Human plasma concentrations of the unchanged drug and metabolites were determined over the first 24h. Peak debrisoquine concentrations (20–50ng/ml) occurred during the first 2–3h and diminished rapidly. Most of the radioactivity was accounted for as metabolites, their rate of disappearance being slower than the parent drug.

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A New Method for the Determination of the Major Metabolite of Prostaglandin F<sub>2α</sub> in Human Urine Based on Stable Isotope Dilution and Gas Chromatography–Mass Spectrometry

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Studies on the biological fate in man of primary prostaglandins of the E and F series have shown that metabolic conversion takes place into a series of side-chain degradation products that are excreted in urine (Samuelsson, 1973). The major metabolite of prostaglandin F<sub>2α</sub> in the urine is 5α,7α-dihydroxy-11-oxotetranorprostane-1,16-dioic acid (referred to below as 'prostaglandin F metabolite'), quantitative determination of which may be used to study the effect of drugs or different physiological conditions on prostaglandin F<sub>2α</sub> turnover in vivo (Hamberg, 1974; Samuelsson, 1973). In connection with our investigations into the role of prostaglandin F<sub>2α</sub> in certain pathological disorders, we have developed a highly specific and sensitive assay for prostaglandin F metabolite, based on stable isotope dilution and combined g.l.c.–mass spectrometry. The internal standard used in this assay is a <sup>2</sup>H-labelled analogue of prostaglandin F metabolite, in which the <sup>1</sup>H atoms occupy positions 4β, 6α and 6β in the cyclopentane ring. This compound was isolated from the urine of a female Rhesus monkey which had received an intravenous infusion of [8β,10α,10β-<sup>2</sup>H<sub>3</sub>, 9β-<sup>3</sup>H]prostaglandin F<sub>2α</sub> (14.5mg). (This labelled precursor was prepared from prostaglandin E<sub>2</sub> by base-catalysed equilibration in [hydroxy-<sup>2</sup>H]carbitol, followed by reduction with NaB<sub>3</sub>H<sub>4</sub>, and chromatographic separation of the prostaglandin F isomers obtained.) Purification of the labelled metabolite (yield 1mg), as its methyl ester derivative was achieved by means of liquid-gel partition chromatography (Nyström & Sjövall, 1973; Brash & Jones, 1974).

Fig. 1 summarizes the sequence of steps in the assay procedure. To a portion (10ml) of a 24h urine collection is added the methyl ester of [<sup>2</sup>H<sub>3</sub>]prostaglandin F metabolite (200ng) followed by 5m-NaOH (2ml). Hydrolysis of this methyl ester and of the δ-lactone form of the endogenous metabolite is allowed to proceed at ambient temperature overnight, resulting in equilibration of the two molecular species of prostaglandin F metabolite as the salt of the dioic acid. The urine sample is then acidified to pH 3 and the prostaglandins are extracted on a small column (2g) of Amberlite XAD-2. Elution of the column with methanol, and esterification of the material so obtained with 2% methanolic tetramethylammonium hydroxide and methyl iodide (Greeley, 1974), affords the metabolite as a mixture of its methyl ester and δ-lactone methyl ester derivatives. These compounds are then converted into the 16-monomethyl ester of prostaglandin F meta-
Fig. 1. Analytical procedure for the measurement of 5α,7α-dihydroxy-11-oxotetranor-1,16-dioic acid (prostaglandin F metabolite) in urine

Abbreviation: t-BDMSO, t-butyldimethylsilyl ether.
bolite by overnight reaction with sodium borate buffer (0.1 M, pH 10), a reagent that we have found to effect hydrolysis not only of the δ-lactone structure (Hamberg, 1973), but also selectively at the C-1 methyl ester grouping in the 'open-chain' (5-hydroxy ester) form of the metabolite. The alkaline solution of the 16-monomethyl ester is washed with dichloroethane to remove basic and neutral components of the urinary extract, and is acidified to pH 2. During a period of 1 h under these conditions, virtually quantitative dehydration of the 16-monomethyl ester takes place (A. R. Brash, T. A. Baillie, R. A. Clare & G. H. Draffan, unpublished work) to yield the δ-lactone methyl ester of prostaglandin F metabolite that is extracted into dichloroethane from aqueous solution at pH 8. The above methylation-selective hydrolysis-back-extraction sequence results in a considerable purification of the sample without recourse to time-consuming chromatographic separations.

A survey of a number of potential derivatives for use in the g.l.c.-mass spectrometric analysis of prostaglandin F metabolite revealed that those structures possessing the δ-lactone moiety exhibited poorer gas-chromatographic behaviour and gave rise to more extensive fragmentation than did derivatives of the 'open-chain' form. Of the latter group, the t-butyldimethylsilyl ether methyl ester (Kelly & Taylor, 1976) appeared particularly suitable in view of its ease of preparation, stability towards hydrolysis and to breakdown on t.l.c., and because of the simplicity of its mass spectrum (base peak at m/e 397, [M—57—132]+, Σ 10%; preparation of this derivative from the δ-lactone methyl ester of prostaglandin F metabolite is carried out by methylation under basic conditions (Greeley, 1974) followed by reaction with t-butyldimethylchlorosilane/imidazole/dimethylformamide (Corey & Venkateswarlu, 1972) and purification by t.l.c. (mobile phase: ethyl acetate/n-heptane, 2:3, v/v). Combined g.l.c.-mass spectrometry is performed by using a Varian series 1400 gas chromatograph coupled via a silicone membrane separator to an AEI MS12 mass spectrometer. A column (2 m x 2 mm) of 1% Dextril-300GC is used at a temperature of 270°C with helium (flow rate 20 ml/min) as carrier gas. The mass spectrometer, modified for operation in the selective ion-monitoring mode (Draffan et al., 1973), is operated at an electron energy of 24 eV and basic accelerating voltage of 8 kV. The ion currents at m/e 397 (unlabelled prostaglandin F metabolite) and at m/e 400 (internal standard) are monitored and the ratio of peak heights in the resulting fragment-ion chromatograms is used to calculate the concentration of endogenous prostaglandin F metabolite in the original urine sample. Values in the normal range of 5–40 ng/ml can be measured with 2% precision, whereas values of 1 ng/ml may be determined with an S.D. of approximately 10%. Values obtained for the 24 h excretion of prostaglandin F metabolite in 18 healthy males (12.5–66.9 μg, mean 22.7 ± 13.1 s.d.) and 16 females (7.2–18.8 μg, mean 11.9 ± 3.6 s.d.) are in good agreement with those reported by other authors (Hamberg, 1973; Granström & Kindahl, 1976).

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