Br. J. Pharmac. (1976), **57**, 589–597

A RAPID METHOD FOR THE ESTIMATION OF PROSTAGLANDIN 15-HYDROXYDEHYDROGENASE ACTIVITY AND ITS APPLICATION TO PHARMACOLOGY

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1 A novel double-isotope assay for prostaglandin 15-hydroxydehydrogenase (PGDH) is described.

2 The assay is simple, rapid, precise, completely specific for PGDH, and free from artefacts. The results obtained correlate well with a conventional assay.

3 The method is versatile, being applicable to estimations of enzyme activity in cell-free systems as well as perfused organs, and is well suited to the assay of PGDH inhibitors.

Introduction

Prostaglandin 15-hydroxydehydrogenase (PGDH) catalyses the initial (and probably rate-limiting) step in the metabolic degradation of prostaglandins (Änggård & Samuelsson, 1964, 1966; Hamberg & Samuelsson, 1971a). The enzyme is a soluble cytoplasmic protein which is widely distributed throughout animal tissues (Änggård, Larsson & Samuelsson, 1971) and has an important role in the metabolism of prostaglandins in animals and man (Gréen & Samuelsson, 1971; Hamberg & Samuelsson, 1971b, 1972). Oxidation of the C-15 alcohol group of prostaglandins, the reaction catalysed by PGDH, occurs extremely rapidly and results in a significant loss of biological activity (Änggård, 1966). The blood or tissue levels of prostaglandins at any given time should therefore be interpreted as the result of a balance between biosynthesis and PGDH activity. Thus PGDH is an important modulator of prostaglandin levels in biological fluids.

Recently we have demonstrated that PGDH has only a short life within the cell (half-life in rat kidney and lung, 45–75 min; Blackwell, Flower & Vane, 1975). Thus, activity depends upon continued enzyme synthesis and this could be very susceptible to hormonal control mechanisms; indeed several authors have now demonstrated alteration of enzyme activity following the administration to animals of steroids or bacterial endotoxins (Nakano & Prancan, 1973; Sun & Armour, 1974; Bedwani & Marley, 1975; Blackwell & Flower, 1976).

The key position in prostaglandin metabolism occupied by PGDH also makes it an interesting target for drugs. Recently it was shown that inhibitors of PGDH produced abortion in animals suggesting a role for this enzyme in the control of parturition (Lerner, Galliani, Carminati & Mosca, 1975) and also that blockade of lung PGDH in rabbits resulted in a significant prolongation of the biological activity of exogenously administered prostaglandins (Crutchley & Piper, 1975).

These factors make the activity of PGDH of considerable interest from the pharmacologist's viewpoint and we have therefore devised a novel method for the assay of PGDH, which can be used for estimation of enzyme activity in cell-free systems or perfused organs. The assay is simple, precise and rapid, and is well suited to the investigation of drug inhibitors of PGDH.

Methods

PGDH catalyses the oxidation of the C-15 alcohol group of prostaglandins and during this NAD^+-dependent reaction there is a concomitant loss of the C-15 hydrogen. The assay described here depends on the synthesis of prostaglandin $F_{2\alpha}$ specifically labelled with tritium at C-15, so that oxidation of this group by the enzyme results in a loss of tritium from the molecule (see Figure 1). After the PGDH reaction the mixture of labelled prostaglandins and unlabelled 15-keto metabolites is extracted from the reaction mixture with organic solvents leaving the ^3H label which has been removed by the enzyme in the aqueous phase. [1-^{14}C]-labelled prostaglandins are added as an internal standard since the label on these prostaglandins is not removed by PGDH. Metabolism can then be

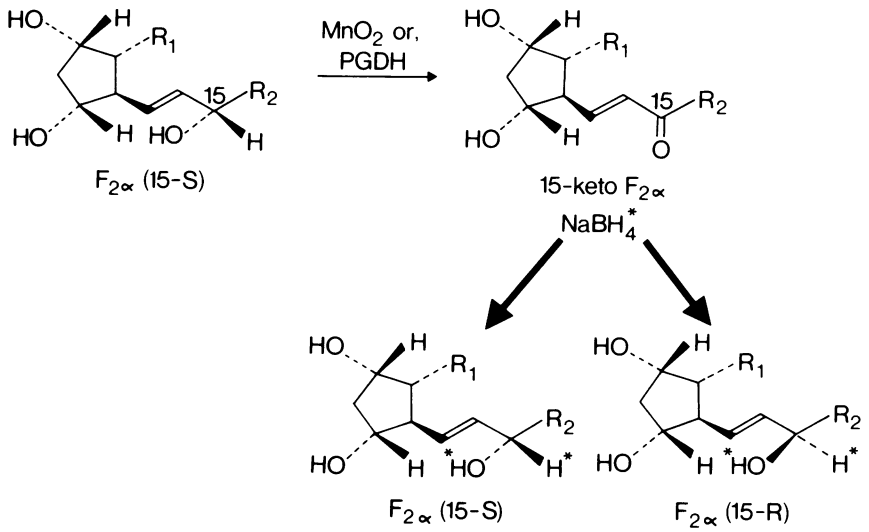

Figure 1 Preparation of the specifically labelled prostaglandin $F_{2\alpha}$. The groups 'R_1' and 'R_2' refer to the hydrocarbon chains appropriate to the bis-unsaturated prostaglandins. After the oxidation of prostaglandin $F_{2\alpha}$ (by MnO_2 or swine lung prostaglandin 15-hydroxydehydrogenase (PGDH)) the 15-keto derivative is reduced with [^{3}H]-$NaBH_4$. The reaction does not proceed stereospecifically and the products may have 'S' or 'R' configuration at C-15. In our hands the 15-R epimer predominates. Although two tritium atoms are incorporated into the molecule only the C(15)-H* tritium is stable. The hydroxylic tritium C(15)-OH* is lost by rapid exchange with the solvent, ultimately therefore only monotritiated prostaglandins are formed.

estimated by calculating the $^3H/^{14}C$ ratio in the organic extract.

Preparation of specifically labelled prostaglandin $F_{2\alpha}$

The specifically labelled substrate [15-^{3}H]-prostaglandin $F_{2\alpha}$ is prepared from 15-keto prostaglandin $F_{2\alpha}$ by reduction with [^{3}H]-sodium borohydride.

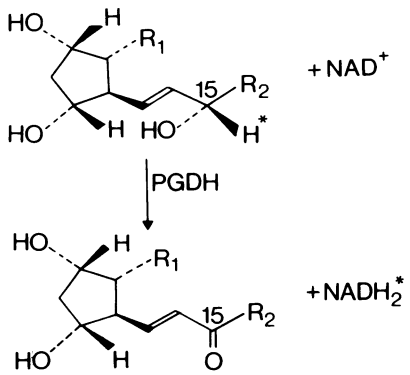

Figure 2 The prostaglandin 15-hydroxydehydrogenase (PGDH) reaction. During oxidation of the C-15 alcohol the tritium label is removed from the molecule.

Unless authentic samples of 15-keto $F_{2\alpha}$ are available, the starting material must first be synthesized from prostaglandin $F_{2\alpha}$. This can be accomplished by oxidation of prostaglandin $F_{2\alpha}$ with manganese dioxide or by a biosynthetic route. In practice we found the latter approach superior because we experienced difficulty in specifically oxidizing the 15-OH group of prostaglandin $F_{2\alpha}$ with manganese dioxide. In most animal tissues the 15-keto metabolite of prostaglandins is rapidly metabolized by the enzyme PGΔ^{13}-reductase to another derivative. However, this enzyme is absent from swine lung (Änggård & Samuelsson, 1966) and we have therefore used homogenates of this tissue to prepare 15-keto prostaglandin $F_{2\alpha}$ biosynthetically. Fresh swine lungs were obtained from a local slaughter house and were stored in dry ice until used later the same day. Lung tissue (100 g) was trimmed free of connective tissue and homogenized in ice cold phosphate buffer (100 mM, pH 7.4) in the ratio of 4 : 1 (v/w) with a Waring blender. The resulting homogenate was filtered through cheesecloth and centrifuged at 100,000 g_{av} in a Beckman 'Spinco' ultracentrifuge for 1 hour. The resulting supernatant (which contains PGDH) was decanted into an Erlenmeyer flask and mixed with 2 mg of prostaglandin $F_{2\alpha}$. The cofactor for this reaction, β-NAD$^+$, was added to give a final concentration of 10 mM. After incubation at 37°C for 30 min the reaction mixture was acidified with 1N HCl to pH 3. Saturating amounts of crystalline

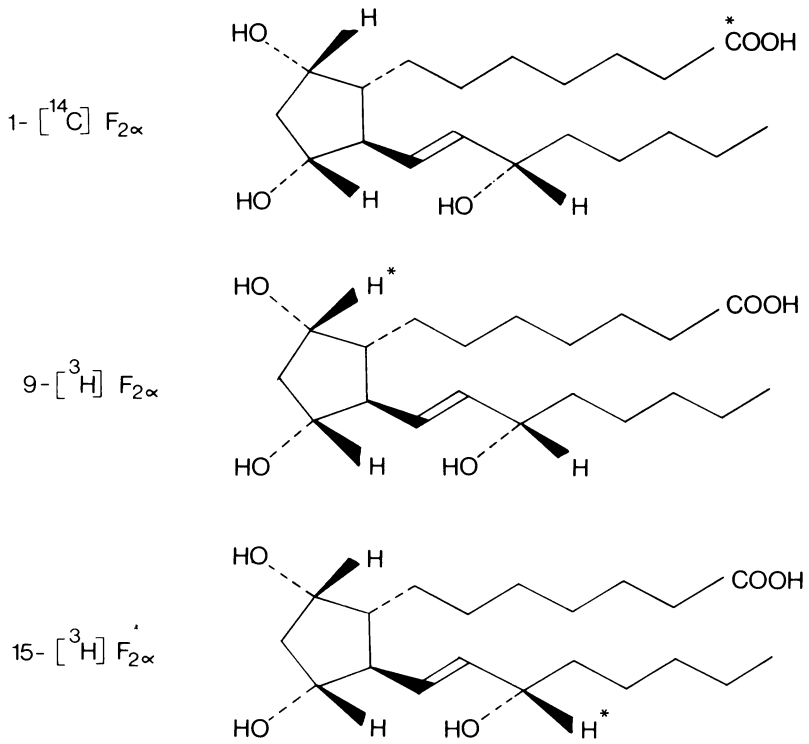

Figure 3 Three different types of specifically labelled prostaglandin $F_{2\alpha}$ molecules used in this study.

NaCl were added (to prevent emulsion formation) and the mixture was extracted with an equal volume of ethyl acetate. After phase separation this organic phase was dried in a rotary evaporator, the residue taken up in 100 µl chloroform–methanol (1 : 1) and streaked (1 cm) onto a silica gel thin layer chromatogram which was subsequently developed to a distance of 20 cm in *iso*-octane, ethyl acetate, acetic acid, water (5 : 11 : 2 : 10). After development the plate was dried and the zones containing prostaglandin $F_{2\alpha}$ and its 15-keto metabolite were located by exposure to iodine vapour. Prostaglandin $F_{2\alpha}$ has an R_F value of about 0.13 in this system and 15-keto prostaglandin $F_{2\alpha}$ an R_F of about 0.27. In order to remove traces of 15-keto prostaglandin $F_{1\alpha}$ which might have been formed from endogenous prostaglandin $F_{1\alpha}$ and which would co-chromatograph with 15-keto-prostaglandin $F_{2\alpha}$, it was necessary to rechromatograph the product on a silica gel plate impregnated with 5% $AgNO_3$. The same solvent system was utilized, the approximate R_F value of 15-keto prostaglandin $F_{2\alpha}$ being 0.19. The final product was eluted from the plate, dissolved in ethanol and stored at −20°C. Yields of up to 1 mg of 15-keto prostaglandin $F_{2\alpha}$ have been obtained in this way.

Reduction of 15-keto prostaglandin $F_{2\alpha}$ with [^{3}H]-sodium borohydride

In a typical preparation, 0.5 mg of 15-keto prostaglandin $F_{2\alpha}$ was dissolved in 0.5 ml absolute ethanol in a small glass tube and placed in an ice bath. Approximately 80 mCi [^{3}H]-sodium borohydride was transferred to a small hand-held homogenizer containing 0.5 ml cooled ethanol. The particle of sodium borohydride was then disintegrated in the ethanol and the resulting partial solution added to the sample in the ice bath. This reaction must be performed in a fume cabinet since tritium is liberated.

After 30 min, 5 ml water was added and 0.1 ml 1N HCl. This acidification decomposes the unreacted [^{3}H]-sodium borohydride with concomitant liberation of labile tritium, and protonates the prostaglandin products so that the sample can then be extracted by shaking with ethyl acetate (5 ml). After the organic layer was removed it was evaporated to dryness and applied to a silica gel t.l.c. plate which was developed in the solvent system already described. After development, the plate was briefly dried and the zones containing radioactivity were located with a radiochromatogram scanner. Two main zones of

radioactivity were seen, one of which corresponded to the authentic prostaglandin $F_{2\alpha}$ standard (R_F 0.13). The chemical reduction of 15-keto prostaglandin $F_{2\alpha}$ does not proceed stereospecifically (see Figure 3) and as well as the naturally occurring 15-S configuration, the 15-R epimer is also found (R_F 0.24) often in larger amounts than the 15-S compound. The 15-R compound is not a substrate for the enzyme.

When fresh [^{3}H]-sodium borohydride was used a very small amount (< 10%) of the radioactivity recovered was found at the origin of the t.l.c. plate. In aged preparations this residual activity may account for 50–60% of the radioactivity recovered; it is apparently a [^{3}H]-sodium borohydride decomposition product. For this reason only fresh preparations of the reducing agent should be used. The batch of [15-^{3}H]-prostaglandin $F_{2\alpha}$ prepared for this study by the method outlined above had a specific activity of 1.34 mCi/mmol. The product was mixed with [1-^{14}C]--labelled prostaglandin $F_{2\alpha}$, and unlabelled prostaglandin $F_{2\alpha}$ and dissolved in ethanol giving a final solution containing 2.7 μg/ml prostaglandin $F_{2\alpha}$ and 5.2×10^6 d/min ^{3}H and 5.5×10^5 d/min ^{14}C/ml. The solution was stored at −20°C until required.

Preparation of cell-free system containing prostaglandin 15-hydroxydehydrogenase

Rat kidneys were chosen as a convenient source of PGDH for routine purposes, and the 100,000 *g* supernatant was prepared as follows: rats (male, Wistar strain, weighing between 150–250 g) were killed by a blow on the head. The kidneys were removed, immediately placed on ice and homogenized within 5 min in 5 vol of ice-cold buffer (100 mM pH 7.5 Tris). The resultant suspensions were then centrifuged at 100,000 g_{av} in a Beckman 'Spinco' ultracentrifuge. The resultant pellet was discarded, and the supernatant, which contained PGDH, was used for assays of prostaglandin metabolism. The supernatant was stored on ice until required for use since enzyme activity was very labile; when kept at room temperature, about 50% activity was lost within 30 min of centrifugation.

Estimation of prostaglandin $F_{2\alpha}$ metabolism by 'single isotope' technique

The results obtained by the double-isotope technique described here were compared with a well established technique for measurement of PGDH activity.

We have employed a radiochromatographic method with [9-^{3}H]-prostaglandin $F_{2\alpha}$ as a substrate.

Reaction sets for this assay comprised the following components: 100,000 *g* supernatant, 1 ml; NAD^+, 756 pmol; [9-^{3}H]-prostaglandin $F_{2\alpha}$, 0.1 μCi; unlabelled prostaglandin $F_{2\alpha}$, 75.6 pmol. After the contents were thoroughly mixed the tubes containing the reaction mixture were incubated in a shaking waterbath at 37°C for various time periods. Inhibitory drugs were added as required. The reaction was terminated by boiling for 1 min, and acidifying the mixture to pH 3 with 1 N HCl. The unreacted prostaglandin $F_{2\alpha}$ and its metabolites were extracted by 'vortex' mixing the preparation for 30 s with 1.5 ml of ethyl acetate. This procedure consistently extracts more than 95% of labelled products. After mixing, the phases were separated by centrifugation, 1 ml of the organic layer was removed and mixed with 5 μg each of unlabelled prostaglandin $F_{2\alpha}$ and 15-keto-prostaglandin $F_{2\alpha}$. The organic solvents were removed in a vacuum desiccator and the dried residue redissolved in 50 μl of ethanol and spotted quantitatively onto a plastic-backed silica gel t.l.c. plate. After development in the solvent system already described, the plates were dried and zones corresponding to authentic prostaglandin markers were visualized by brief exposure to iodine vapour and cut out with scissors. After spontaneous evaporation of the iodine, the radioactivity in each zone was estimated by liquid scintillation counting.

Estimation of prostaglandin $F_{2\alpha}$ metabolism by the 'double isotope' technique

When the double isotope technique was used, reaction sets were prepared in an identical fashion to those for the single isotope method except that the substrate was a mixture of the [1-^{14}C]-prostaglandin $F_{2\alpha}$ and the [15-^{3}H]prostaglandin $F_{2\alpha}$; 10 μl of the ethanol mixture was used per reaction set.

After incubation, the samples were processed as before, except that the sample was extracted with 1 ml ether. After centrifugation in a bench centrifuge (to facilitate phase separation) convenient aliquots of ether were transferred into scintillation counting vials. The solvent was removed under a stream of N_2, in a vacuum aspirator, or was allowed to evaporate spontaneously. When dry, 10 ml of scintillation counting fluid was added to each vial and the ^{3}H/^{14}C ratio estimated.

Liquid scintillation counting procedures

The radioactivity in the samples was estimated with a Beckman LS-150 liquid scintillation counter. The scintillation counting fluid used was Beckman 'Cocktail D': 5 g PPO and 100 g naphthalene per litre dioxane. The double-isotope method depends on accurate counting so all samples were counted such that $2\sigma \not> 0.5\% \bar{x}$. All ct/min values were converted to d/min by the AES ratio method. The ^{14}C 'spillover' into the ^{3}H channel and appropriate correction to obtain the true ^{3}H d/min was calculated by a conventional method with an Olivetti P602 computer.

Calculation of results obtained by the double-isotope method

The $^3H/^{14}C$ ratio in boiled 'control' samples was arbitrarily set at 1.000 and the isotope ratios of the 'experimental' samples normalized accordingly. The amount of prostaglandin $F_{2\alpha}$ oxidized is then given by the formula:

$$\text{Units prostaglandin } F_{2\alpha} \text{ oxidized} = \left(1 - \frac{^3\text{H d/min}}{^{14}\text{C d/min}}\right)$$

x Units prostaglandin $F_{2\alpha}$ in reaction mixture.

where '^{3}H d/min' and '^{14}C d/min' refer to the experimental sample. In this paper these values are corrected for protein content of sample and/or incubation time.

Students *t* test (independent) was used for statistical analysis of significance.

Reagents and drugs

The following compounds were used: cycloheximide, *N*-ethyl maleimide and β-NAD$^+$ (Sigma); ethacrynic acid and indomethacin (Merck, Sharpe & Dohme); diphloretin phosphate (Leo); frusemide (Hoechst); [9-^{3}H]-prostaglandin $F_{2\alpha}$, batch 6, 15 Ci/mmol, and [^{3}H]-sodium borohydride, batch 38, 271 mCi/mmol (Radiochemicals, Amersham); [1-^{14}C]-prostaglandin $F_{2\alpha}$, cat no. 769-213, 52.5 mCi/mmol (New England Nuclear); unlabelled prostaglandin $F_{2\alpha}$ (Cambrian Chemicals); type 6060 Silica gel plastic backed t.l.c. plates (Eastman Kodak); glass-backed silica gel t.l.c. plates (Anachem). All other reagents were of 'Analar' grade, or the highest purity obtainable.

Results

Change in $^3H/^{14}C$ ratio during incubation with rat kidney supernatant

In the first set of experiments the $^3H/^{14}C$ ratio of (extracted) prostaglandins in the ether extract of rat kidney supernatant was measured at different times. Figure 4 shows the fall in $^3H/^{14}C$ ratio which was observed. The loss of ^3H was linear for up to 4 min after which the reaction rate began to decline. The enzyme is completely specific for the 'S' configuration of the 15-OH group (Nakano, Änggård & Samuelsson, 1969) and the specifically labelled 15-R-prostaglandin $F_{2\alpha}$ was not oxidized.

Comparison of 'single' and 'double isotope' methods for estimation of prostaglandin metabolism

During the reaction catalysed by PGDH the C-15 alcohol group must bind to the enzyme and the C(15)-H bond must be broken. In this assay the C(15) hydrogen of prostaglandin $F_{2\alpha}$ is replaced by a tritium atom. Because of the greater mass of the tritium atom, the specifically labelled prostaglandin $F_{2\alpha}$ might not bind to, or be oxidized by, the enzyme

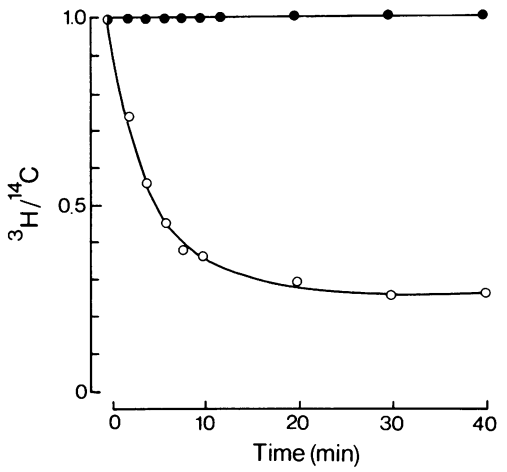

Figure 4 Decline with time of the $^3H/^{14}C$ ratio of extracted prostaglandin $F_{2\alpha}$ incubated with rat kidney prostaglandin 15-hydroxydehydrogenase (PGDH) preparations. Only the 15-S-epimer (O) is oxidized. Each point on the 15-S-$F_{2\alpha}$ curve is a mean of five observations, and each point on the 15-R-$F_{2\alpha}$ curve (●) is a mean of two observations. The s.e. mean values are too small to show on the graph and have been omitted.

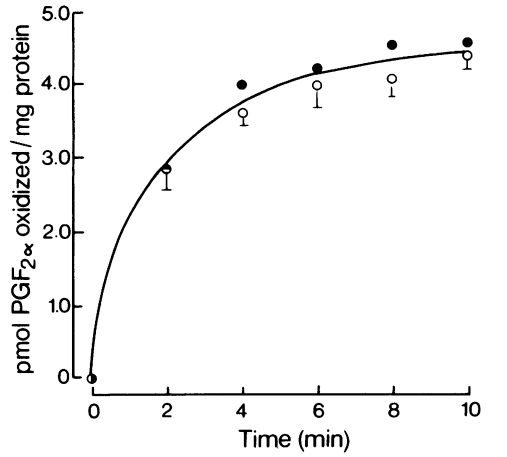

Figure 5 A comparison between the single (O) and double (●) isotope methods for estimation of prostaglandin oxidation in rat kidney. Each point is the mean of five observations. The s.e. mean values for the double-isotope assay are too small to show on the graph and have been omitted (but see Table 1).

Table 1 Comparison of double isotope and single isotope estimations of PGDH in rat kidney

	Single isotope method	*Double isotope method*
Readings obtained (pmol $F_{2\alpha}$ oxidized/mg protein)	3.624	4.566
	4.781	4.354
	4.242	4.538
	3.957	4.522
	3.406	4.369
Mean	4.002	4.469
Standard deviation	0.539	0.100
Standard error	0.241	0.044
Range	3.406–4.781	4.354–4.566

in exactly the same manner as the native molecule, thus giving rise to spurious results. To exclude the possibility of such a 'kinetic isotope effect' the results obtained by the double isotope method were compared with those compared with a conventional single isotope radiochromatographic technique.

Figure 5 shows the rate curves obtained with the two methods; there is no statistical difference between the results obtained by either technique. Table 1 is an analysis of the data at one time point (8 min) on both curves. Inspection of the double isotope data shows the standard deviation and standard error of the double isotope assay is considerably smaller than that of the single isotope assay; the range is also much smaller. However, there is very good overall agreement between the two methods, and the difference between the two sets of results is not statistically significant.

Table 2 Data (mean ± s.e. mean) for β-oxidation experiment

Incubation time (min)	*$^{14}C/^{3}H$ ratio*
Non-incubated control	1.000
5	1.056 (±0.010)
10	1.010 (±0.006)
15	1.037 (±0.010)
20	1.033 (±0.023)
25	1.063 (±0.013)
30	1.008 (±0.008)

β-Oxidation experiment

Prostaglandins and their metabolites are further oxidized *in vivo* by the fatty acid β- and ω-oxidation systems (Samuelsson, Granström, Gréen & Hamberg, 1971; Johnson, Davison & Ramwell, 1972). When metabolized by β-oxidation systems the carboxylic side chain of prostaglandins is successively degraded in a stepwise fashion finally yielding tetranorprostane derivatives. If such a process occurred in tissue homogenates during measurement of metabolism by the double-isotope method the internal standard [1-^{14}C]-prostaglandin $F_{2\alpha}$ would lose its [1-^{14}C] label and again spurious results could be obtained. To discover whether this effect obtains under the circumstances of our assay we measured the ratio between [1-^{14}C]-prostaglandin $F_{2\alpha}$ and a titrium

Table 3 Effect of pretreatment with cycloheximide on prostaglandin $F_{2\alpha}$ metabolism in rat kidney: comparison of assays

Group	*Single isotope*		*Double isotope*		
	$F_{2\alpha}$ *metabolized (pmol)*	*% Inhibition*	*$^{3}H/^{14}C$ ratio*	$F_{2\alpha}$ *metabolized (pmol)*	*% Inhibition*
Control ($n=2$)	5.461	—	0.776	6.286	—
Cycloheximide ($n=2$)	1.096	79.93	0.949	1.422	77.4

labelled species of prostaglandin $F_{2\alpha}$ ([9-^{3}H]-prostaglandin $F_{2\alpha}$) in which the tritium label would not be removed enzymatically; thus if β-oxidation were occurring there would be a fall in the $^{14}C/^3H$ ratio. β-Oxidation probably occurs in microsomes so we used unseparated kidney homogenates in these experiments. Although metabolism plateaus after 6–8 min (see Figure 3) we measured the $^{14}C/^3H$ ratio for up to 30 min (see Table 2) but found no fall in the $^{14}C/^3H$ ratio.

Effect of pretreatment of rats with cycloheximide

Tissue activity of PGDH in rat kidneys declines rapidly after treatment with the protein synthesis inhibitor cycloheximide (Blackwell *et al.*, 1975). In one experiment we estimated the fall in activity the administration of cycloheximide and compared the results obtained by both 'single' and 'double isotope' assays. Each rat received cycloheximide (20 mg/kg) and was killed 2 h later. The kidneys were removed and processed, and metabolism estimated as already described. Table 3 shows that the rate of control metabolism and the decline following cycloheximide when measured by the double-isotope methods were in very good agreement with rates obtained using the single isotope method.

Measurement of the effect of inhibitors of prostaglandin 15-hydroxydehydrogenase

The applicability of the double-isotope method to the study of inhibitors was tested with five drugs, frusemide, ethacrynic acid, *N*-ethyl maleimide, indomethacin and diphloretin phosphate, all of which have been previously shown to affect PGDH activity (Paulsrud & Miller, 1974; Crutchley & Piper, 1974). All drugs except *N*-ethyl-maleimide showed high activity against the enzyme at 1 mM, the descending order of potency being, diphloretin phosphate, frusemide, indomethacin, and ethacrynic acid. The inhibition by *N*-ethyl maleimide (1.57%) was not significant.

Measurement of prostaglandin metabolism by perfused lungs

In one experiment we compared the double-isotope method with bioassay as a measurement of prostaglandin $F_{2\alpha}$ metabolism in perfused lungs. Guinea-pig lungs were perfused with Krebs solution (10 ml/min) through the pulmonary artery (Piper & Vane, 1969). The effluent from the lungs superfused a rat stomach strip and a rat colon in cascade (Ferreira & Vane, 1967). Injections of 4 μg prostaglandin $F_{2\alpha}$ containing the labelled species were made into the pulmonary artery, and the contractions produced by the unmetabolized prostaglandin $F_{2\alpha}$ in the effluent were matched by injections of authentic prostaglandin $F_{2\alpha}$ directly over the tissues. In addition, the lung effluent was collected and the ratio of $^3H/^{14}C$ prostaglandin $F_{2\alpha}$ estimated as described. With the double-isotope assay the metabolism of prostaglandin $F_{2\alpha}$ was estimated as 71% during a single passage through the lungs. With the rat fundic strip as an assay organ, metabolism was estimated as 57%, but on the rat colon (a more reliable assay organ for prostaglandin $F_{2\alpha}$ than the fundic strip), metabolism was estimated at 71%; an extraordinarily close correlation with the radio isotope method.

Discussion

The double isotope assay which has been developed for the estimation of PGDH activity has the advantages of simplicity, complete specificity, rapidity and sensitivity. It is free from artefacts due to β-oxidation or kinetic isotope effects. The results obtained with the assay showed excellent agreement with those obtained by a conventional radiochromatographic method. We believe the assay to be superior in specificity, precision or sensitivity to other existing methods for estimating PGDH activity such as the spectrophotometric determinations of 15-keto metabolites or of $NADH_2$ generation (see Änggård,

Table 4 Data (mean ± s.e. mean) for inhibition of rat kidney prostaglandin 15-hydroxydehydrogenase (PGDH) by drugs (1 mM). ($n = 5$)

Group	*$^3H/^{14}C$ ratio*	*$F_{2\alpha}$ oxidized (pmol)*	*% Inhibition*
Control	0.531 (±0.024)	35.13 (±1.83)	—
Frusemide	0.926 (±0.008)	5.47 (±0.64)	84.44 (±1.82)
Ethacrynic acid	0.804 (±0.031)	16.88 (±0.49)	51.95 (±1.39)
N-ethyl maleimide	0.537 (±0.025)	34.58 (±1.89)	1.57 (±5.40)
Indomethacin	0.882 (±0.005)	8.74 (±0.45)	75.13 (±1.37)
Diphloretin phosphate	0.973 (±0.007)	3.44 (±0.96)	90.24 (±2.74)

1971) or the radiochromatographic method we have also described in this paper.

Amongst the disadvantages of the assay are the expense of the radioisotopes, the need to synthesize the specifically labelled substrate from a starting material which may be difficult to obtain, and the necessity for careful double-isotope counting. Perhaps the major drawback is that only prostaglandin $F_{2\alpha}$ can be used as a substrate in this assay; this is because the 9-keto group of prostaglandin E_2 would also be reduced together with the 15-keto group so that it is not easily possible to prepare specifically labelled prostaglandin E_2 in the same straightforward way in which prostaglandin $F_{2\alpha}$ may be prepared. However, both prostaglandin E_2 and $F_{2\alpha}$ are good substrates for the enzyme, the relative reaction rates on the swine lung enzyme being 97% (of prostaglandin E_1 rate) for prostaglandin E_2 and 62% for $F_{2\alpha}$, (Änggård & Samuelsson, 1966).

The method is suitable for the screening of inhibitors of the enzyme and we would expect this to be a major application; that it is also suitable for the measurement of metabolism in perfused organs confirms its versatility and suggests new ways of using the technique. For example, we have recently shown that the assay is applicable to the estimation of metabolism *in vivo* (Blackwell & Flower, unpublished). By giving intravenous injections of the isotopes into anaesthetized rats and taking small serial blood samples from a catheter in the aorta it is possible to estimate rapidly and accurately the amount of prostaglandin $F_{2\alpha}$ metabolized at any time.

It is possible that PGDH will emerge as a major determinant of blood/tissue levels of prostaglandins and that the assay described here will be an extremely useful tool for the measurement of its activity both *in vitro* and *in vivo*.

We wish to express our gratitude to Dr K. Crowshaw for a sample of authentic 15-keto-$F_{2\alpha}$ (Ono) and to Ms A. Harvey for performing the bioassays.

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(Received February 13, 1976. Revised March 1, 1976.)