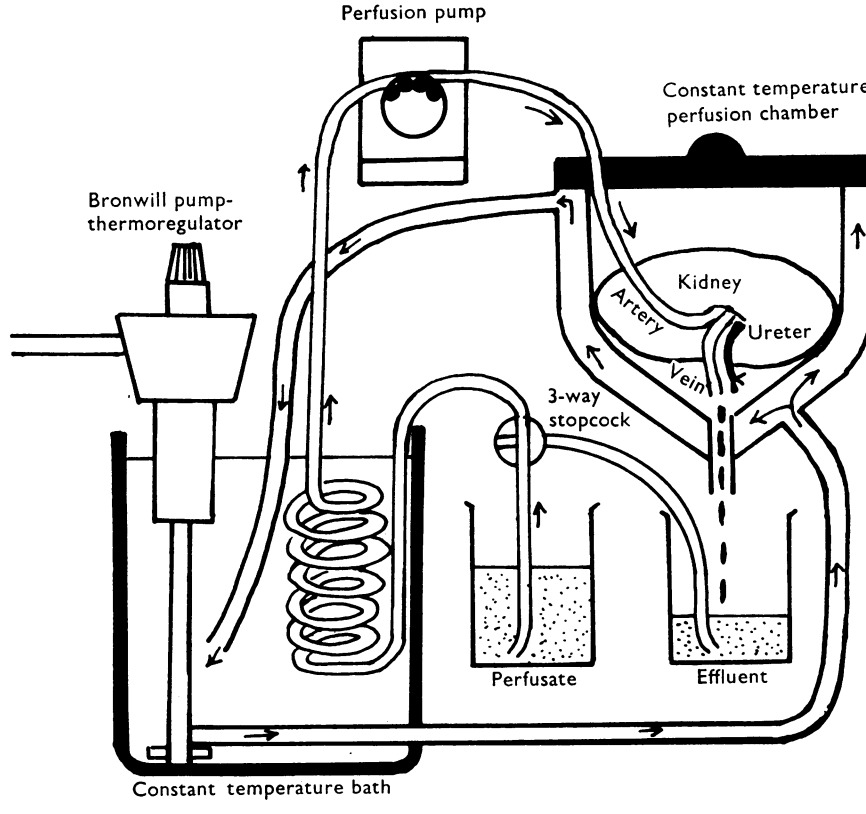

FIG. 1. Technique used for kidney perfusion.

Perfusion technique

Twelve dogs, weighing between 10 and 12 kg, were lightly anaesthetized by the intravenous administration of 35 mg/kg of sodium pentobarbital. After a mid-line laparotomy, a kidney was removed and perfused by the technique depicted in Fig. 1. The kidney was placed on a funnel-shaped stainless steel sieve in a constant temperature chamber (37° C). After cannulating the renal artery, the kidney was perfused at a constant rate (30 ml/min) with 500 ml of oxygenated Tyrode solution (pH 7·4, 37° C), using a Buchler Polystalic pump.

In eight experiments the control perfusate contained 1 μCi/ml of ^{3}H-PGE_1 and 50 ng/ml of PGE_1. The perfusion pressure was approximately 80 mmHg (1 mmHg $\equiv$1·333 mbar). The renal venous outflow (effluent) was collected until the whole of the solution in the reservoir had been perfused. The effluent solution was then re-perfused through the kidney via the renal artery. This procedure was repeated until twenty passages through the kidney had been made. An aliquot (10 ml) of control perfusate and each effluent solution were pipetted before each passage for the chromatographic separation of and the determination of the radioactivity of ^{3}H-PGE_1 and its metabolites.

In order to obtain larger amounts of PGE_1 metabolites in eight isolated kidneys, using a similar procedure, each kidney was perfused at a constant rate (30 ml/min) with 400 ml of Tyrode solution (pH 7·4) containing 1 μCi/ml of ^{3}H-PGE_1 and 50 μg/ml of PGE_1. The perfusate was recirculated continuously through the kidney for 2 h. At the end of the experiment, PGE_1 and its metabolites were extracted and separated by chromatographic techniques described below.

Extraction of prostaglandins and their metabolites

Prostaglandins and their metabolites in the renal vein effluents of the dogs were extracted by a slight modification of the method described by Änggård (1965). Aliquots (10 ml) of the effluent solutions were pipetted, and they were acidified to pH 3 with 1 N HCl. The solution was then twice extracted with half its volume of ethyl acetate. The aqueous phase was discarded, and the combined ethyl acetate phases were at once extracted several times with small portions of 0·2 M phosphate buffer at pH 8 until less than 10% of the biological activity was left in the ethyl acetate phase. The combined buffer phases of pH 8 were then washed with a small quantity of ethyl acetate. After acidification to pH 3 with 2–6 N HCl, the buffer was extracted several times with one-fiftieth of its volume of ethyl acetate. The combined ethyl acetate phases were then washed with distilled water and evaporated to dryness under reduced pressure. The material was dissolved in equal volumes of petroleum ether and ethanol-water (2 : 1). The combined ethanol phases were evaporated at reduced pressure with a nitrogen leak at 40° C, the pH adjusted to 3 and extracted three times with one volume of ethyl acetate. The combined extracts were washed with distilled water and were evaporated to dryness.

Separation of PGE_1 and its metabolites by silicic acid column chromatography

^{3}H-PGE_1 and its metabolites were separated by silicic acid column chromatography using a modification of the method described by Samuelsson (1963). In this study the solvent mixture of ethyl acetate-toluene was used for elution instead of that of ethyl acetate-benzene, since the dielectric constant of toluene (2·379 at

20° C) is almost identical to that of benzene (2·284 at 20° C) (*Handbook of Chemistry and Physics,* 1967) and also since the former is less toxic than the latter (*The Merck Index,* 1968). The absorbant, silicic acid (Unisil, Clarkson Chemical Co., Williamsport, Pennsylvania) (100–200 mesh) was activated at 110° C for 12 h. The amount of silicic acid used in a single column varied 1–5 g. The loading factor was usually less than 1 : 100. The volume of the fractions was kept at 5–10 ml/g of silicic acid. The solvent (moving phase) flowed spontaneously without employing a backward pressure. A discontinuous gradient elution with ethyl acetate in toluene was used, the ethyl acetate concentration being increased stepwise from 20 to 100%. This system separates the least potent metabolite(s) of PGE_1 with 20% ethyl acetate in toluene, 15-keto-PGE_1 with 40% ethyl acetate in toluene, and PGE_1 with 60% ethyl acetate in toluene (Fig. 2). In the experiments with

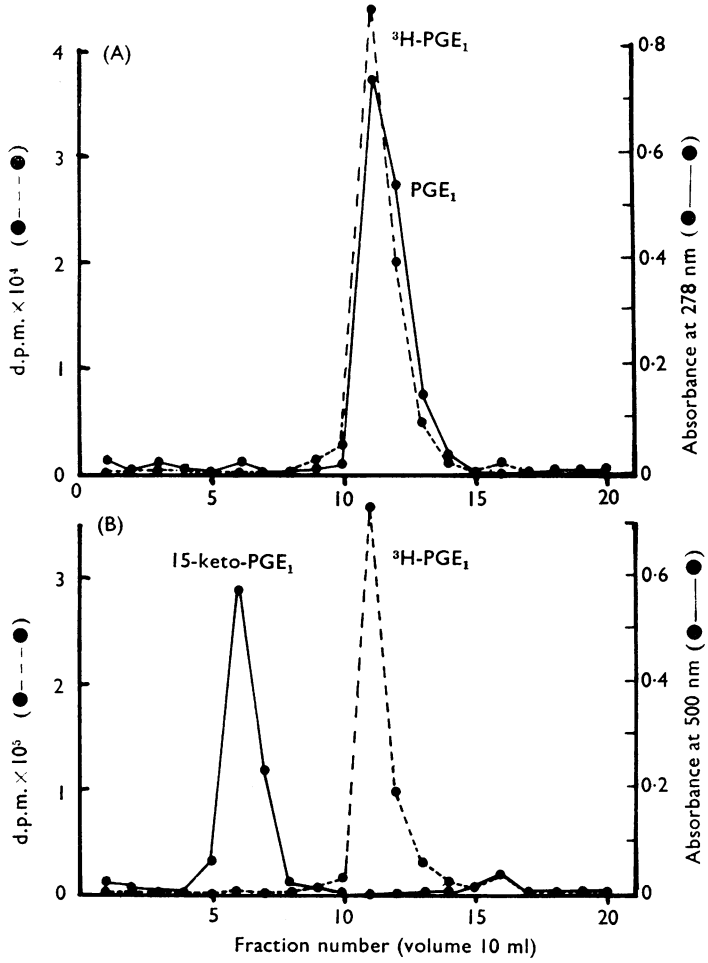

FIG. 2. Separation of PGE_1 and 15-keto-PGE_1 by silicic acid column chromatography. Column, 1 g silicic acid ; fractions, 10 ml. Fractions 1 to 5: ethyl acetate : toluene, 20 : 80 ; fractions 6 to 10: ethyl acetate-toluene, 40 : 60 ; fractions 11 to 15: ethyl acetate-toluene, 70 : 30 ; fractions 16 to 20: ethyl acetate alone. (A), Aliquots of the fractions were treated with 0·5 N NaOH in 50% ethanol, at 37° C for 40 min, and the absorbance at 278 nm was measured. References: ^{3}H-PGE_1 and PGE_1 (0·5 mg). (B), The absorbance of aliquots of the fractions was measured at 500 nm. References: ^{3}H-PGE_1 and 15-keto-PGE_1 (0·1 mg).

further separation of the PGE_1 metabolites, an elution method with a smaller stepwise increase in the ratio of ethyl acetate-toluene mixture, ranging from 10 : 90 to 40 : 60, was employed.

Measurements of radioactivity

For the study of the separation of ^{3}H-PGE_1 and its metabolites, an aliquot of each chromatography fraction was pipetted into a counting vial, and then 15 ml of the counting solution (toluene-methanol 400 ml:300 ml containing 4 g of PPO (2,5-diphenyloxazole) and 100 mg of POPOP (1,4-*bis*-2-5-phenyloxazolyl-benzine) was added. Radioactivity of each sample was counted within a 5% counting error in a Packard Tri-Carb liquid scintillation spectrometer (model 3000 series). Automatic external standard (^{226}Ra) was used to correct for quenching. Counting efficiencies for sample solution averaged 20%.

Separation of PGE_1 and its metabolites by gas-liquid chromatography

The ethyl acetate extracts of the fractions which showed the distinctly separated four peaks were subjected to the gas-liquid chromatographic analysis using a Hewlett Packard (F & M) gas chromatograph (model 402) equipped with ionization detector. After the active fractions were grouped and evaporated separately under reduced pressure, PGE_1 and its metabolites were converted into the O-methyloxime trimethylsilyl ether derivatives and were chromatographed by the method described by Vane & Horning (1969). The column was packed with 1% OV-17 (Applied Science Laboratories, Inc., College Station, Pennsylvania) and 1% SE-30 (General Electric) on Gas-Chrom P (Mesh 100–120). The flash heater and detector were kept at a temperature 30° C above that of the column (220° C). Inlet pressure was 50 (pounds/inch^2)/foot^2 and carrier gas (nitrogen) 50 ml/min.

The data in this study were analysed statistically using the *t* test (Snedecor, 1956). *P* values of less than 0·05 were accepted as indicating a significant difference between compared values.

Results

The biotransformation of PGE_1 was studied in eight isolated dog kidneys perfused with Tyrode solution containing 1 μCi/ml of ^{3}H-PGE_1 and 50 ng/ml of PGE_1. A silicic acid column chromatograph of the control perfusate and the renal venous effluents from a representative experiment is shown in Fig. 3. The control perfusate shows a single peak representing PGE_1 in fractions 11 to 14, which were eluted with the solvent mixture, ethyl acetate-toluene, 70 : 30. On the other hand, after one pass through the kidney, this PGE_1 peak decreased significantly, as a less polar metabolite I peak appeared in fractions 6 to 10, which were eluted with the solvent mixture, ethyl acetate-toluene, 40 : 60. After ten passes, the PGE_1 peak decreased further as the metabolite I peak increased. In addition, the least polar metabolite II appeared in fractions 1 to 5, which were eluted with the solvent mixture, ethyl acetate-toluene, 20 : 80. As summarized in Fig. 4, a single passage through the kidney resulted in a conversion of $43 \pm 0{\cdot}8$% of PGE_1 into less polar metabolites. As recirculation of the perfusate continued, PGE_1 was converted rather rapidly into metabolite I but more slowly into metabolite II. Usually six passages elapsed before PGE_1 was metabolized maximally in this kidney perfusion system.

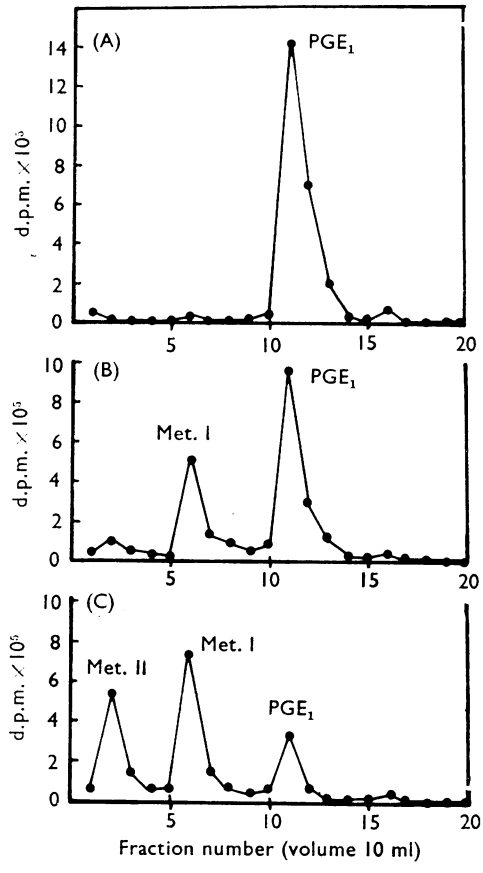

FIG. 3. Silicic acid column chromatography of ^{3}H-PGE$_1$ and its metabolites in the control perfusates (A) and the renal vein effluents after one pass (B) and after ten passes (C) through the dog kidney. The procedure for elution was the same as that in Fig. 2. Met I and Met II denote the less polar and least polar metabolites of PGE$_1$, respectively.

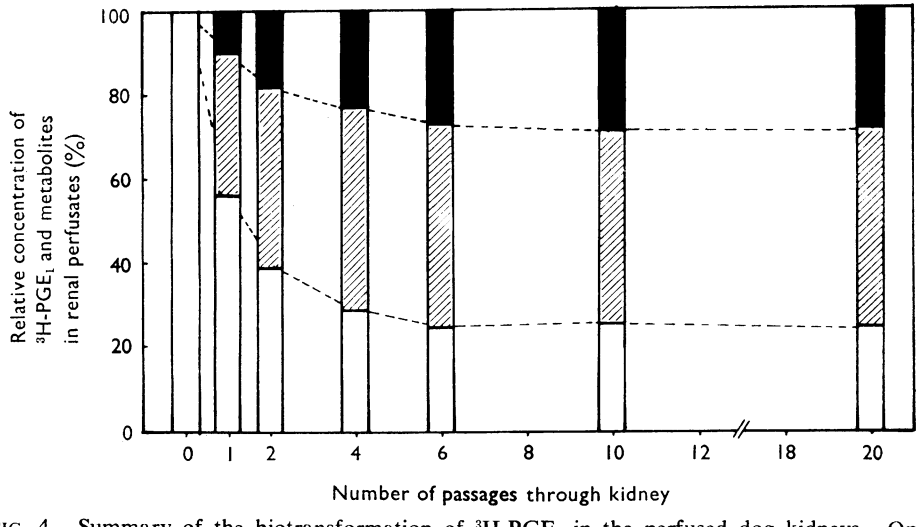

FIG. 4. Summary of the biotransformation of ^{3}H-PGE$_1$ in the perfused dog kidneys. Open columns, ^{3}H-PGE$_1$; hatched columns, metabolite I; filled columns, metabolite II.

In an attempt to separate further the metabolites, the combined fractions 1 to 10 were re-chromatographed with a smaller, stepwise, increase in the ratio of ethyl acetate-toluene mixture (Fig. 5). As shown in Fig. 5, metabolite II was further separated into metabolite IIa (fractions 6 to 8) and into metabolite IIb (fractions 11 to 22). A further attempt was made to separate the fractions representing the three metabolites—metabolite I, metabolite IIa and metabolite IIb—with gas-liquid chromatography. For this purpose, as described in **Methods**, larger amounts (more than 0·5 mg) of the metabolites were obtained by perfusing eight kidneys with

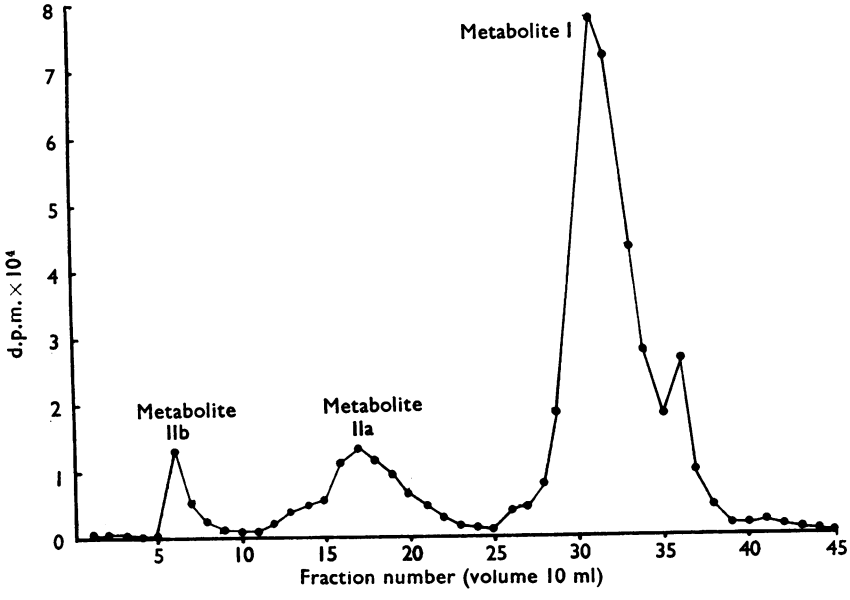

FIG. 5. Silicic acid column chromatography of ^{3}H-PGE_1 metabolites. Fractions 1 to 5: toluene alone; fractions 6 to 15: ethyl acetate-toluene, 15 : 85; fractions 16 to 25: ethyl acetate-toluene, 20 : 80; fractions 26 to 30: ethyl acetate-toluene, 30 : 70; fractions 31 to 35: ethyl acetate-toluene, 40 : 60; fractions 36 to 45: ethyl acetate-toluene, 70 : 30.

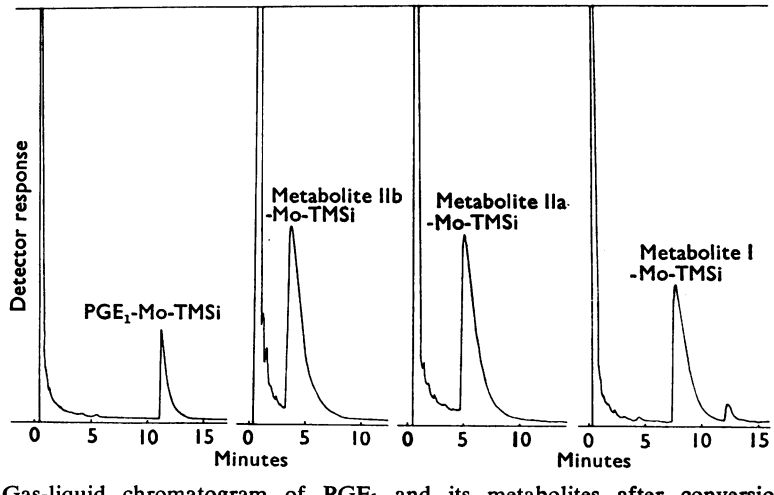

FIG. 6. Gas-liquid chromatogram of PGE_1 and its metabolites after conversion to the O-methyloxime trimethylsilyl ether derivatives. Column: 1·8 m×5 mm; 1% OV-17 on 100–120 mesh Gas-Chrom P. Temperature, 220° C.

Tyrode solution containing 50 μg/ml of PGE_1. PGE_1 and three metabolites were converted into the O-methyloxime trimethylsilyl ether derivatives and separated with 1% OV-17 on Gas-Chrom P (mesh 100–120). As illustrated in Fig. 6, gas-liquid chromatography made no further separation of the three metabolites which were obtained by silicic acid column chromatography (Fig. 5). It showed three individual peaks with different retention times corresponding to each metabolite.

Discussion

Änggård & Samuelsson (1964) showed that PGE_1 was converted into 15-keto-PGE_1 by the oxidation of the secondary alcohol group at 15-C in swine lungs, whereas PGE_1 was converted into dihydro-PGE_1 and 15-keto-dihydro-PGE_1 by the reduction of Δ^{13} double bond in guinea-pig lungs. The enzyme which catalyses the oxidation of PGE_1 was identified as NAD^+-dependent 15-hydroxy-prostaglandin dehydrogenase and was purified from swine lungs (Änggård & Samuelsson, 1966). Nakano *et al.* (1969) found that this enzyme is specific to prostaglandins. However, no study has been made on the distribution of this enzyme. Nissen & Andersen (1968) showed histochemically the existence of prostaglandin dehydrogenase in the rat renal tubules. The present study also shows that PGE_1 is rather rapidly converted into three metabolites in the dog kidney, although the chemical structure of these metabolites has not been identified. All or at least one of these metabolites are most likely 15-keto PGE_1 metabolites. Samuelsson (1964), Granström, Inger & Samuelsson (1965), Green (1969) and Hamberg (1968) demonstrated that prostaglandins were also metabolized into dinor- and tetranor-prostaglandins by β-oxidation in the rat liver mitochondria. Although no study has been made on the β-oxidation of prostaglandins in kidneys, it is reasonable to assume that this would take place. Geyer, Matthews & Stare (1949) and Felts (1964) demonstrated that octanoate-^{14}C and palmitate-1-^{14}C were more rapidly oxidized into $^{14}C\,O_2$ in kidney slices than in liver slices. From these previous studies, therefore, it seems that the three metabolites separated in this study could be 15-keto-PGE_1, 15-keto-dihydro-PGE_1, dinor-PGE_1, tetranor-PGE_1, dinor-15-keto-PGE_1 or tetranor-15-keto-PGE_1. Obviously, mass spectrometric analysis is required to determine the precise chemical structures of these metabolites.

Lee *et al.* (1965) and Daniels *et al.* (1967) showed that PGE_2, PGA_2 and $PGF_{2\alpha}$ exist in the rabbit renal medulla. The physiological roles of renal prostaglandins are rather controversial and remain to be elucidated (Bergström, Carlson & Weeks, 1968 ; Horton, 1969). Orloff & Handler (1965) postulated that PGE_1 antagonizes the adenyl cyclase activating action of vasopressin in the renal tubules, thus exerting the diuretic action. Herzog, Johnston & Lauler (1968) showed that PGE_1, PGE_2 and PGA_1 caused a significant increase in renal plasma flow, urine sodium excretion, urine volume excretion, osmolar excretion and free water clearance in dogs. In addition to the physiological actions of PGE_1 on the kidney, Edwards *et al.* (1969) showed that prostaglandin-like substances were increased in the renal venous blood in dogs and patients with renal hypertension. Hence, many investigators have postulated the interrelationship between the pathogenesis of renal diseases and prostaglandins (Bergström *et al.*, 1968 ; Horton, 1969). It is of interest to study whether the metabolic degradation of prostaglandins in kidney may be altered in the individuals with renal diseases.

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