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SHORT COMMUNICATION

CONFIRMATION OF THE PRESENCE OF DIHYDROTESTOSTERONE IN HUMAN PLASMA BY MASS SPECTROMETRY

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SUMMARY

The presence of dihydrotestosterone in human blood has been confirmed by mass spectrometry.

INTRODUCTION

ON THE basis of competitive protein-binding studies, it was suggested in 1968 that dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) might account for some of the androgenic material present in serum[1]. This was tentatively confirmed by studies of plasma fractionated by Sephadex column chromatography[2]. Further evidence has been provided recently by the studies of Ito and Horton[3] and of Tremblay *et al.*[4] which have depended on thin layer or paper chromatographic separation. However other more definitive criteria of identity have been lacking. Recently we have been able to obtain data by mass spectrometry which clearly indicate the presence of this steroid in human plasma.

Pooled human plasma (from apparently healthy men and women) 1·3 litres, to which purified, tritiated dihydrotestosterone (DHT) (3·8 ng, specific activity 46·5 Ci/mmol) had been added, was extracted with 1·5 volumes diethyl ether. The extract was washed with 0·1M sodium hydroxide, adjusted to pH 7 and reduced to dryness *in vacuo*. It was then dissolved in 1 ml of the solvent system consisting of heptane : chloroform : ethanol : water (50 : 50 : 1 : water to incipient turbidity) and applied to a column (43 cm × 2·1 cm) of Sephadex LH20, as described elsewhere[2, 5].

Two hundred fractions of 5 ml vol. were collected, pooled according to the elution pattern of the labelled steroid, and subjected without further purification to mass spectrometric analysis (A.E.I. Ms. 902) along with standards of various androgens. The mass spectral data for fractions containing the bulk of the [^{3}H-] dihydrotestosterone and for the standard dihydrotestosterone are shown in Fig. 1. These spectra are almost identical and are compatible with that obtained for the same compound by others[6]. 17β-Hydroxy-5β-androstan-3-one exhibits distinctly different mass spectral behavior and can thus be excluded from structural consideration[6]. The electron impact-induced fragmentation reactions of

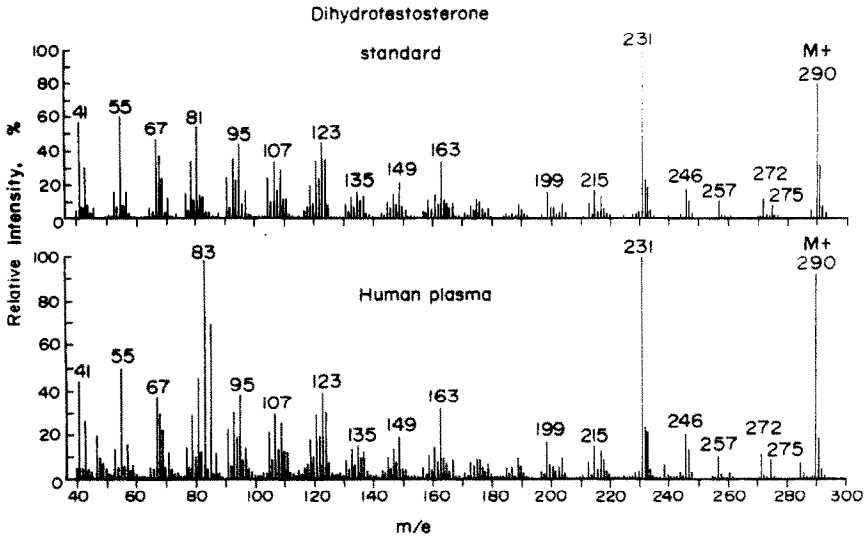

Fig. 1.

17β-hydroxysteroids have recently been studied by Spiteller-Friedmann and Spiteller [6]. The major fragment ion of DHT is the rupture of the C-13/C-17 and C-14/C-15 bonds with the loss of C_3H_7O to give the base peak at m/e = 231. The amount of DHT was approximately equivalent to 0·25 ng/ml. This corresponds to the amount to be expected in a mixed male and female sample since our studies and those of others [3, 4] indicate that the normal mean level in women is approximately 0·15 ng/ml and in men, 0·50 ng/ml.

These data provide therefore the first unequivocal proof of the presence of DHT in human blood. Mass spectral analysis of the other fractions, which have also confirmed the presence of testosterone, dehydroepiandrosterone, androstenedione and androstanediol will be published separately [7].

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