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ABSTRACTS OF PAPERS

Jerusalem, Israel, 5–10 September 1982

1. ANALYTICAL METHODS: I

1. ASSESSMENT OF THE TWO 20-DIHYDROISOMERS OF CORTISOL IN HUMAN URINE
Schöneshöfer, M. and Weber, B. - Klin.-Chemisches Zentrallabor, Klinikum Charlottenburg, Free University of Berlin, FRG

Preliminary data on cortisol-like substances in urine of hypercortisolemic patients have pointed out that urinary free 20α- and 20ß- dihydrocortisol (20α-, 20ß-DHF) are obviously more elevated than urinary free cortisol itself. Using solid-phase extraction and automatic high performance liquid chromatography with UV-detection, these cortisol metabolites were quantitated in urine of normal subjects and patients with hypercorticoidism. Reference ranges (nmol/24h) in normal subjects were 89-455 for 20α-DHF and 79-235 for 20ß-DHF. Values were highly elevated in hypercortisolemic states. The method has been shown to provide values probably more specific than do direct immunological quantitation techniques.

2. ANALYSIS OF C_{19}-STEROID METABOLISM BY HPLC AND IN-LINE MONITORING OF RADIOACTIVITY.
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Assays of androgen metabolism often use TLC as the chromatographic method for separation of various metabolites. TLC-based assays are generally time-consuming and laborious. HPLC usually offers increased resolution and reproducibility compared to TLC. Recently, radioactivity monitors (RM) for continous monitoring of radioactivity in eluents from liquid chromatographs have been introduced.
We have developed a method for separation of biologically important C_{19}-steroids based on HPLC employing a high performance reverse phase column (Supelcosil LC-18) and a three component eluent (methanol/acetonitrile/H_2O, 33/26/41, v/v/v)(J.Chromatogr. 1982, in press). The eluent from the HPLC column is led to a radioactivity monitor (Isoflo, Nuclear Enterprises). The signals from the RM-unit are fed to a minicomputer (ABC-80, Scandia-Metric). Parallell studies of androgen metabolism in the rat prostate using both TLC-based and HPLC-RM based assays have been carried out. The two different assays gave almost identical results, however, the HPLC-RM-based assay was faster, had higher resolution and demanded far less manual work than TLC based assay. With addition of an automatic sample injector, a combined HPLC-RM unit may represent a fast, reproducible and fully automatic procedure for separation and quantitation of a varity of labelled compounds.

3. TRILOSTANE: INTERFERENCE WITH ASSAYS FOR STEROID HORMONES
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Assessment of the adrenal blocking agent Trilostane (Sterling) has been hindered, for the drug appears to interfere with assays used to monitor its action. We have examined the effect of trilostane(T) and its 17-keto derivative(KT) on routine steroid assays and have analysed samples from 10 normal men who took T in doses up to 240 mg qds.
When added to serum both T and KT (100μM) increased the measured fluorimetric cortisol by >350 nmol/l ($p < 0.01$). Volunteers taking 960 mg T showed an increase of 175 nmol/l in fluorimetric serum cortisol. An alkali wash(0.2N) fully removed the interference from T but only partly from KT. Volunteers taking T showed a large rise in fluorimetric urinary cortisol despite an alkali wash step. Neither T nor KT(100 μM) interfere with the Amerlex cortisol RIA kit. Both T and KT cross-react in parallel fashion with our antisera for testosterone and androstenedione and the measured level of both these androgens rose in subjects taking T ($p < 0.01$). Column chromatography of a lipid extract of serum prior to RIA eliminated this interference. T and KT do not interfere with our assays for serum aldosterone, dehydroepiandrosterone sulphate or oestradiol.
We conclude that fluorimetric cortisol assays are unsuitable for monitoring patients on T and that androgen assays should be checked before being applied to these samples.