# Interconversion of $\Delta^1$-Cortisone and $\Delta^1$-Hydrocortisone in Man.* (22924)

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The interconversion of cortisone (E) and hydrocortisone (F) in man is well established(1,2,3). The dramatic therapeutic effect of their $\Delta^1$-analogs, first described by Bunim *et al.*(4) has stimulated investigations into the study of the *in vivo* metabolism of these compounds. Recent preliminary reports have indicated that $\Delta^1$-cortisone ($\Delta^1$E) is converted to $\Delta^1$-hydrocortisone ($\Delta^1$F)(5,6). The present study extends the applicability of the method of determining individual adrenocortical hormones, developed in this laboratory(7,8), to the urinary corticosteroid excretion in patients treated with $\Delta^1$E and $\Delta^1$F.

*Methods.* Details of urine extraction and partitioning of extracts are given elsewhere (8). In principle, the 24-hr urine sample is adjusted to pH 1, extracted with chloroform, and the concentrated extract partitioned on a water-impregnated silicic acid column, using gradient elution with petroleum ether containing increasing amounts of dichloromethane. The dried eluate fractions are dissolved in 95% ethanol, an aliquot is analyzed by ultraviolet (u.v.) absorption at 240 m$\mu$ and another aliquot by Blue Tetrazolium (B. T.) reduction(9). Excretion patterns are obtained by plotting tube numbers against optical density for both assays. The identity of isolated chromatographic bands is confirmed by paper chromatography.

*Results.* A man with rheumatoid arthritis was given 15 mg of $\Delta^1$F/day for 4 days. The 24-hr urine extracts of this period were pooled and gave the pattern in Fig. 1. Paper chromatography of Fraction 130-180 in the toluene-propylene glycol system of Zaffaroni(10) for 96 hrs gave a spot with a mobility of 7.0-10.1 cm compared with mobilities of 9.3-12.0 cm for E and 7.0-10.0 cm for $\Delta^1$E. In the toluene-ethyl acetate-methanol-water system of Bush(11), this fraction gave one spot with an Rf of 0.48 compared with 0.48 for $\Delta^1$E and 0.55 for E. F and $\Delta^1$F could not be separated in 96 hrs in the Zaffaroni system. However, in the Bush system Fraction 200-250 gave a spot with an Rf value of 0.21 as compared to 0.23 for $\Delta^1$F and 0.31 for F. Both fractions exhibited an absorption maximum near 240 m$\mu$ and reduced both Tollens and B. T. reagents. After spraying the chromatograms with 10% aqueous NaOH and drying them for 1 hr at 60°C neither fraction gave the primrose fluorescence shown by E and F (11). The qualitative tests and chromatographic comparisons with known compounds indicate the identity of Fraction 130-180 with $\Delta^1$E and of Fraction 200-250 with $\Delta^1$F.

Quantitative estimations were made by referring absorbances to standard curves for the u.v. and B.T. assays of pure $\Delta^1$E and $\Delta^1$F. The amount of $\Delta^1$E was 2.0 mg by u.v.

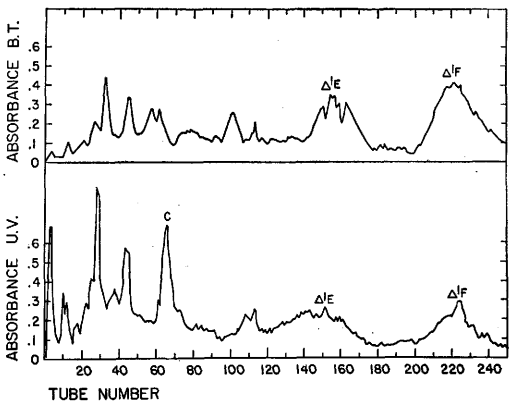


FIG. 1. Corticosteroid excretion pattern after administration of $\Delta^1$-hydrocortisone.

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While this manuscript was in preparation, 2 papers (12,13) on this subject have appeared which confirm our observations.

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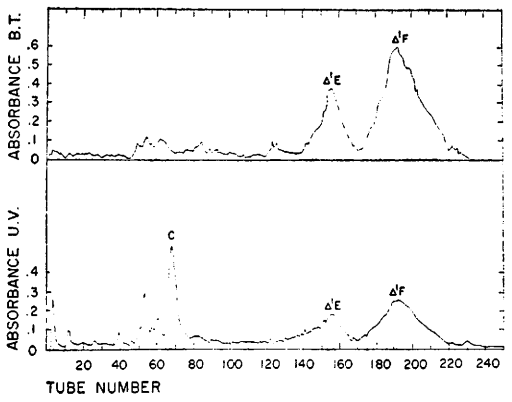


FIG. 2. Corticosteroid excretion pattern after administration of $\Delta^1$-cortisone.

assay and 1.1 mg by B.T. For $\Delta^1$F the amount was 1.6 mg by u.v. assay and 1.3 mg by B.T.

Fig. 2 represents the corticosteroid excretion pattern of a woman with multiple myeloma who received 50 mg of $\Delta^1$E. Paper chromatography of Fraction 135-170 in the Bush system gave one spot with an Rf of 0.48 compared with 0.48 for $\Delta^1$E and 0.55 for E. In the same system, Fraction 171-220 gave one spot with an Rf of 0.25 compared with 0.26 for $\Delta^1$F and 0.34 for F. Qualitative tests were carried out as described above. The amount of $\Delta^1$E was 0.9 mg by u.v. assay and 0.6 mg by B.T. The amount of $\Delta^1$F was 1.5 mg and 1.6 mg by u.v. and B.T. assays respectively. The high value in the u.v. assays of $\Delta^1$E indicates in both cases interference with the analysis by this method.

The peak labeled C in both figures had a maximum ultraviolet absorption at 273 m$\mu$ and was identified as caffeine(8).

*Discussion.* The high therapeutic and glucocorticoid activity of $\Delta^1$E led Gray *et al.*(5) to postulate that this effect might be due to *in vivo* conversion of $\Delta^1$E to E. However, they obtained paper chromatographic evidence of the excretion of unchanged $\Delta^1$E and $\Delta^1$F, but not E or F. Subsequent work by Vermeulen(6) confirmed the conversion of $\Delta^1$E to $\Delta^1$F. The results of our experiments furthermore indicate that $\Delta^1$E and $\Delta^1$F are interconvertible in the body and that the order of magnitude of excreted free $\Delta^1$E and $\Delta^1$F is similar after the administration of either compound. Analogous results have been reported in metabolic studies of the parent compounds E and F(1,2,3). Our results as well as others(5,6) indicate no excretion of E and F as a result of reduction of $\Delta^1$E and $\Delta^1$F. This does not exclude the possibility that E and F are formed. If formed, they may be further reduced to the tetrahydro derivatives which are excreted in conjugated form. Glucuronidates were not hydrolyzed in this study.

*Summary.* A method of determining individual adrenocortical hormones by partition chromatography on columns is applied to the study of urinary corticosteroid excretion in patients treated with the synthetic steroids $\Delta^1$-cortisone and $\Delta^1$-hydrocortisone. Chromatographic evidence indicates *in vivo* interconversion of the two compounds in man.

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