# Development of a radioimmunoassay for measuring 6-oxo-prostaglandin $F_{1\alpha}$

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6-oxo-$PGF_{1\alpha}$ (5 mg) was conjugated to 16.2 mg thyroglobulin by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride in water at pH 5.5. Following the 5 h reaction, the solution was dialyzed. The retentate was dissolved in 0.03 M sodium carbonate solution, centrifuged (900 *g* for 20 min), and the supernatant withdrawn and lyophilized to produce the dried conjugate. By incorporating 3 μCi [$^3$H]-prostaglandin $F_{2\alpha}$ (sp. act. 150 Ci/mmol; Amersham) into the conjugating reaction, it was calculated that 60 moles of prostaglandin were bound to 1 mole of thyroglobulin.

Conjugate (4 mg) was dissolved in 4 ml normal saline and emulsified with 4 ml Freund's complete adjuvant. Four rabbits were each immunized with 2 ml emulsified conjugate, injected intradermally at 50 sites. The rabbits were boosted at 6 weekly intervals with 2 ml emulsified conjugate, injected subcutaneously at 4 sites. 50 ml blood were collected 8 days after boosting, and the serum treated and stored, as described by Dighe, Emslie, Henderson, Rutherford & Simon (1975).

[$^3$H]-6-oxo $PGF_{1\alpha}$ (tracer) was synthesized by reacting 25 mCi octa-tritiated arachidonic acid (sp. act. 120 Ci/mmol; Amersham) with 200 mg sheep uterus microsomal enzyme preparation in 5 ml Krebs' solution (containing 1 mg/ml tryptophan and 10 μg/ml haemoglobin), aerated with 5% $CO_2$ in $O_2$, at 37°C for 60 minutes. The pH was then lowered to 4.5 and the prostaglandins extracted with ethyl acetate. The extract was evaporated to dryness, re-dissolved in 67% ethanol, washed with petroleum ether (b.p. 60–80°C), and taken to dryness again. The residue was further purified by straight-phase liquid–gel partition column chromatography (on Lipidex 1000 eluted with hexane, 1,2-dichloroethane, ethanol, acetic acid, 100:100:15:0.2), followed by reversed-phase high-performance liquid chromatography (on Partisil ODS eluted with acetonitrile, water, acetic acid, 40:60:0.1). The major radioactive substance isolated co-chromatographed with non-tritiated 6-oxo-$PGF_{1\alpha}$ on thin-layer chromatography using two solvent systems, FVI (Anderson, 1969) and 1a (Cottee, Flower, Moncada, Salmon & Vane, 1977).

**Table 1** % Cross-reactivities of various prostaglandins with antiserum raised against 6-oxo-$PGF_1$, taken at 40% binding of tracer

| *Prostaglandin* | *Rabbit No. 2* *Bleed No. 2* | *Bleed No. 3* |
|---|---|---|
| $PGF_{2\alpha}$ | 0.90 | 1.0 |
| $PGE_2$ | 1.2 | 0.76 |
| $PGD_2$ | 0.38 | 0.18 |
| $PGA_2$ | 0.05 | 0.08 |
| $TXB_2$ | 0.11 | 0.12 |
| $PGF_{1\alpha}$ | 0.36 | 0.35 |
| $PGE_1$ | 2.2 | 2.7 |
| 15-oxo-$PGE_2$ | <0.05 | <0.05 |
| 13,14-dihydro-15-oxo-$PGE_2$ | <0.05 | <0.05 |
| 15-oxo-$PGF_{2\alpha}$ | <0.05 | <0.05 |
| 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ | <0.05 | <0.05 |

Dilution curves were set up using the serum and prepared tracer (30 pg; sp. act. 90–105 Ci/mmol). Serum from rabbit 2 (2nd and 3rd bleeds) produced 60% binding at 1/640 dilution, using the double-antibody method of separation. Good standard curves were obtained with these 2 antisera, the working range being 20 to 600 pg. Cross-reactivities are shown in Table 1. These 2 antisera are being developed for assaying endogenous 6-oxo-$PGF_{1\alpha}$, and the rabbits boosted further hopefully to produce an antiserum with a higher titre.

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