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SHORT COMMUNICATION

TECHNICAL IMPROVEMENTS TO A METHOD FOR DETERMINATION OF TESTOSTERONE

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SUMMARY

A number of small modifications have been made to a recently presented method [1] for plasma testosterone determination by the competitive protein-binding (CPB) technique. When compared with a radioimmunoassay procedure including the same purification steps a correlation coefficient of 0·95 was found when samples from female and male subjects were analysed.

SINCE the submission of our method (June 1971), the following observations and modifications have been made:

(1) Stability of late twin pregnancy plasma: Three such plasma samples have been kept at −20°C for a period of more than 1000 days. All the samples were found to have kept their original testosterone-binding properties and to be fit for use.

(2) Stability of diluted protein/radioactive testosterone: Such a solution (0·5% of late twin pregnancy plasma with testosterone-binding capacity of more than $6{\cdot}5 \times 10^{-7}$ mol/l in water containing tritiated testosterone approximately 40,000 c.p.m. per ml) is used in the competitive protein-binding assay. When this solution was kept for 2–4 days at +4°C it was found to be losing its binding properties. However, these properties were unchanged when the solution was kept for up to six weeks at −70°C (Revco ultra-low temperature freezer).

(3) Two modifications have been made in the radioactive material employed: (a) The use of [1, 2, 6, 7-^{3}H]-testosterone (N.E.N. Corp.) of S.A. 312 mCi/mg instead of the previously used [1, 2-^{3}H]-testosterone (Radiochemical Centre, Amersham, England) of S.A. 153 mCi/mg, reduces the mass interference (from about 0·3 to 0·15 ng/sample) of the labelled steroid with the endogenous steroid in the sample [2]. (b) For determination of recovery, [1, 2-^{3}H]-epitestosterone (N.E.N. Corp.) of S.A. 192 mCi/mg is now used. Its behaviour in the method, including column chromatography on alumina, was the same as that of testosterone. This modification also reduces the interference with the "marker" steroid in the protein-binding solution [2]. The affinity of epitestosterone for SBP is negligible, approximately 1%, [3, 4], a fact which we have confirmed. Epitestosterone has been used for the same purpose in one previously presented method [5].

(4) In an attempt to improve the standard curve, the endogenous steroids have been eliminated by treatment of the late twin pregnancy plasma with activated charcoal (Norit A) according to the method of Heyns *et al.* [6]. With our usual dilution and plasma (0·5%, late twin pregnancy) no significant improve-

ment was achieved. On the other hand, in 1–2% dilutions of some normal late pregnancy plasmas slight improvements were observed after such treatment (on the slope and linearity of the standard curve).

(5) With the same aim a non-ionic liquid surfactant, Triton-X-100 (octyl phenoxypolyethoxyethanol) B.D.H., has been added to the plasma solution used for the binding assay. Triton concentrations in the region of 1–0·05% (Triton to water) produced a flat standard curve. However, with much lower concentrations (the optimum for each plasma being found experimentally) in the region of 0·00001% for 1% plasma dilution, it was found that the standard curve occasionally improved in linearity and slope. However, following statistical analyses of 32 different standard curves with and without addition of Triton-X-100 it was found that this phenomenon was not statistically significant. As a consequence this modification was not included in the method.

(6) Addition of human γ-globulin (Finnish Red Cross, for clinical use) to the dilute plasma protein solution used for CPB (10 mg/ml) did not decrease the blank values and flattened the standard curve. It seems that this modification does not offer any improvement with respect to the CPB assay of testosterone in contrast to the results reported by G. Leyendecker *et al.*[7] who used this device in order to minimize the blank value in radioimmunoassay of oestradiol-17β and CPB assay of progesterone.

(7) The testosterone fraction obtained from column chromatography is collected in tubes siliconized before use (1% Dimethyldichlorosilan–Fluka–in distilled toluene). This has been satisfactory, especially when old glassware was used, as adsorption on the glass was minimized.

(8) Some new batches of alumina produced higher blanks than previously reported (0·3–0·6 ng/sample). This problem has been solved by cleaning the alumina column with the usual 10 ml of n-hexane followed by 15 ml of 5% CH_3OH/n-hexane and 10 ml n-hexane. With this procedure we have obtained lower blanks than before, i.e. 0·1–0·4 ng/sample.

(9) The present method was compared to a radioimmunoassay (RIA) procedure including exactly the same steps. The final quantitation was carried out by RIA using anti-testosterone-3-oxime-BSA-serum (Lot #667, provided through the courtesy of Dr. G. D. Niswender, University of Michigan). The correlation coefficient was found to be 0·95 for 17 parallel analyses of samples from female subjects and the same result was found for 16 parallel analyses of samples from male subjects.

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