Method for the Determination of Binding Capacity and Endogenous Concentrations of Steroids

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Several steroid hormones are known to be tightly bound to plasma proteins (Sandberg, Rosenthal, Schneider & Slaunwhite, 1966). The association constant for cortisol binding to transcortin has been determined, although it has been pointed out that progesterone also has a high affinity for this protein (Seal & Doe, 1966). Murphy (1964) has presented a method of assay for minute quantities of hormone that utilizes the binding characteristics of plasma for particular hormones. The main limitation of this method is its non-specificity owing to competition for binding sites by other endogenous steroids, and its dependence on a precharacterized plasma. To overcome these limitations the following method for endogenous steroid determination is presented.

In considering cortisol binding in plasma, the endogenous concentration of cortisol (S_E) may be taken as a total of free cortisol (S_F), loosely bound cortisol (S_A) and transcortin-bound cortisol (S_T).

If a small amount of radioactively labelled cortisol, Z , is added to a sample of this plasma, it will distribute itself in such a way that the transcortin-bound radioactivity, Z_T , obeys the relationship:

$$\frac{Z_T}{Z} \cdot (S_E) = (S_T)$$

We can determine the transcortin-bound radioactivity by use of gel-filtration, realizing, as Doe, Fernandez & Seal (1964) have shown, that the loosely bound steroid dissociates under these separation conditions.

If a large excess of unlabelled cortisol ($10 \mu M$) is then added to a sample of this plasma with the same amount of radioactively labelled cortisol, then the transcortin-bound radioactivity, Z_T^* , obeys the relationship:

$$\frac{Z_T^*}{Z} \times 10^{-5} = (S_T^*)$$

where (S_T) represents the maximum transcortin-binding capacity of the plasma.

The fraction of transcortin sites bound in the original plasma will then be given by:

$$\frac{(S_T)}{(S_T^*)} = \frac{Z_T(S_E)}{Z_T^* \times 10^{-5}} = K_1(S_E)$$

We can use the experimentally determined values of transcortin-bound radioactivity to evaluate K_1 , and can now show that this provides sufficient information to allow the determination of the endogenous steroid concentration.

The association constant for the transcortin-cortisol binding will be given by:

$$K = \frac{(S_T)}{[(S_T^*) - (S_T)](S_F)}$$

Hence:

$$\frac{(S_T)}{(S_T^*)} = \frac{K(S_F)}{1 + K(S_F)} = \frac{K_2(S_E)}{1 + K_2(S_E)}$$

where K_2 is related to K by the ratio of the free labelled cortisol, Z_F , to the total labelled cortisol, Z .

The endogenous cortisol concentration will be given by:

$$K_1(S_E) = \frac{K_2(S_E)}{1 + K_2(S_E)}$$

$$(S_E) = \frac{1}{K_1} - \frac{1}{K_2}$$

Normally (S_E) will be much more sensitive to K_1 than to K_2 , which has a value of the same order as the transcortin-cortisol association constant. However, the value of K_2 may be determined by repeating the experiment with a standard amount of unlabelled cortisol added to the plasma, the transcortin-cortisol association constant K being obtained by determination of the concentration of free labelled cortisol by ultrafiltration (Chen, Mills & Bartter, 1961), by equilibrium dialysis (Sandberg, Slaunwhite & Carter, 1960) or by adsorption methods (Murphy, 1967). For plasma cortisol binding we can use values already determined for these constants, although it is recommended that an experimentally determined value of K_2 is obtained for all samples. In such a way reliable determination of binding capacities and endogenous steroid concentrations may be obtained even where the binding protein has not been identified.

The method offers an opportunity for detailed description of the binding characteristics and endogenous concentrations of several steroids in

plasma or target tissue (Snart, 1967; Sanyal & Snart, 1968). It is presented as a simple, specific and accurate method for hormonal assay.

Chen, P. S., Mills, I. H. & Bartter, F. C. (1961). *J. Endocrin.* **23**, 129.

Doe, R. P., Fernandez, R. & Seal, U. S. (1964). *J. clin. Endocrin. Metab.* **24**, 1029.

Murphy, B. E. P. (1964). *Nature, Lond.*, **201**, 679.

Murphy, B. E. P. (1967). *J. clin. Endocrin. Metab.* **27**, 973.

Sandberg, A. A., Rosenthal, H., Schneider, S. L. & Slaunwhite, W. R. (1966). In *Steroid Dynamics*, p. 1. Ed. by Pincus, G., Nakao, T. & Tait, J. F. New York: Academic Press Inc.

Sandberg, A. A., Slaunwhite, W. R. & Carter, A. C. (1960). *J. clin. Invest.* **39**, 1914.

Sanyal, N. N. & Snart, R. S. (1968). *J. Endocrin.* **40**, xvi.

Seal, U. S. & Doe, R. P. (1966). In *Steroid Dynamics*, p. 63. Ed. by Pincus, G., Nakao, T. & Tait, J. F. New York: Academic Press Inc.

Snart, R. S. (1967). *Biochim. biophys. Acta*, **135**, 1056.