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# A NOVEL PROSTAGLANDIN 11-KETO REDUCTASE FOUND IN RABBIT LIVER

DIANE F REINGOLD, AKIYOSHI KAWASAKI and PHILIP NEEDLEMAN

*Department of Pharmacology, Washington University Medical School, 660 South Euclid Avenue, St Louis, MO 63110 (U S A )*

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## Summary

The prostaglandin 11-keto reductase of rabbits, which catalyzes the conversion of prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$, is only found in the liver. This enzyme, which is primarily localized in the soluble fraction, requires NADPH for activity, its $K_m$ value for prostaglandin $D_2$ is approx. 200 $\mu$M. [$^{14}$C]Prostaglandin $D_2$ administered to rabbits via the portal vein escapes from the liver unchanged, suggesting that exogenous prostaglandin $D_2$ may be inaccessible to the enzyme. The possible role of this enzyme in the metabolism of endogenous hepatic prostaglandin $D_2$ is suggested by the demonstration that the liver synthesizes prostaglandin $D_2$ from arachidonic acid.

## Introduction

Prostaglandin $D_2$ is a metabolite of arachidonic acid found in several organs and cell types [1—6]. Its function in most of these tissues is, as yet, unknown. Prostaglandin $D_2$ has, however, been shown to be a potent inhibitor of platelet aggregation [7,8]. We investigated possible routes of metabolism of the D prostaglandins by various tissues. We report the presence of an enzyme, prostaglandin 11-keto reductase, which catalyzes the conversion of prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$ and its unique localization to the liver. Further studies demonstrate that the liver has the capacity to metabolize arachidonic acid to prostaglandin $D_2$, which may provide an endogenous substrate for the enzyme.

Abbreviation EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid

## Materials and Methods

*Materials* [$^{14}$C]Arachidonic acid (spec act. 55 Ci/mol) and [$^{3}$H]prostaglandin $D_2$ (spec. act. 130 Ci/mmol) were obtained from New England Nuclear (Boston, MA) [$^{3}$H]Prostaglandin $E_2$ (spec act 100 Ci/mmol) was obtained from Amersham Radiochemicals (Arlington Heights, IL). Prostaglandin $D_2$ and prostaglandin $E_2$ were kindly supplied by Dr. John Pike of the Upjohn Co. NADPH and EGTA were purchased from Sigma Chemical Co. (St. Louis, MO), fatty acid-depleted albumin from Calbiochem (La Jolla, CA) and thin-layer chromatography (TLC) plates from Brinkman Instruments (Westbury, NY). [$^{14}$C]Prostaglandin $D_2$ was prepared as follows [$^{14}$C]prostaglandin $H_2$ was prepared from [$^{14}$C]arachidonic acid incubated with an acetone/pentane powder of sheep seminal vesicles. [$^{14}$C]Prostaglandin $H_2$ was incubated for 30 min at 37° C in 0 1 M potassium phosphate buffer containing 0 3% bovine serum albumin. Under these conditions, the major prostaglandin formed is prostaglandin $D_2$. Prostaglandin $D_2$ was separated from other prostaglandins by TLC in system $A_9$ [9], the organic phase of ethyl acetate/acetic acid/2,2,4-trimethylpentane/water (110 20 50 100, v/v) Prostaglandin $D_2$ was eluted from TLC plates with methanol, concentrated and stored at −20°C until use.

*Preparation of liver enzyme* Rabbit livers were homogenized (1 3, w/v) in 100 mM potassium phosphate buffer (pH 7.4) (buffer 1) using a Tekmar homogenizer. The homogenate was centrifuged at 4°C in a Sorvall RC2-B centrifuge at 10 000 × *g* for 20 min. The supernatant was then dialyzed at 4°C against buffer 1 for 16 h (three buffer changes). The dialyzed supernatant was centrifuged in a Beckman ultracentrifuge for 90 min at 100 000 × *g*.

*Measurement of 11-keto reductase activity in vitro.* The activity of 11-keto reductase was determined by measuring the conversion of [$^{3}$H]prostaglandin $D_2$ to [$^{3}$H]prostaglandin $F_{2\alpha}$ The standard reaction mixture contained the following 2 μg prostaglandin $D_2$ (5.64 nmol), 30 000 cpm [$^{3}$H]prostaglandin $D_2$, 300 nmol NADPH; liver supernatant (approx. 0.2 mg protein) in a final volume of 0.3 ml. Reactions were carried out at 37°C for 15 min and were terminated by addition of 2 M formic acid to bring the pH to 3.0—3.5. The reaction mixture was extracted twice with 2 vol. ethyl acetate. The ethyl acetate was evaporated under $N_2$, the samples were plated on silica gel plates and developed in solvent system $A_9$ to separate prostaglandin $F_{2\alpha}$ from prostaglandin $D_2$. The position of the prostaglandins on the TLC plates was determined by adding unlabelled prostaglandin standards to each of the samples and visualizing them with iodine vapor. The location of radioactivity on each plate was determined using a Vangard scanner. The appropriate silica gel zones were scraped into siliconized glass tubes and the radioactivity was extracted twice with 1 ml methanol. The methanol was added to scintillation fluor and the vials were counted in a Packard Tri-Carb 460C Counter. Recovery of counts was between 35 and 40%. Results are not corrected for recovery.

*Measurement of 11-keto reductase activity in vivo.* Male, New Zealand white rabbits, weighing 2—3 kg, were anesthetized with 30 mg/kg Nembutal and 250 U/kg heparin was administered To administer prostaglandin $D_2$, a cannula (PE 50 tubing) was inserted into a branch of the hepatic portal vein. Thus, the flow of blood in the hepatic portal vein was not interrupted by the catheter. To col-

lect blood samples, a cannula (PE 160 tubing) was inserted into the inferior vena cava just above the liver. To minimize dilution of the labelled compound into the blood, the following blood vessels were tied off common carotid arteries, jugular veins, aorta — below the level of the superior mesenteric artery, and vena cava — above the level of the renal vein. Two types of experiment were performed. In one set, a bolus injection of [$^{14}$C]prostaglandin $D_2$ (50 $\mu$g, 1.5 $10^6$ cpm) was given and serial 5-ml blood samples were collected over the next 5 min. Saline was than given to the animal and an additional 5 min were allowed to elapse before the final 5 ml blood sample was taken. In a second set of experiments, a bolus injection was given and the blood was allowed to circulate for 15 min prior to sampling. At this point, a 35 ml blood sample was removed. Extraction of label from the blood was accomplished essentially as described in Ref. 10. Briefly, blood was centrifuged to remove red blood cells The plasma proteins were precipitated by addition of 2 vol. ice-cold acetone and then were removed by centrifugation. The remaining supernatant was extracted twice with 1 vol. petroleum ether (35—60°C). The aqueous layer was acidified to pH 3 0—3 5 with 2 M formic acid and extracted twice with 2 vol. ethyl acetate The ethyl acetate was evaporated under $N_2$ and samples were chromatographed as described for measurement of 11-keto reductase activity in vitro

*Measurement of 11-keto reductase activity by bioassay*. The biological activity of the prostaglandin $F_{2\alpha}$ formed in 11-keto reductase incubation mixtures was studied by measuring contraction of rat stomach strips in a superfusion cascade. Rat stomach strips were superfused at 37°C with Krebs-Henseleit medium, at a flow rate of 10 ml/min. The tissues were also superfused with a mixture of antagonists to histamine, catecholamines and acetylcholine to increase their sensitivity [11], and with indomethacin. Dose-response curves to standards of prostaglandin $F_{2\alpha}$ and prostaglandin $D_2$ were obtained. Aliquots of the incubation mixtures to be described were then applied to the tissues and the response was compared to prostaglandin $F_{2\alpha}$ standards. Changes in smooth muscle tension were measured with a Harvard smooth muscle transducer (Harvard Apparatus Co., Inc., Millis, MA). The standard reaction mixture, as described above, was used to determine conversion of prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$ radiochemically. In parallel, incubations were carried out, containing all components of the standard reaction mixture, except [$^3$H]prostaglandin $D_2$. The latter samples were incubated for 6, 15, 30 or 60 min and were acidified and extracted into ethyl acetate as described above. The ethyl acetate was dissolved under $N_2$ and the residue was re-dissolved in 0.25 ml saline A 15 $\mu$l aliquot of the residue (equivalent to 6% of the original starting material) was applied to rat stomach strips and the contractions were measured

*Arachidonic acid metabolism of rabbit liver*. Rabbits were anesthetized and heparin was administered as described above. The portal vein was cannulated (PE 160) and saline was used to clear the liver of as much blood as possible. The liver was homogenized (1 : 3) using a Tekmar homogenizer in 100 mM potassium phosphate buffer (pH 8.0) (buffer 2) containing EGTA (10 mM) and bovine serum albumin (1.0%). The homogenate was centrifuged at 8000 $\times g$ in a Sorvall RC2B centrifuge for 15 min and the supernatant was then centrifuged in a Beckman ultracentrifuge for 60 min at 100 000 $\times g$. The resulting micro-

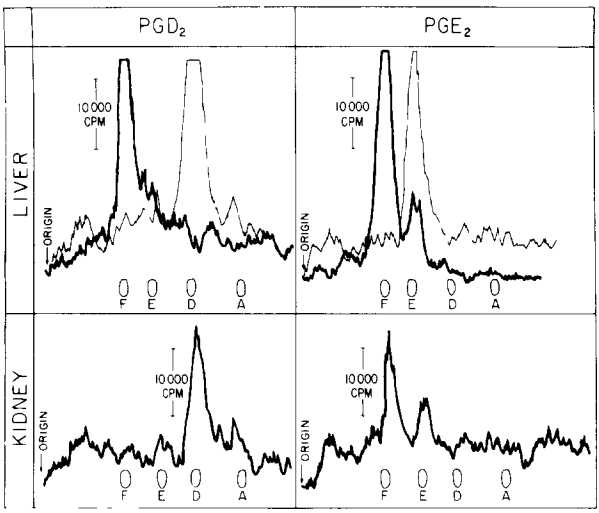
somal pellet was washed three times with buffer 2 and was resuspended in the same buffer (volume = 1/4 of the original tissue weight). Incubations contained the following 1-[$^{14}$C]arachidonic acid (1 $\mu$g, 300 000 cpm), epinephrine (150 nmol) and 0.1 ml (approx 300 $\mu$g protein) of microsomes in a final volume of 0.15 ml. When present, glutathione was used at a final concentration of 1.0 mM. Incubations were carried out, with stirring, at 37°C for 30 min. Reactions were terminated by addition of 0.05 ml of 4 M formic acid. The reaction mixture was extracted twice with 2 vol. of ethyl acetate The ethyl acetate was evaporated under $N_2$ and the residue applied to silica gel TLC plates. The plates were developed in solvent system C (chloroform/methanol/acetic acid/water, 90 8 1 0 0 8, v/v) [12] Positions of the prostaglandins was determined by adding unlabelled standards to the samples and visualizing with iodine vapor. The position of radioactivity was determined using a Vangard No. 930 scanner. Localization of the radioactivity in selected samples was determined by autoradiography. In this case, the TLC plate was placed on a piece of Kodak X-Omat R X-ray film and was kept in the dark for 48 h. After developing, the position of the bands was compared to that of the standards as visualized by iodine vapor. The appropriate lanes were cut and were counted in scintillation fluor (4a20-RPI Corp.) in a Beckman LS-230 scintillation counter.

## Results

A possible pathway in the metabolism of prostaglandin $D_2$ would be its conversion to prostaglandin $F_{2\alpha}$ by a presumed prostaglandin 11-keto reductase. Therefore, several tissues were examined for their ability to convert [$^3$H]prostaglandin $D_2$ to [$^3$H]prostaglandin $F_{2\alpha}$. Under the conditions used, the liver quantitatively converts either prostaglandin $D_2$ or prostaglandin $E_2$ to prostaglandin $F_{2\alpha}$. In Fig 1 are shown typical radiochromatograms from incubations of liver or kidney 100 000 $\times g$ supernatant with labelled prostaglandin $D_2$ or prostaglandin $E_2$. Following dialysis of the 100 000 $\times g$ supernatant, no conversion is detectable, addition of NADPH to incubation mixtures restores activity (Fig 1). Identity of the product as prostaglandin $F_{2\alpha}$ was confirmed by demonstrating that the product of the reaction comigrates with authentic prostaglandin $F_{2\alpha}$ in chromatographic system $A_9$ (Fig. 1) as well as in chromatographic systems C and benzene/1,4-dioxane/acetic acid (60 . 30 : 3, v/v) (results not shown). Less than 5% of the activity of the liver 11-keto reductase was detectable in several other tissues, including heart, kidney cortex, kidney medulla, brain, lung, whole blood and plasma. Prostaglandin 9-keto reductase [13], which catalyzes conversion of prostaglandin $E_2$ to prostaglandin $F_{2\alpha}$, is probably not responsible for the conversion of prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$, since incubation of [$^3$H]prostaglandin $D_2$ with kidney supernatant did not result in conversion to prostaglandin $F_{2\alpha}$, whereas prostaglandin $E_2$ was metabolized to prostaglandin $F_{2\alpha}$ by either kidney cortex or medulla supernatant (Fig. 1).

11-Keto reductase activity is found almost exclusively in the soluble fraction of liver. Enzyme activities of the 10 000 $\times g$ supernatant, the 100 000 $\times g$ supernatant and of the microsomal fraction are 0 22 ± 0.01, 0.25 ± 0.7 and 0.03 ± 0.01 nmol/min per mg protein, respectively.

Enzyme activity was determined as a function of substrate, cofactor and



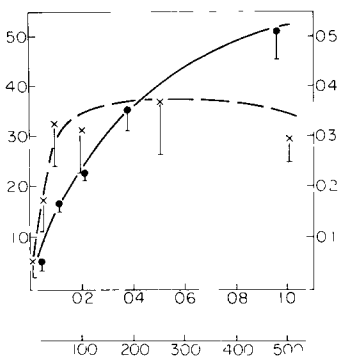




Fig 1 Prostaglandin 11- and 9-keto reductase activity in rabbit liver or kidney supernatant. 11- or 9-keto reductase activity of a dialyzed 100 000 × *g* supernatant of either rabbit liver or kidney was determined using [$^3$H]prostaglandin $D_2$ or [$^3$H]prostaglandin $E_2$, respectively Reaction mixtures contained 2 μg of prostaglandin $D_2$ (or prostaglandin $E_2$), 30 000 cpm of [$^3$H]prostaglandin $D_2$ (or prostaglandin $E_2$) and 0 1 M potassium phosphate buffer, pH 7 4 (buffer 1), in a final volume of 0 3 ml NADPH was either present, at a concentration of 1 0 mM (heavy lines), or was absent (light lines) Reactions were carried out for 15 min at 37°C Reactions were acidified, extracted, concentrated, and reactants and products were separated by TLC system $A_9$ Localization of radioactivity was determined using a Vangard scanner F, prostaglandin $F_{2\alpha}$, E, prostaglandin $E_2$, D, prostaglandin $D_2$, A, prostaglandin $A_2$

Fig 2 Effect of substrate and cofactor concentration on prostaglandin 11-keto reductase activity Prostaglandin 11-keto reductase activity was determined using a dialyzed preparation of rabbit liver 100 000 × *g* supernatant When the prostaglandin $D_2$ concentration was varied (•———•, lower scale) reaction mixtures contained enzyme (approx 0 08 mg protein), NADPH (1 0 mm), buffer 1, and prostaglandin $D_2$ at a concentration of 24 to 470 μM (left-hand ordinate) When the NADPH cofactor concentration was varied (X———X, upper scale) reaction mixtures contained enzyme (approx. 0 2 mg protein), buffer 1, prostaglandin $D_2$, 18 8 μM, and NADPH at a concentration of 0 01 to 1 0 mM (right-hand ordinate) All reactions were carried out at 37°C for 15 min Results presented are expressed as nmol/min per mg protein and are the mean ± S E for seven experiments

enzyme concentrations and as a function of time. Enzyme activity was tested with prostaglandin $D_2$ at a concentration of 24—470 μM (Fig. 2). The $K_m$ value was determined to be between 180 and 350 μM. Hepatic 11-keto reductase exhibits a preference for NADPH as its cofactor, as shown in Fig. 2. The minimum effective concentration of NADPH to activate 11-keto reductase is between 0.01 and 0.05 mM, maximum activation is obtained at 0.2 mM In contrast, NADH is much less effective as a cofactor, requiring 5-fold greater amounts to achieve the same effect as NADPH. Enzyme activity is linear with up to 500 μg of protein per incubation and up to 30 min of incubation at 37°C. No conversion of prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$ was observed in the absence of tissue or in incubations using enzyme which had been boiled for 2 min.

Additionally, we confirmed the identity of the product of the reaction as prostaglandin $F_{2\alpha}$, using a bioassay system. In these experiments, advantage is taken of the fact that prostaglandin $F_{2\alpha}$ is much more potent in contracting

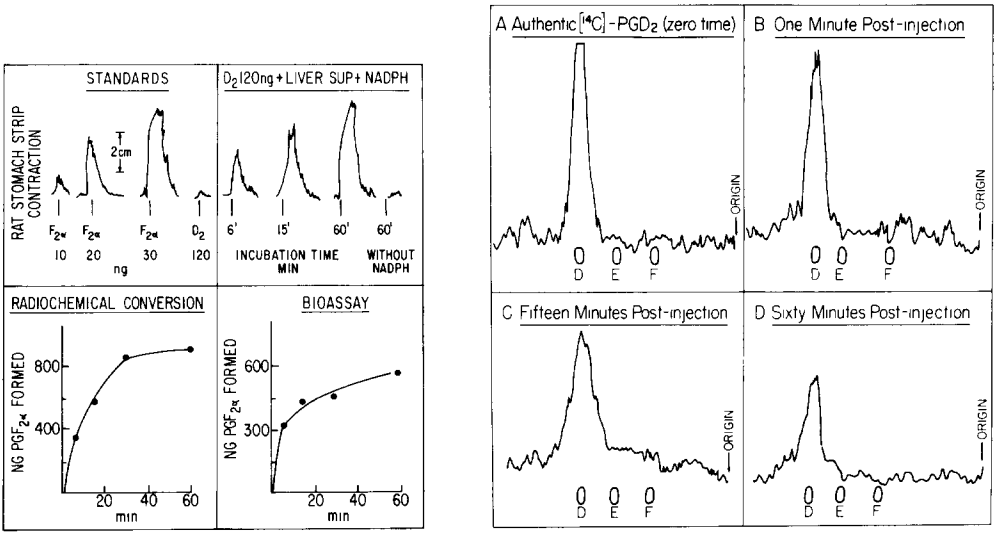



Fig 3 Determination of 11-keto reductase activity using rat stomach strip bioassay 11-Keto reductase activity was determined using liver supernatant, approx 0 2 mg protein (LIVER SUP), prostaglandin $D_2$, 2 $\mu$g, [$^3$H]prostaglandin $D_2$ (30 000 cpm, radiochemical experiments only), buffer 1 m in the presence or absence of NADPH, 1 0 mM Reactions were carried out for 6, 15, 30 or 60 min at 37°C Reaction mixtures were acidified, extracted and concentrated An aliquot equivalent to 6% of the original reaction mixture was applied to rat stomach strips and contraction was measured Dose-response curves were determined with standard prostaglandin $F_{2\alpha}$ ($F_{2\alpha}$) and prostaglandin $D_2$ ($D_2$) In the radiochemical experiments, the concentrated reaction mixture was applied to TLC plates and the appropriate zones were scraped and counted Results presented are the average of two experiments

Fig 4 Determination of 11-keto reductase activity in vivo [$^{14}$C]Prostaglandin $D_2$ ($PGD_2$) (1 5 $10^6$ cpm, 50 $\mu$g) was injected as a bolus into the portal vein of a prepared rabbit Blood samples were collected at 1 (B), 15 (4C) and 60 min (D) after injection of prostaglandin $D_2$ Blood samples were centrifuged free of red cells and plasma proteins were precipitated with acetone Prostaglandins were recovered from the acetone by acidification, extraction into ethyl acetate and were then separated on TLC system $A_9$ Localization of radioactivity was determined using a Vangard scanner In A is shown an authentic sample of the [$^{14}$C]prostaglandin $D_2$ which was chromatographed in system $A_9$ F, prostaglandin $F_{2\alpha}$, E, prostaglandin $E_2$, D, prostaglandin $D_2$

smooth muscle than is prostaglandin $D_2$. The tissue used was rat stomach strip, which was calibrated to known amounts of prostaglandin $D_2$ and prostaglandin $F_{2\alpha}$. When enzyme, substrate and cofactor are incubated together, the product is biologically active. Generation of a biologically active substance requires the presence of cofactor (Fig. 3). Neither substrate (prostaglandin $D_2$) nor enzyme alone induces contraction of the tissue. The time dependence of the reaction is also evident with longer incubation periods producing more of the contractile substance (Fig. 3). Parallel incubations using [$^3$H]prostaglandin $D_2$ and the identical preparation of the enzyme contain increasing amounts of [$^3$H]prostaglandin $F_{2\alpha}$ with increasing incubation time (Fig. 3). Thus, liver supernatant converts prostaglandin $D_2$ to a substance with both the identical chromatographic mobility and biological activity of prostaglandin $F_{2\alpha}$

The presence of 11-keto reductase in liver homogenates prompted us to assess if the activity of this enzyme could be detected in vivo. [$^{14}$C]Prostaglandin $D_2$ was injected as a bolus into the portal vein of an anesthetized rabbit

and serial 5-ml samples were collected over the next 5 min from the vena cava. Radiochemical analysis of the blood showed that, surprisingly, prostaglandin $D_2$ appears to leave the liver unmetabolized (Fig. 4). In other experiments, 15 min were allowed to elapse between time of injection and removal of blood. Even under these conditions, the only radioactively labelled prostaglandin which was recovered is prostaglandin $D_2$. This indicated that exogenous prostaglandin $D_2$ was not accessible to 11-keto reductase.

An alternative function of prostaglandin 11-keto reductase would be to metabolize endogenous hepatic prostaglandin $D_2$. Experiments were undertaken to determine if liver microsomes could convert arachidonic acid to prostaglandin $D_2$. Incubation of [$^{14}$C]arachidonic acid with liver microsomes without any cofactors resulted in the formation of prostaglandin $D_2$, prosta-

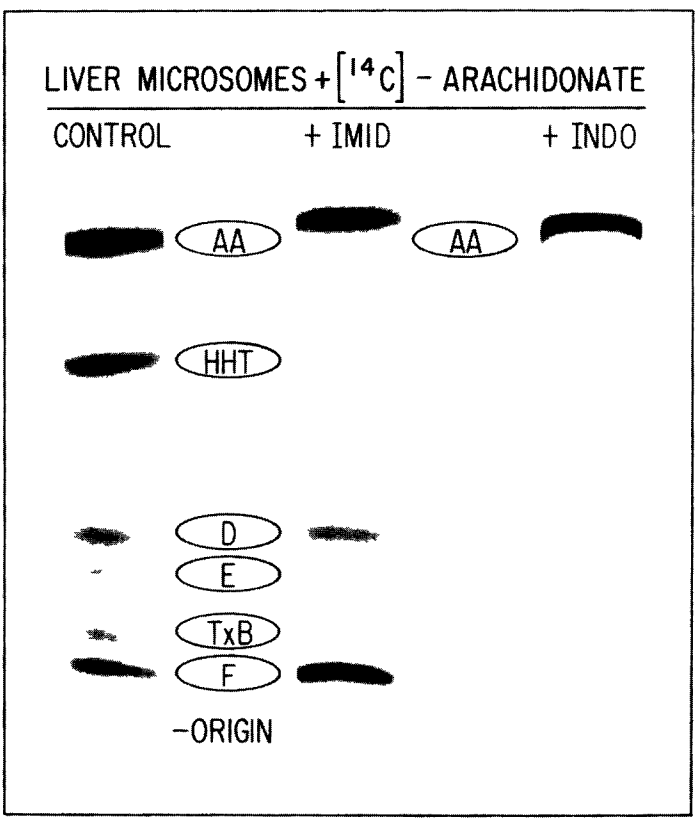



Fig 5 [$^{14}$C]Arachidonate conversion by rabbit liver microsomes Liver microsomes (approx 0 3 mg protein) were incubated in a final volume of 0 15 ml at 37°C for 30 min in the presence of the following (final concentration) [$^{14}$C]arachidonic acid, 1 μg, 300 000 cpm (21 9 μM), epinephrine (1 0 mM), glutathione (1 0 mM), potassium phosphate buffer, 0 1 M, pH 8 0 Prostaglandins were separated on TLC in solvent system C and were then placed on X-ray film in cassettes for 2 days Appropriate zones were scraped and counted to determine radioactivity present In the control, counts found in each zone were prostaglandin $F_{2\alpha}$ (F) 27 329, thromboxane $B_2$ (TxB) 2588, prostaglandin $E_2$ (E) 4524, prostaglandin $D_2$ (D) 6257, 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) 9327, and arachidonic acid (AA) 31 833 In the presence of 5 mM imidazole the counts were as follows F, 31 043, TxB, 658, E, 5903, D, 8733, HHT, 2477, AA, 29 682 In the presence of 5 μg/ml indomethacin, less than 0 5% of the counts were found in any of the peaks corresponding to prostaglandin products

glandin $F_{2\alpha}$, prostaglandin $E_2$ and thromboxane $B_2$. No prostacyclin synthesis was measurable Conversion was markedly enhanced by the addition of epinephrine (1.0 mM) to incubations. Addition of glutathione (1.0 mM) further enhacned total arachidonate metabolism with preferential production of prostaglandin $F_{2\alpha}$. In Fig. 5 are shown autoradiographs obtained from incubations of [$^{14}$C]arachidonic acid with liver microsomes in the presence of both epinephrine and glutathione. In the presence of imidazole, a selective inhibitor of thromboxane synthetase [14], thromboxane $B_2$ and 12-hydroxy-5,8,10-heptadecatrienoic acid production is virtually abolished, concomitantly, more prostaglandin $F_{2\alpha}$ and prostaglandin $D_2$ are formed. In the presence of indomethacin (5 $\mu$g/ml), no prostaglandin formation was seen (Fig. 5).

## Discussion

Interest in prostaglandins of the D series has been generated recently by the demonstration that both prostaglandin $D_2$ and its triene congener prostaglandin $D_3$ are potent inhibitors of platelet aggregation [7,8,15,16]. The potential usefulness of prostaglandin $D_3$ as a circulating antithrombotic agent prompted the present study of possible routes of metabolism of the D prostaglandins. Prostaglandin $D_2$ had previously been shown not to be a substrate for lung 15-hydroxy-prostaglandin dehydrogenase in vitro [17]. There had been a report of conversion of both prostaglandin $D_2$ and prostaglandin $E_2$ to prostaglandin $F_{2\alpha}$ by fetal sheep blood, but no further characterization of this activity had been forthcoming [18]. In the present studies we could detect no conversion of prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$ by either whole blood or plasma. This may be due to species differences. The only organ with detectable levels of 11-keto reductase in the rabbit is the liver. Enzyme activity is enhanced by NADPH, which is much more potent than NADH, the $K_m$ value of prostaglandin $D_2$ for the enzyme is approx. 200 $\mu$M. Since the liver contains both prostaglandin 9- and 11-keto reductase activity, it is possible that 9-keto reductase is responsible for the conversion of prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$. There are several similarities between the properties of prostaglandin 11-keto reductase reported here and those of 9-keto reductase as previously described. These include $K_m$ [19—21], subcellular localization [19,22] and preference for NADPH as cofactor [20,22]. However, despite the similarities between the 11- and 9-keto reductases, there are several reasons which make it unlikely that the conversion of prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$ is catalyzed by prostaglandin 9-keto reductase. The first is that purified chicken heart 9-keto reductase does not metabolize prostaglandin $D_2$ [23]. In addition, both kidney medulla and cortex can convert prostaglandin $E_2$ to prostaglandin $F_{2\alpha}$, whereas under the same conditions, prostaglandin $D_2$ is not metabolized (Fig. 1). Thus, if the reduction of prostaglandin $E_2$ to prostaglandin $F_{2\alpha}$ in liver and kidney is catalyzed by the same enzyme, then clearly the conversion of prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$ requires the presence of a separate enzyme. Wong [24] has simultaneously discovered the 11-keto reductase activity. He purified the liver soluble enzyme and demonstrated that radioactively labelled prostaglandin $E_2$ was not a substrate for the purified 11-keto reductase (personal communication).

In most cases, metabolism of the primary prostaglandins results in the formation of less biologically active products The finding that, in vitro, prostaglandin $D_2$ is converted to prostaglandin $F_{2\alpha}$ is, therefore, of potential importance. Prostaglandin $F_{2\alpha}$ is less active than prostaglandin $D_2$ as an inhibitor of platelet aggregation, but is much more active than prostaglandin $D_2$ in contracting smooth muscle (Fig. 3) Therefore, the conversion of prostaglandin $D_2$ (or prostaglandin $D_3$) to its prostaglandin $F_{2\alpha}$ counter part could increase side effects (i.e., gastrointestinal distress and pulmonary constriction) and limit therapeutic efficacy. It was of interest to determine if, indeed, prostaglandin $D_2$ could be converted to prostaglandin $F_{2\alpha}$ in vivo Studies in which prostaglandin $D_2$ was injected into the portal vein of an anesthetized rabbit indicated, however, that prostaglandin $D_2$ escapes from the liver unchanged. Thus, exogenous D prostaglandins may not be converted by the liver to more active products, at least in a short-term experiment. These results are in contrast to those obtained by Ellis et al. [25] in monkey. In their experiments, prostaglandin $D_2$ was converted to both prostaglandin $F_{2\alpha}$ and several of its metabolites, in fact, prostaglandin F-ring metabolites accounted for over 2/3 of the radioactivity recovered in the urine. In the monkey experiments, prostaglandin $D_2$ was infused for 6.5 h at the rate of 300 $\mu$g/h and urine was collected for 24 h. Such an exposure is considerably longer than the time used in the present experiments. In addition, although prostaglandin $F_{2\alpha}$ was recovered in the urine, it accounted for only 1% of the recovered radioactivity which would probably be below our limit of detection. In the discussion of their results, Ellis et al. [25] suggested the presence of an 11-keto reductase but did not confirm its existence in any monkey tissues. Our results suggest that it may be found in the monkey liver. The contribution of prostaglandin F-type metabolites to the therapeutic efficay (or side effects) of administered prostaglandin $D_2$ still remains to be determined.

An alternative function of hepatic 11-keto reductase would be the conversion of hepatic prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$. Therefore, we studied the conversion of [$^{14}$C]arachidonic acid to labelled products to determine if the liver has the capacity to form prostaglandin $D_2$. We determined that prostaglandin $D_2$ is a normal metabolite of arachidonic acid incubated with rabbit liver microsomes. Prostaglandin $D_2$ and prostaglandin $F_{2\alpha}$ are the major arachidonic acid metabolites formed by rabbit liver microsomes, in addition, prostaglandin $E_2$ and thromboxane $B_2$ are formed. Previous reports, using rat liver, had demonstrated that the only products of arachidonic acid metabolism in liver microsomes are prostaglandin $E_2$ and prostaglandin $F_{2\alpha}$ [26]. We confirmed this work (results not shown). Thus, there seems to be a species difference in arachidonic acid metabolism. It was of interest, therefore, to determine if rat liver contained 11-keto reductase activity. In three separate experiments we could not detect 11-keto reductase activity in rat liver. Thus, rabbit liver, which has the capacity to form prostaglandin $D_2$, can also metabolize it, whereas rat liver can neither form nor metabolize prostaglandin $D_2$.

We have demonstrated that the liver contains the novel enzymatic activity prostaglandin 11-keto reductase, which converts prostaglandin $D_2$ to prostaglandin $F_{2\alpha}$ in the presence of NADPH. Although this enzyme does not appear to metabolize exogenous prostaglandin $D_2$, it may serve to metabolize hepatic

prostaglandin $D_2$, formed from endogenous arachidonic acid, to prostaglandin $F_{2\alpha}$. Prostaglandin $F_{2\alpha}$ was also the major product of microsomal [$^{14}$C]arachidonic acid metabolism. Thus, the liver has a substantial capacity to form prostaglandin $F_{2\alpha}$, both from endogenous arachidonic acid and from products of arachidonic acid cyclo-oxygenation. The rate of production of prostaglandin $F_{2\alpha}$ by the isolated perfused liver has been reported to increase following hypoxia [28]. The involvement of prostaglandin $F_{2\alpha}$ in other liver functions has not yet been established.

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## References

1 Pace-Asciak, C and Nashat, M (1976) J Neurochem 27, 551—556
2 Oelz, O , Oelz, R , Knapp, H R , Sweetman, B J and Oates, J A (1977) Prostaglandins 13, 225—234
3 Sun, F F., Chapman, J P and McGuire, J C (1977) Prostaglandins 14, 1055—1075
4 Friesinger, G C , Oelz, O , Sweetman, B J , Nies, A S and Data, J L (1978) Prostaglandins 15, 969—981
5 Jakschik, B A , Lee, L A , Shuffer, G and Parker, C W (1978) Prostaglandins 16, 733—748
6 Knapp, H R , Oelz, O , Sweetman, B J and Oates, J A (1978) Prostaglandins 15, 751—757
7 Smith, J B , Silver, M J , Ingerman, C M and Koesis, J J (1974) Thromb Res 5, 291—299
8 Nishizawa, E E , Miller, W L , Gorman, R R , Bundy, G L , Svensson, J and Hamberg, M (1975) Prostaglandins 9, 109—121
9 Hamberg, M and Samuelsson, B (1966) J Biol Chem 241, 257—263
10 Green, K , Hamberg, M , Samuelsson, B and Frolich, J C (1978) Adv Prostaglandin Thromboxane Res 5, 15—38
11 Ferreira, S H and Vane, J R (1967) Nature 216, 868—872
12 Nugteren, D H and Hazelhof, E (1973) Biochim. Biophys Acta 326, 448—461
13 Leslie, C A and Levine, L (1973) Biochem Biophys Res Commun 52, 717—724
14 Needleman, P , Raz , A , Ferrendelli, J A and Minkes, M (1977) Proc Natl Acad Sci U S A 74, 1716—1720
15 Whitaker, M O , Wyche, A , Fitzpatrick, F , Sprecher, H and Needleman, P (1979) Proc Natl Acad Sci U S A 76, 5919—5923
16 Greglewski, R J , Salmon, J A , Ubatuba, F B , Weatherly, B C , Moncada, S and Vane, J R (1979) Prostaglandins 18, 453—478
17 Sun, F F , Armour, S B , Bockstantz, V R and McGuire, J C (1976) Adv Prostaglandin Thomboxane Res 1, 163—169
18 Hensby, C N (1974) Prostaglandins 8, 369—375
19 Thuy, L P and Carpenter, M P (1978) Biochem Biophys Res Commun 81, 322—328
20 Stone, K J and Hart, M (1975) Prostaglandins 10, 273—288
21 Toft, B S and Hansen, H S (1979) Biochim Biophys Acta 574, 33—38
22 Lee, S -C and Levine, L (1974) J Biol Chem 249, 1369—1375
23 Lee, S -C and Levine, L (1975) J Biol Chem 250, 4549—4555
24 Wong, P Y -K (1981) Biochim. Biophys Acta 659, 169—178
25 Ellis, C K , Smigel, M D , Oates, J A , Oelz, O and Sweetman, B J (1979) J Biol Chem 254, 4152—4163
26 Morita, I and Murota, S -i (1978) Eur J Biochem 90, 441—449
27 Flynn, J T (1979) Prostaglandins 17, 39—52