Title

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LETTER TO THE EDITOR

An Economical Method for Determining Tritium Content in Competitive Protein Binding Assays for Steroid Hormones

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QUANTITATION of steroid hormones by competitive protein binding analysis (CPBA) and radioimmunoassay (RIA) techniques depends upon the measurement of tritium activity.[1] The only exception to this generalization is RIA in which ^{131}I is coupled to the steroid by tyrosine methyl ester linkage.[2] During the past few years of tremendous growth in CPBA and RIA techniques, the use of ^{3}H has far surpassed the use of ^{131}I. The major expenses for CPBA determinations are counting supplies (vials and cocktails) if one can ignore the labor cost. Because of an increasing number of samples, spiraling costs, and reductions in research funds, it became imperative to search for areas in which economical measures could be instituted without sacrificing accuracy and reliability.

CPBA is used in our laboratory for the routine determination of cortisol, 11-desoxycortisol, and testosterone. The methods of MURPHY[3] are used and bound and free steroid are separated with florisil. In all instances, 0·5 ml of the bound steroid are used for tritium determination in a Nuclear Chicago Mark I liquid scintillation counter. For routine assay, this aliquot is pipetted directly into a 1-dram glass shell vial fitted with a plastic snap-cap (Brockway Glass Co., Vial # 15045 AW) and 4 ml commercial scintillation cocktail (Aquasol, New England Nuclear) is added. The contents of the shell vials are mixed thoroughly. The shell vials then are placed in regular 20-ml scintillation vials and are counted in the normal manner. After counting, the shell vials are discarded and the counting vials are reused. ^{3}H counting efficiencies were determined by the use of a tritium internal standard (Nuclear Chicago radioactive solution standard, ^{3}H-benzene in toluene, Lot # E392). Ten lambda of this standard solution was added to vials with a Hamilton syringe.

To evaluate the validity of this shell vial counting technique, ^{3}H counting efficiencies were determined with our counter using a tritium internal standard. The efficiencies of various vial/cocktail volume combinations were checked. Counting efficiencies for tritium in regular scintillation vials which contained 10 ml cocktail or in shell vials which contained smaller quantities of cocktail (2, 3 and 4 ml) were essentially equivalent (range: 30·5–32·4%). In a similar comparison, samples to which 0·5 ml protein solution (0·5% plasma in barbital–sodium acetate buffer, pH 9·1) was added showed very similar counting efficiencies (range: 22·6–24·0%). The use of a regular scintillation vial which contained 10 ml cocktail did not enhance the ^{3}H counting efficiency.

Figure 1 illustrates standard curves for cortisol and 11-desoxycortisol as observed in routine clinical assays in which shell vials and 4 ml cocktail were used. Percent bound $[(B/B + F) \times 100]$ versus steroid dose yields the typical curvilinear dose response curve. In all cases, the zero dose was set at 100 per cent. The counting method also is applicable

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11-desoxycortisol as observed in routine clinical assays in which shell vials and 4 ml cocktail were used. Percent bound $[(B/B - F) \times 100]$ versus steroid dose yields the typical curvilinear dose response curve. In all cases, the zero dose was set at 100 per cent. The counting method also is applicable to testosterone determination by CPBA as shown in Fig. 2. To analyze the testosterone responses more closely, calculations were done by the methods proposed by RODBARD *et al.*[4] Non-specific binding of testosterone by the plasma proteins was calculated by including tubes in each assay to which had been added 200 ng testosterone in a manner as has been applied to the analysis of 17α-hydroxyprogesterone.[5] Figure 2 represents the graphic analysis of seven testosterone standard curves: Fig. 2(a) shows the curvilinear dose response curve represented by the mean value of seven determinations at each point and the brackets define ± three standard errors of the mean for each point. Figure 2(b) illustrates the logit transform of these data. Again brackets define ± three standard errors of the mean for each dose. Linearity was observed between 25–83% bound which falls within the normally accepted usuable range of the curves in such assays. Below and above these points, the relative error is large.

The data suggest that CPBA assays can be counted in a more economical manner. A cost comparison at current market prices indicates that a cost reduction of between 68 and 79 per cent in counting supplies can be realized. 4 ml cocktail has been chosen for routine assays because of the physical appearance of the resultant cocktail aqueous protein solution. Tritium efficiencies suggest that one may just as confidently work with less cocktail. The major disadvantage of the glass shell vials is the higher background count which has averaged to about 38 cpm. This probably is due to the higher potassium content of the glass from which the shell vials are made. This can be a problem when working with extremely low activities, but this is usually not the case in routine clinical binding assays. Various lots of shell vials have been used and all have proved satisfactory.

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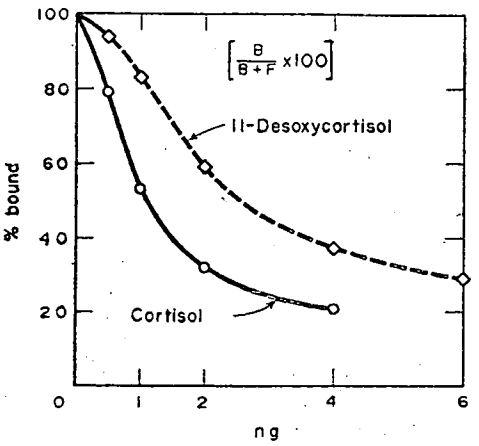

FIG. 1. CPBA standard curves for cortisol (solid line) and 11-desoxycortisol (dashed line).

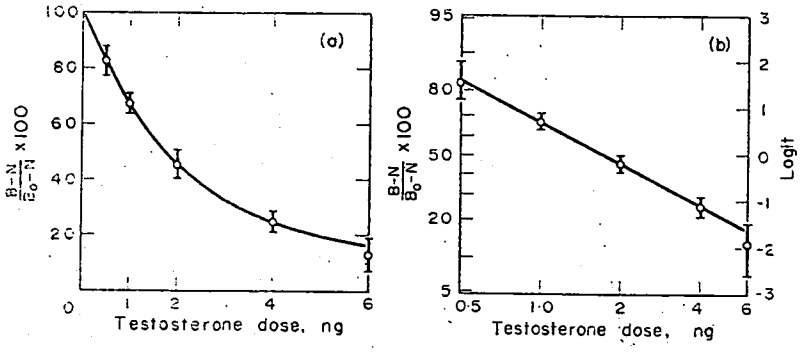

FIG. 2. (a) Standard curve for testosterone. Each point is the mean of seven points and brackets represent three standard errors of the mean. (b) Plot of the same data (as shown in (a) after log–logit transformation.