The Catabolism of Prostaglandins by Rat Skin

Richard CAMP and Malcolm W. GREAVES
Department of Pharmacology, Institute of Dermatology, Homerton Grove, London E9 6BX, U.K.

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The activities of NAD+-dependent 15-hydroxy prostaglandin dehydrogenase in soluble fractions of rat skin and lung were compared by using a radiochemical assay method. Tritiated prostaglandin \( F_{2\alpha} \) was incubated with NAD\(^+\) and 120000 g supernatant of tissue homogenate. Extracted prostaglandin substrate and reaction products were separated by t.l.c. and quantitatively determined by liquid-scintillation counting. With skin 120000 g supernatant, 10 mm-NAD\(^+\) and an incubation time of 15 min, the mean \( V_{\text{max}} \) was 5.5 nmol of prostaglandin \( F_{2\alpha} \) converted/s per litre of reaction mixture. With lung 120000 g supernatant, 60 mm-NAD\(^+\) and an incubation time of 5 min, the mean \( V_{\text{max}} \) was 26.9 nmol/s per litre, demonstrating 5-fold greater dehydrogenase activity in lung per unit wet weight of tissue. However, the total wet weight of skin was about 23 times that of lung, on dissection of individual rats, indicating that the entire skin may contain 4.5 times the total 15-hydroxy prostaglandin dehydrogenase activity of the lungs. Skin may thus be an important organ of prostaglandin catabolism.

The prostaglandins are a group of biologically active acidic lipids that are widely distributed in mammalian tissues (Piper, 1973a). They have a wide range of biological actions (Nakano, 1973), many of which are considerably diminished by oxidation of the 15(S)-hydroxy group (Anggard & Samuelsson, 1966; Nakano, 1972; Crutchley & Piper, 1975; Wasserman, 1975). This oxidation is catalysed by NAD+-dependent 15-hydroxy prostaglandin dehydrogenase (11α,15α-dihydroxy-9-oxoprost-13-en-10-one–NAD\(^+\) 15-oxido-reductase, EC 1.1.1.141), and represents the first step in the catabolism of the parent prostaglandins (Anggard & Larsson, 1971; Anggard et al., 1971; Hamberg & Samuelsson, 1971). This enzyme is active in particle-free fractions of many mammalian tissues (Hansen, 1976), but there have been few studies directly comparing its activities in different tissues of the same species. One such study (Anggard et al., 1971) demonstrated that its activity was highest in soluble fractions of kidney, spleen and lung of the pig, but skin was not assayed. Preparations of kidney, placenta and lung of various species have high activity, and are the usual sources of purified enzyme (Hansen, 1976). The importance of lung as an organ of prostaglandin catabolism is borne out by studies made in vivo and by experiments involving perfusion of isolated organs (Piper, 1973b). Activity of NAD+-dependent 15-hydroxy prostaglandin dehydrogenase has been demonstrated in human (Jonsson & Anggard, 1972), newborn mouse (Wilkinson & Rabinowitz, 1976) and rat (Camp et al., 1978) skin. As skin is by weight a very large organ, it was decided to compare the properties of the enzyme in rat skin and lung, in order to assess the relevance of skin as an organ of prostaglandin catabolism.

Experimental

Materials

Unlabelled prostaglandins were a gift from Dr. J. Pike (The Upjohn Co., Kalamazoo, MI, U.S.A.). NaB\(^3\)H\(_4\) (555–661 mCi/mmol) was from The Radiochemical Centre (Amersham, Bucks., U.K.). NAD\(^+\) (free acid, grade 1) and pig heart lactate dehydrogenase [specific activity approx. 300 units (μmol of...
lactate transformed/min)/mg) were from Boehringer Corp. (London) (Lewes, East Sussex, U.K.). Sigma (London) Chemical Co. (Poole, Dorset, U.K.) supplied 2-mercaptoethanol. Sephadex LH-20 was from Pharmacia (Great Britain) (London W.5, U.K.) and Lipidex 5000 from Packard Instrument Co. (Caversham, Berks., U.K.). Silica gel G1500 t.i.c. plates (0.25 mm thickness) were manufactured by Schleicher und Schüll (Dassel, West Germany) and were supplied by Anderman and Co. (East Molesey, Surrey, U.K.). Organic solvents used in extraction and chromatographic procedures were from BDH Chemicals (Poole, Dorset, U.K.) and were distilled before use. Chloroform was distilled over anhydrous CaCl₂ and stabilized with 2% (v/v) ethanol. Liquid-scintillant chemicals included 2-ethoxyethanol, sulphur-free toluene, naphthalene (from BDH Chemicals) and 2,5-diphenyloxazole (from Intertechnique, Uxbridge, Middlesex, U.K.). Materials used in g.l.c.—mass spectrometry have been described previously (Black et al., 1978). All other chemicals used were obtained from BDH Chemicals and were of AnalR grade or of the highest purity available. Water was distilled and de-ionized before use.

Laboratory animals

Albino C.F.Y. rats were purchased from Anglia Laboratory Animals (Alconbury, Huntingdon, U.K.) and a colony was maintained at the Institute of Dermatology, London. Males weighing 300–400 g were used in all experiments.

Total skin and lung weights were determined in four animals. Each animal was killed by a blow to the head and cervical dislocation, and was shaved with Oster clippers from the base of the skull to the tail. The entire skin was removed excluding that of head and tail, subcutaneous fat was excised and the skin was weighed. Lungs from the same animals were removed, blotted dry and weighed.

Preparation of subcellular fractions

Animals were killed as described above, and the abdominal skin was shaved with Oster clippers, excised and freed of subcutaneous fat. Thereafter all procedures were carried out at 0–4°C. The skin was chopped with scissors and homogenized in 0.11 M-sodium phosphate buffer (0.09 M-Na₂HPO₄/0.02 M-NaH₂PO₄), pH 7.3, containing 0.06% (v/v) 2-mercaptoethanol. A fixed ratio of 2 g of tissue to 5 ml of buffer was always used. The homogenizer (type X1020; Internationale Laboratoriums-Aparate G.m.b.H., Ballrichten-Dottingen, West Germany) was fitted with a 1 cm-diameter shaft and was operated at half-maximum speed for four 15 s periods, each period being interrupted by an interval of 15 s to avoid overheating. In experiments to determine subcellular enzyme distribution, the homogenate was centrifuged at 600 g for 10 min at 3°C in an MSE Mistral 6L centrifuge (rₑ 23 cm). In all other experiments the homogenate was centrifuged at 2500 g under otherwise similar conditions. The supernatant was re-centrifuged at 120000 g for 60 min at 3°C in an MSE Superspeed 75 ultracentrifuge with a 10 x 10 ml titanium angle rotor (rₑ 6.435 cm), and the supernatant was used in subsequent experiments. In the experiments to determine subcellular enzyme distribution, the 120000 g pellet derived from 3 ml of 600 g supernatant was suspended in buffer and re-centrifuged at 12000 g for a further 60 min at 3°C. The supernatant was discarded and the pellet resuspended in 1 ml of the above buffer, in a hand-held glass homogenizer. This suspension was used in subsequent experiments.

When lung was to be processed, rats that had no overt signs of respiratory disease were selected. The resected lungs were briefly rinsed with distilled de-ionized water and dried with filter paper. Those showing prominent inflammatory changes were discarded. The lungs were then processed exactly as described above, with the same tissue/buffer ratio on homogenization. Subcellular fractions were freshly prepared for each experiment, and incubations were carried out immediately after centrifugation.

Preparation of substrate

[9β-³H]Prostaglandin F₂α was prepared by reduction of prostaglandin E₂ with NaB₃H₄, by using the method described by Samuelsson (1964). The epimeric reduction products, [9β-³H]prostaglandin F₂α and [9α-³H]prostaglandin F₂β, were converted into methyl esters with ethereal methanolic diazo-methylane and separated by Lipidex 5000 straight-phase gel partition chromatography as described by Hensby (1975). Radioactivity in the column effluent fractions was determined by liquid-scintillation counting. The fractions containing [9β-³H]prostaglandin F₂α methyl ester were pooled and evaporated in vacuo, and the residue was demethylated by reaction with 30 ml of methanolic 1.0 M-KOH for approx. 15 h at room temperature (approx. 24°C). This mixture was then diluted to 300 ml with distilled de-ionized water, acidified to pH 3 with 0.5 M-HCl and extracted with 3 equal volumes of ethyl acetate. The pooled organic phases were evaporated in vacuo and vacuum-desiccated, and the residue was subjected to reversed-phase gel partition chromatography with the Sephadex LH-20 system previously described (Hensby, 1975). This chromatographic step yielded [9β-³H]prostaglandin F₂α of at least 98% radiochemical purity as determined by the three t.l.c. systems described below. The [9β-³H]-prostaglandin F₂α was added to unlabelled prostaglandin F₂α to give the desired specific radioactivity.
[11β-3H]Prostaglandin F2α was similarly prepared by reduction of prostaglandin D2, with NaB₃H₄. The [11β-3H]prostaglandin was separated from its [11α-3H]prostaglandin epimer and purified as described above, yielding [11β-3H]prostaglandin F2α with radiochemical purity of 99% on t.l.c. system A described below. It was added to unlabelled prostaglandin F2α to give the appropriate specific radioactivity. Both substrates were stored at −20°C in methanol at known concentrations.

Reaction conditions

Initially incubation volumes of 1.5 and 0.75 ml were used, but these were later scaled down to 187.5 μl without altering the relative proportions of reactants. The procedures adopted for the small incubation volume are described below. [9β-3H]- and [11β-3H]-Prostaglandin F2α substrates were prepared to give specific radioactivities of 4.33 and 4.27 Ci/mol respectively. [11β-3H]Prostaglandin F2α was required in a few experiments only, [9β-3H]prostaglandin F2α, being otherwise routinely used. Exact quantities of the methanolic stock solutions were added to test tubes, and the solvent was evaporated in vacuo. To each was added 62.5 μl of 0.11 M sodium phosphate buffer, pH 7.3, containing 0.06% (v/v) 2-mercaptoethanol and the desired amount of NAD+. Reactions were initiated by the addition of 125 μl of subcellular fraction, the test tubes were incubated in a Grant shaking water bath, and reactions were terminated by acidification to approx. pH 3 with 37.5 μl of 0.5 M HCl. In some experiments lactate dehydrogenase and pyruvic acid were added to the reaction mixture. In determining blank rates at a range of [9β-3H]prostaglandin F2α concentrations, acidification was carried out before the addition of high-speed supernatant, and extraction was performed immediately.

Extraction

Saturating amounts of solid NaCl were added to the acidified reaction mixtures. This simplified subsequent extraction by diminishing protein emulsification. After the addition of 0.8 ml of ethyl acetate, each tube was vortex-mixed and briefly centrifuged at maximum speed in an MSE Minor centrifuge. The ethyl acetate was removed, the extraction was repeated and the pooled organic phases were evaporated under a stream of N2 at 42°C. After vacuum-desiccation for 20 min the samples were either subjected to t.l.c. or stored at −20°C for 1–2 days before chromatography.

T.l.c.

Sample residues were dissolved in 40 μl of methanol and applied to 10 cm × 20 cm t.l.c. plates as a narrow streak of approx. 2.5 cm length by using a microsyringe. A further application of sample in 40 μl of methanol was made. Two different samples were applied to each plate, and plates were developed to a distance of 15 cm. Three t.l.c. systems were used. In system A, the solvent mixture contained ethyl acetate/acetone/acetic acid (90:10:1, by vol.) as described by Andersen (1969), and plates were each developed twice. In system B (Sun & Armour, 1974), plates were subjected to a single development in chloroform/methanol/acetic acid (18:3:1, by vol.). In initial experiments, 0.5 cm sections of the developed plates were scraped into scintillation vials. The central section of each plate, where reference prostaglandins had been spotted, was left intact and the positions of the standards were detected with I2 vapour. In later experiments, unlabelled standard prostaglandin F2α, 15 oxoprostaglandin F2α and 15 o xo-13,14-dihydroprostaglandin F2α were spotted directly onto the applied residues. After development in solvent system B, the plates were briefly exposed to I2 vapour and the positions of the three standards were marked. The I2 staining was allowed to fade under a stream of air, and the three sections of the plate corresponding to the positions of the three reference standards were scraped into scintillation vials.

Scintillation counting

Liquid scintillant was prepared in the proportions 1500 ml of toluene, 900 ml of 2-ethoxyethanol, 112.5 g of naphthalene and 10.5 g of 2,5-diphenylloxazole, the final mixture containing 2% (v/v) methanol. An LKB Ultratbeta 1210 scintillation counter was used, d.p.m. being calculated by the external-standard channels-ratio method. The radioactivities of the 0.5 cm t.l.c. scrapings were counted in 10 ml of scintillant for 5 min each, or in the later method the radioactivities of the three sections corresponding to reference prostaglandins were each counted in 14 ml of scintillant to a ±2 standard error of 2.5%. In both cases counting of the radioactivity of the external standard was for 1 min.

Quantitative determination of endogenous prostaglandins in skin 120000 g supernatant

Portions (50 μl) of 120000 g supernatant were equilibrated with mixtures of 100 ng each of tetra-deuterated prostaglandins E2 and F2α in 1 ml of ethanol/water (1:19, v/v) saturated with indomethacin. The mixtures were acidified to pH 4 with 0.5 M HCl, extracted and subjected to t.l.c. as described above, with a single development with solvent system A. Sections of the plates corresponding to the
positions of reference prostaglandins \( E_2 \) and \( F_{2\alpha} \), developed in parallel, were eluted with methanol. This was evaporated and the endogenous prostaglandins \( E_2 \) and \( F_{2\alpha} \) in the residues were measured quantitatively by multiple-ion-detection g.l.c.-mass spectrometry. Details of this method have been described previously (Hensby et al., 1977; Black et al., 1978).

**Determination of protein**

This was carried out by the method of Lowry et al. (1951), with human plasma albumin as standard. This was supplied in aqueous solution by the Blood Products Laboratory, Lister Institute, Elstree, Herts., U.K.

**Results and Discussion**

Representative \( R_F \) values obtained for the relevant prostaglandin standards, with the three described t.l.c. systems, are listed in Table 1.

**Enzyme activity in 120000 g supernatant of skin homogenate**

Under various conditions (0.5–20 mM \( \text{NAD}^+ \), 1.9–136 \( \mu \text{M} \)-prostaglandin \( F_{2\alpha} \), and incubation times from 5 to 60 min at 32–50 \(^\circ\)C), \([9\beta^3\text{H}]\)prostaglandin \( F_{2\alpha} \) was converted into two less-polar metabolites, which, on t.l.c. system B, had \( R_F \) values corresponding to those of authentic 15-oxoprostaglandin \( F_{2\alpha} \) and 15-oxo-13,14-dihydroprostaglandin \( F_{2\alpha} \). As indicated in Table 1, t.l.c. system B does not separate adequately prostaglandins \( E_2 \) and \( D_2 \) from these two metabolites, nor 13,14-dihydprostaglandin \( F_{2\alpha} \) from prostaglandin \( F_{2\alpha} \). T.l.c. systems A and C were thus used to exclude the possibility of concurrent oxidation of 9\( \alpha \)- and 11\( \alpha \)-hydroxy groups, and the further conversion of 15-oxo-13,14-dihydroprostaglandin \( F_{2\alpha} \) into 13,14-dihydroprostaglandin \( F_{2\alpha} \). In these experiments an incubation time of 15 min and \( \text{NAD}^+ \) and prostaglandin \( F_{2\alpha} \) concentrations of 10 mM and 56.5 \( \mu \text{M} \) respectively were used. With t.l.c. system A, concurrent oxidation of the 11\( \alpha \)-hydroxy group was excluded, as there was no evidence of conversion of \([9\beta^3\text{H}]\)prostaglandin \( F_{2\alpha} \) into prostaglandin \( D_2 \), which is clearly separated from the 15-oxo and 15-oxo-13,14-dihydro metabolites of prostaglandin \( F_{2\alpha} \) (Table 1). To exclude concurrent oxidation of the 9-hydroxy group of prostaglandin \( F_{2\alpha} \), substrate tritiated in the 11\( \beta \)-position was required. Such oxidation of \([9\beta^3\text{H}]\)prostaglandin \( F_{2\alpha} \) would result in loss of the radiolabel and consequent inability to detect the resulting 9-oxo compound(s) on t.l.c. By using \([11\beta^3\text{H}]\)prostaglandin \( F_{2\alpha} \) and t.l.c. system A, it could be shown that no prostaglandin \( E_2 \) or its 15-oxo or 15-oxo-13,14-dihydro metabolites were being formed. With t.l.c. system C, which separates 13,14-dihydroprostaglandin \( F_{2\alpha} \) from prostaglandin \( F_{2\alpha} \) and its other two metabolites (Table 1), it was evident that no dihydro metabolite was being formed.

These results indicate that, under the conditions used, two prostaglandin-catabolizing enzymes were active in skin 120000 g supernatant, a 15-hydroxy prostaglandin dehydrogenase and a prostaglandin \( \Delta^{13} \)-reductase. These two enzymes commonly coexist in other tissues (Hansen, 1976), and oxidation of the 15-hydroxy group has been shown to precede saturation of the 13,14-double bond (Anggard & Larsson, 1971; Anggard et al., 1971; Hamberg & Samuelsson, 1971). It was possible to confirm this enzymic sequence in the present reactions. When incubations were carried out with 82.8 \( \mu \text{M} \)-\([9\beta^3\text{H}]\)-prostaglandin \( F_{2\alpha} \) and 10 mM \( \text{NAD}^+ \) for 15 min at 37 \(^\circ\)C, t.l.c. system B, which separates the two metabolites adequately, showed that the ratio of radioactivity corresponding to 15-oxoprostaglandin \( F_{2\alpha} \) to 15-oxo-13,14-dihydroprostaglandin \( F_{2\alpha} \) respectively was approximately 3:7. Addition of 50 mM-pyruvic acid and 0.94 unit of lactate dehydrogenase to incubation mixtures that were otherwise identical resulted in a reverse of this product ratio to approximately 2:1, without significantly affecting the total mass of products formed. These results suggest that NADH, formed by the action of 15-hydroxy prostaglandin dehydrogenase, is being bound in the enzymic conversion of pyruvate into lactate, reducing available NADH and inhibiting

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>T.l.c. system A</th>
<th>T.l.c. system B</th>
<th>T.l.c. system C</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F_{2\alpha} )</td>
<td>0.15</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>( 13,14)-Dihydro-( F_{2\alpha} )</td>
<td>N.D.</td>
<td>0.23</td>
<td>0.33</td>
</tr>
<tr>
<td>( E_2 )</td>
<td>0.31</td>
<td>0.33</td>
<td>N.D.</td>
</tr>
<tr>
<td>15-Oxo-( F_{2\alpha} )</td>
<td>0.41</td>
<td>0.39</td>
<td>0.46</td>
</tr>
<tr>
<td>15-Oxo-( 13,14)-dihydro-( F_{2\alpha} )</td>
<td>0.48</td>
<td>0.52</td>
<td>0.53</td>
</tr>
<tr>
<td>( D_2 )</td>
<td>0.57</td>
<td>0.41</td>
<td>N.D.</td>
</tr>
<tr>
<td>15-Oxo-( E_2 )</td>
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<td>0.60</td>
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<tr>
<td>15-Oxo-( 13,14)-dihydro-( E_2 )</td>
<td>0.71</td>
<td>0.65</td>
<td>N.D.</td>
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</table>
prostaglandin Δ13-reductase activity. The resulting accumulation of 15-oxoprostaglandin F2a and diminished 15-oxo-13,14-dihydroprostaglandin production, as demonstrated, could occur only if oxidation of the 15-hydroxy group was the first step. Activity of the 15-hydroxy prostaglandin dehydrogenase could thus be determined by calculating the percentage conversion of prostaglandin F2a into total products, and velocities were expressed as nmol of prostaglandin F2a converted/s per litre of reaction mixture, after subtraction of blank rates. Velocities were not expressed in terms of the protein content of the reaction mixtures, as the intention was to refer enzyme activity to wet weight of tissue. The total protein concentration of lung 120000 g supernatant was 22.2 ± 5.2 mg/ml and that of skin 120000 g supernatant was 9.2 ± 1.0 mg/ml (means ± s.d., n = 11). Expression of velocities in terms of protein would thus bias results.

The effects of temperature, NAD+ concentration and incubation time were studied, with [9β-3H]prostaglandin F2a and t.l.c. system B (Figs. 1–3). Incubations were performed in duplicate, as shown, and repeat experiments gave similar results. Fig. 1 demonstrates increases in enzyme activity up to 50°C, with decrease in rate above this temperature. Fig. 2 illustrates an experiment to determine the NAD+ concentration required for enzyme saturation. The NAD+ concentrations used were not in the range required for satisfactory determination of a Km value for NAD+, but nevertheless it was established that a concentration of 10 mM approaches enzyme saturation. Fig. 3 demonstrates that prostaglandin F2a conversion increased in a near-linear fashion up to 15 min. A lower prostaglandin F2a concentration (15 μM) was used in the illustrated time-course experiments because the rate-limiting effects of prostaglandin F2a depletion would be most evident. With a higher prostaglandin F2a concentration of 60.3 μM, similar linearity was obtained. A 10 mM-NAD+ concentration and incubation time of 15 min were thus used in subsequent experiments. Shorter incubation times resulted in smaller differences between background and product radioactivity on t.l.c., especially at higher [9β-3H]prostaglandin F2a concentrations, and were thus avoided. Probable lack of absolute linearity would result in slight under-estimations of Vmax, which is therefore referred to as ‘apparent’. A representative prostaglandin F2a saturation curve and corresponding

![Fig. 1. Dependence of skin 15-hydroxy prostaglandin dehydrogenase activity on incubation temperature](image)

Prostaglandin F2a (60.3 μM) was incubated with 120000 g skin supernatant and different concentrations of NAD+ for 60 min at 37°C. Experimental details are given in the text. Reaction velocity (v) is expressed as in Fig. 1.

![Fig. 2. Dependence of skin 15-hydroxy prostaglandin dehydrogenase activity on NAD+ concentration](image)

Prostaglandin F2a (60.3 μM) was incubated with 120000 g skin supernatant and different concentrations of NAD+ for 60 min at 37°C. Experimental details are given in the text. Reaction velocity (v) is expressed as in Fig. 1.

![Fig. 3. Dependence of 15-hydroxy prostaglandin dehydrogenase activity on incubation time](image)

Prostaglandin F2a (15 μM) was incubated for different time periods at 37°C. Experimental details are given in the text. Enzyme activity is expressed as nmol of prostaglandin F2a converted per litre of reaction mixture. (a) Incubation mixtures included skin 120000 g supernatant and 10 mM-NAD+; (b) incubation mixtures included lung 120000 g supernatant and 60 mM-NAD+.
Hanes plot are shown in Figs. 4 and 5, and demonstrate Michaelis-Menten kinetics. In five such experiments, the apparent \( K_m \) for prostaglandin \( F_2a \) at 10 mM-NAD\(^+\) was 38.0 ± 10.2 \( \mu \)M (mean ± s.d.), and the apparent \( V_{max} \) was 5.5 ± 1.3 nmol/s per litre of reaction mixture (mean ± s.d.). Straight lines were constructed by simple linear regression, and the mean \( r \) value was 0.9824 ± 0.0069 (s.d.). Slight deviations from linearity were occasionally noted at lower prostaglandin \( F_2a \) concentrations, and were investigated. A range of seven different \( [9\beta-^3H] \) prostaglandin \( F_2a \) concentrations (1.9–13.2 \( \mu \)M) were incubated with 10 mM-NAD\(^+\) and skin 120000 g supernatant for 15 min at 37°C. Inconsistent minor deviations from linearity were noted, and were assumed to be of methodological origin.

**Enzyme activity in particulate fractions of skin homogenate**

Suspensions of 120000 g pellet were prepared as described, and incubated with 56.5 \( \mu \)M-[\( 9\beta-^3H \)] prostaglandin \( F_2a \) and 10 mM-NAD\(^+\) for 60 min at 37°C. T.l.c. revealed that no conversion of substrate had occurred, confirming the soluble nature of the enzyme.

**Enzyme activity in 120000 g supernatant of lung homogenate**

By using the three t.l.c. systems it was shown that, in the presence of NAD\(^+\), \( [^3H] \) prostaglandin \( F_2a \) was again converted into two less-polar metabolites with \( R_p \) values corresponding to those of 15-oxoprostaglandin \( F_2a \) and 15-oxo-13,14-dihydroprostaglandin \( F_2a \). In these experiments an incubation time of 5 min and NAD\(^+\) and prostaglandin \( F_2a \) concentrations of 60 mM and 56.5 \( \mu \)M respectively were used. By using the same combination of t.l.c. systems it was possible to exclude concurrent oxidation of 9- or 11-hydroxy groups and further conversion of 15-oxo-13,14-dihydroprostaglandin \( F_2a \) into 13,14-dihydroprostaglandin \( F_2a \). T.l.c. radio-profiles showed that, of the two metabolites, radioactivity with the same \( R_p \) value as reference 15-oxoprostaglandin \( F_2a \) formed the major peak. Activity of 15-hydroxy prostaglandin dehydrogenase was calculated and expressed as described above.

The effects of incubation time and NAD\(^+\) concentration were studied, with \( [9\beta-^3H] \) prostaglandin \( F_2a \) and t.l.c. system B (Figs. 3 and 6). Incubations were performed in duplicate and repeat experiments gave similar results. As shown in Fig. 6, a higher concentration of NAD\(^+\) of 60 mM was required to approach enzyme saturation, but increasing the concentration to 100 mM resulted in a large decrease in the rate. Again, the NAD\(^+\) concentrations used were not in the range required for satisfactory \( K_m \) determination, but determination of an adequate saturating concentration was possible. An incubation time of 5 min provided near-linear reaction rates (Fig. 3), but a shorter time than this was not adopted.

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**Fig. 4.** Dependence of 15-hydroxy prostaglandin dehydrogenase activity on prostaglandin \( F_2a \) concentration

Different concentrations of prostaglandin \( F_2a \) were incubated at 37°C. Experimental details are given in the text. Reaction velocity (\( v \)) is expressed as in Fig. 1. (a) Incubations of mixtures containing skin 120000 g supernatant and 10 mM-NAD\(^+\) were carried out for 15 min; (b) incubations of mixtures containing lung 120000 g supernatant and 60 mM-NAD\(^+\) were carried out for 5 min.

**Fig. 5.** Linear plot of the effect of prostaglandin \( F_2a \) concentration on reaction velocity

Experimental details are as described in Fig. 4. Plots of \( [\text{prostaglandin } F_{2a}] / v \) versus \( [\text{prostaglandin } F_{2a}] \) are demonstrated for skin (a) and lung (b) 15-hydroxy prostaglandin dehydrogenase. The mean kinetic constants obtained in a number of such experiments are stated in the Results and Discussion section.
for the reasons discussed above. A representative prostaglandin F$_{2a}$ saturation curve and corresponding Hanes plot are shown in Figs. 4 and 5, and again demonstrate Michaelis–Menten kinetics. In three such experiments the apparent $K_m$ for prostaglandin F$_{2a}$ at 60 mM-NAD$^+$ was 44.7 $\pm$ 10.2 $\mu$M (mean $\pm$ s.d.) and the apparent $V_{\text{max}}$ 26.9 $\pm$ 3.0 nmol/s per litre of reaction mixture (mean $\pm$ s.d.). Straight lines were constructed by simple linear regression and the mean $r$ value was 0.9824 $\pm$ 0.0103 (s.d.). Slight deviations from linearity were again noted at lower prostaglandin F$_{2a}$ concentrations and were again investigated. The same range of prostaglandin F$_{2a}$ concentrations (1.9–13.2 $\mu$M) were incubated with 60 mM-NAD$^+$ and lung 120,000 g supernatant for 5 min at 37°C. Inconsistent minor deviations from linearity again occurred, and were assumed to be of methodological origin.

Recovery of radioactivity in all the described experiments was between 80 and 98%.

**Conclusion**

The purpose of this study was to compare maximal 15-hydroxy prostaglandin dehydrogenase activities in skin and lung. Crude enzyme preparations were used, containing associated prostaglandin $\Delta^{13}$ reductase activity. This activity is particularly significant in skin 120,000 g supernatant, and would interfere with 15-hydroxy prostaglandin dehydrogenase assays based on measurement of NADH or 15-oxo-prostaglandin production. A series of purification steps was required to separate prostaglandin $\Delta^{13}$ reductase from 15-hydroxy prostaglandin dehydro-

The concentration, considerable decrease in total recoverable enzyme activity occurs (Hansen, 1976), complicating accurate determination of enzyme activity in terms of original wet weight of tissue.

In the present radiochemical assay method, with unpurified enzyme, $K_m$ values were obtained that correspond to those obtained for prostaglandin F$_{2a}$ with purified 15-hydroxy prostaglandin dehydro-

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References