Addition, together with antigen, of compound I.C.I. 74917 (0.1 μg/ml) to chopped sensitized lung, preincubated with D,L-isoprenaline for 3 min, resulted in extra inhibition of histamine release as seen in Fig. 2(a). Comparison of the total inhibition with individual values for compound I.C.I. 74917 and isoprenaline show that there is addition rather than synergism, i.e. if compound I.C.I. 74917 inhibits release by y% then isoprenaline could only act on the remaining (100-y)% and inhibit it by x% and vice versa (Fig. 2b).

It has been shown that compound I.C.I. 74917 is an inhibitor of cyclic AMP phosphodiesterase from rat mast cells (Barrett-Bee & Henderson, 1976). At the concentrations used here, however, which were much less than the \( K_i \) for mast-cell phosphodiesterase, the data indicate that compound I.C.I. 74917 was not acting as a phosphodiesterase inhibitor and potentiating the inhibition by isoprenaline, but it was merely adding to it, i.e. it was acting by a different mechanism.

The experiments shown in Fig. 2 demonstrate that the inhibitions of allergic histamine release by compound I.C.I. 74917 and isoprenaline are independent of each other. This would not be expected if compound I.C.I. 74917 was acting as a phosphodiesterase inhibitor. Any inhibition of histamine release by isoprenaline, caused by its increasing the concentration of cyclic AMP by stimulation of the adenyl cyclase, would be potentiated by the presence of a phosphodiesterase inhibitor. Since potentiation does not occur with compound I.C.I. 74917, it seems unlikely, therefore, that the anti-allergic properties of compound I.C.I. 74917 involve its activity as an inhibitor of cyclic AMP phosphodiesterase.

Disodium cromoglycate, which itself at a concentration of 500 μg/ml caused no inhibition of histamine release, increased the total inhibition by isoprenaline (Fig. 2a). This suggests that, at high concentrations, poor phosphodiesterase inhibitors such as cromoglycate are able to potentiate the inhibition of histamine release by isoprenaline. However, their normal mode of anti-allergic action does not depend upon this property.

Further evidence for this belief is provided by the experiments that indicate that the time of addition of compound I.C.I. 74917 or disodium cromoglycinate relative to addition of antigen is critical (Fig. 1b). Maximal inhibition is observed when the compound is added with antigen rather than before, as with isoprenaline or theophylline. This observation would indicate that a build-up of cyclic AMP is not required for compound I.C.I. 74917 to inhibit histamine release.

Barrett-Bee, K. J. & Green, L. R. (1975) Prostaglandins 10, 589–598

The Metabolism of Debrisoquine in Rat and Man

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Although debrisoquine (2-amidino-1,2,3,4-tetrahydroisoquinoline) sulphate has been used therapeutically as an anti-hypertensive agent for a number of years, little was known until recently about its metabolism. Allen et al. (1975) identified the major metabolites of the drug in rat and human urines and, in the same year, Angelo et al. (1975) noted that although the drug appeared to be well absorbed in a number of human subjects, the degree of oxidative metabolism varied widely and was a major determinant of responsiveness.

\(^{[14]}\text{C}\)Debrisoquine hydrochloride was synthesized from K\(^{14}\)CN labelled in the amidino carbon atom, and rats and male human volunteers were given the drug orally in
Table 1. Metabolites of [14C]debrisoquine in man and rat, expressed as a percentage of the dose in 0–24 h urine

<table>
<thead>
<tr>
<th>Metabolite RF†‡</th>
<th>Probable identity</th>
<th>Human subjects</th>
<th>Rat†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>See text</td>
<td>3.3 6.4 1.3 10.1</td>
<td>9.1 (7.2–11.6)</td>
</tr>
<tr>
<td>0.14</td>
<td>See text</td>
<td>1.9 2.6 0.2 1.1</td>
<td>1.9 (1.6–2.0)</td>
</tr>
<tr>
<td>0.28</td>
<td>4-Hydroxy-debrisoquine</td>
<td>24.1 16.7 5.4 15.1</td>
<td>34.9 (29.9–40.1)</td>
</tr>
<tr>
<td>0.60</td>
<td>Unknown</td>
<td>22.8 25.4 7.3 32.7</td>
<td>11.5 (7.8–13.4)</td>
</tr>
<tr>
<td>0.77</td>
<td>Debrisoquine</td>
<td>18.5 23.1 60.8 16.5</td>
<td>8.8 (6.9–12.2)</td>
</tr>
<tr>
<td>Total 14C in 0–24 h urine</td>
<td>75.2 75.7 71.3 76.7</td>
<td>66.9 (60.8–75.8)</td>
<td></td>
</tr>
</tbody>
</table>

* Descending paper chromatography on Whatman no. 1 paper with butan-2-ol/formic acid/water (100:12:10, by vol.).
† Mean values with range in parentheses for three female Wistar albino rats.
‡ RF values for standards were debrisoquine sulphate, 0.73; 5-, 6-, 7- and 8-hydroxydebrisoquine, 0.58; 4-hydroxydebrisoquine, 0.28, and the compounds were made visible with alkaline sodium nitroprusside (in H2O2) spray reagent (Hofman & Wünsch, 1958).

Most of the radioactivity was excreted by the rat in the first day (about 70% in urine and 10% in the faeces), whereas the human subjects excreted the drug more slowly (about 80% in urine in 2 days, and about 6% in faeces in 3 days).

Paper chromatography of the first day's urine and radiochromatogram scanning revealed a similar metabolic pattern in both species (Table 1). Each of the radioactive peaks was quantified by cutting up the chromatogram into bands and determining the radioactivity in a liquid-scintillation counter. The peaks of RF 0.61 and 0.77 tended to merge, therefore the peak corresponding to unchanged drug (RF 0.77) was estimated separately by reverse isotope dilution and subtracted from the composite peak to give a value for the peak of RF 0.61.

The identity of each peak was determined by separation of rat urine on an XAD-2 polystyrene bead column (Mule et al., 1971). Paper chromatography (Table 1) of the aqueous eluate yielded a single peak (RF 0.00) which on hydrolysis with β-glucuronidase, (Ketodase) yielded a major peak of RF 0.14. Derivatization with acetylacetone, extraction and g.l.c.–mass spectrometry (Allen et al., 1975) of the origin peak after hydrolysis showed that the peak consisted largely of dihydroxydebrisoquine and two acidic metabolites resulting from hetercyclic-ring cleavage. In addition, traces of the parent drug, 4-hydroxydebrisoquine and 6-hydroxydebrisoquine were detected. These latter compounds had probably been retained on the column and at the origin of the paper chromatogram, although essentially quantitative elution was seen to occur with authentic compounds.

The methanol eluate from the XAD-2 column gave three radioactive peaks on paper chromatography (Table 1) of RF 0.28, 0.60 and 0.77. The former and latter peaks had RF values that corresponded to 4-hydroxydebrisoquine and unchanged drug respectively, and elution and derivatization with acetylacetone as described above showed that this was the case, since they corresponded both in retention time and mass spectrum to the authentic compounds. So far the metabolite of RF 0.60 has not been identified.

Extraction on XAD-2 resin and paper chromatography followed by derivatization and g.l.c. showed the metabolites of debrisoquine in man to have the same identity as those in the rat. However, the quantities of the metabolites do vary markedly between human subjects much more so than in the rat, which does appear to metabolize the drug to a greater extent than man.
Human plasma concentrations of the unchanged drug and metabolites were determined over the first 24 h. Peak debrisoquine concentrations (20–50 ng/ml) occurred during the first 2–3 h and diminished rapidly. Most of the radioactivity was accounted for as metabolites, their rate of disappearance being slower than the parent drug.

We are grateful to Dr. T. R. Marten for determination of certain of the mass spectra and Dr. J. G. Allen of Roche Products Ltd., Welwyn Garden City, Herts., U.K. for a sample of 4-hydroxydebrisoquine, and to the Joint Standing Research Committee of St. Mary's Hospital and the Wellcome Trust for financial support.

Hofman, E. & Wünsch, A. (1958) Naturwissenschaften 45, 338

A New Method for the Determination of the Major Metabolite of Prostaglandin F2α 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 F2α 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 F2α turnover in vivo (Hamberg, 1974; Samuelsson, 1973). In connection with our investigations into the role of prostaglandin F2α in certain pathological disorders, we have developed a highly specific and sensitive assay for prostaglandin F metabolite, based on stable isotope dilution and combined gas chromatography/mass spectrometry. The internal standard used in this assay is a 2H-labelled analogue of prostaglandin F metabolite, in which the 4H 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-2H3, 9β-3H]prostaglandin F2α (14.5 mg).

Fig. 1 summarizes the sequence of steps in the assay procedure. To a portion (10 ml) of a 24 h urine collection is added the methyl ester of [2H3]prostaglandin F2α (200 ng) followed by 5 M-NaOH (2 ml). 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 (2 g) 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-