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MEASUREMENT OF PLASMA CORTICOIDS: A COMPARISON OF SPECTRO-FLUOROMETRIC AND COMPETITIVE PROTEIN BINDING METHODS[1]

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Measurement of Plasma Corticoids: A Comparison of Spectrofluorometric and Competitive Protein Binding Methods

Measurement of plasma corticoids is a determination which now is available in most clinical laboratories. Methods which specifically measure cortisol, the most abundant and biologically active corticoid in man, involve a number of chromatographic separations and consequently are not feasible for routine clinical use. Two relatively simple techniques for the measurement of cortisol have achieved widespread popularity. Spectrofluorometric assays (1, 2, 3, 4) are simple and quick, but they may suffer from the problem of non-specific fluorescence which results in falsely elevated values (5,6). Recently, Murphy (7, 8) described a more specific method for the measurement of plasma cortisol which utilizes the principle of competitive protein binding analysis (CPBA). Because the time factor is comparable for each assay, it seemed important to compare these two techniques in an effort to determine the most valid technique for a simple and rapid corticoid determination.

It has been reported that insulin-induced hypoglycemia results in a marked elevation of plasma corticoids (9). During a study of the response of healthy subjects to insulin tolerance, this observation was confirmed, and it was apparent that a very wide range of corticosteroid values were present. The opportunity allowed us to compare and contrast plasma corticosteroid determinations by the spectrofluorometric and CPBA methods.

Ten healthy adults reported at 8 A.M. after an overnight fast for the insulin tolerance test (ITT). After resting in bed for 30 minutes, the fasting subjects received a single I.V. injection of regular insulin (0.1 unit per kg body wt). Three baseline blood samples were drawn before and at 10, 20, 30, 45, 60, and 90 minutes after insulin administration.

Blood was analyzed for corticoid concentrations by the two methods. The spectrofluorometric assay of Mattingly (1) was employed, and the CPBA technique of Murphy (8) was used with minor modifications. In addition, plasma from a number of healthy individuals was pooled. Corticosteroid determinations were performed in duplicate on aliquots of this pooled plasma on ten different occasions by the CPBA and spectrofluorometric assays. This feature allowed us internal control on the performance of each assay.

The results of the comparison of corticosteroid determinations by the two methods are listed in Table 1. Examination of the data for the pooled plasma samples shows that the fluorometric analysis resulted in a slightly higher mean value than that obtained by CPBA, but this difference was not significant statistically. The corticoid data obtained during the ITT strengthen this observation. At nine different time periods when blood was analyzed, the mean corticoid values determined by the fluorometric method were higher than those obtained by the CPBA method. Although significant differences were not noted in the baseline and 30 minute values, all other differences were significant. An excellent correlation ($r=0.99$) was observed between the two techniques when group comparisons were made on the mean values at the various time intervals ($Y= -0.76 + 0.8X$). Closer examination of individual results of low values (less than 6 μg%) and high values (greater than 20 μg%) shows disparity. When 11 individual values which were less than 6 μg% (by CPBA) were paired with their respective fluorometric values and analyzed, a very poor correlation was observed ($r= 0.15$, $Y= 3.59 + 0.06X$). When values greater than 20 μg% (by CPBA) were paired with their respective fluorometric values, a correlation coefficient of 0.61 was obtained

(Y= 5.1 + 0.56X). For the entire array of data, CPBA analysis yields data which are 23.1% lower in numerical value than that obtained by the fluorometric method. For the respective high and low values, these differences were greater, 26.2% and 57.3%

It is highly probable that these differences are due to a non-specific fluorescent response in the method. Rudd et al (10) suggested that the non-specific fluorogenic material may consist of di-and triglycerides. Recently, impure methylene dichloride which reacted with a benzyl alcohol preservative in heparin used as an anticoagulant has been incriminated for high values obtained by spectrofluorometry (11, 12). In addition, spironolactone has recently been shown to cause abnormally high values by fluorometric method. (13, 14).

For corticoid values, which are elevated or are in the upper normal range, a total accuracy is not clinically important. Furthermore, this would often prompt a more thorough analysis by other means. In situations where values are low, an interferring fluorogenic substance can assume considerable importance and may lead to false security. Values up to 10 µg% have been reported in conditions in which the naturally occurring plasma corticosteroids could be expected to approach zero, i.e., during therapy with high doses of corticosteroids, in hypopituitarism and Addison's disease, and after adrenalectomy (1, 15, 16). With this problem as a distinct possibility, it would seem advisable to do corticosteroid determinations by the CPBA method.

In summary, it must be stated that both methods lack total steroidal specificity. The fluorometric assay measures both cortisol and corticosterone. Although human plasma contains 8 to 16 times as much cortisol as corticosterone (17), the fluorometric intensity of corticosterone

is approximately 3 times that of cortisol. The CPBA, as used in our laboratory, will be affected by other steroids: corticosterone, 11-desoxy-cortisol, 17-hydroxyprogesterone, and to a much lesser extent by cortisone, progesterone and testosterone (18). The CPBA provides a more useful and accurate estimate of plasma corticosteroid values, particularly when the levels fall outside the usually accepted normal range (6 - 20 μg%). This gains special significance in case of lower values as discussed previously. Finally, unless total specificity of the CPBA method is achieved by chromatographic separations, it would seem advisable to report values as plasma corticosteroids rather than plasma cortisol as is often the practice.

References

1. Mattingly, D., J. Clin. Path. 15, 374, 1962.

2. Steenburg, R.W., and B.H. Thomasson, J. Clin. Endocr. 24, 875, 1964.

3. Gantt, C.L., D.E. Maynard and G.G. Hamwi, Metabolism 13, 1327, 1964.

4. Spencer-Peet, J., J.R. Daly and V. Smith, J. Endocr. 31, 235, 1965.

5. Braunsberg, H., and V.H.T. James, J. Endocr. 25, 309, 1962.

6. Daly, J.R., and J. Spencer-Peet, J. Endocr. 30, 255, 1964.

7. Murphy, B.P., Nature, 201, 679, 1964.

8. Murphy, B.P., J. Clin. Endocr. 27, 973, 1967.

9. Nakagawa, K., Y. Horiuchi and K. Mashimo, J. Clin. Endocr. 29, 35, 1969.

10. Rudd, B.T., P. Sampson, and B.N. Brooke, J. Endocr. 27, 317, 1963.

11. Werk, E.E. Jr., K.E. Theiss, Y.K. Choi, and R.T. Marnell, J. Clin. Endocr. 27, 1350, 1967.

12. Kendall, J.W., M.L. Egans and A.K. Stott, J. Clin. Endocrn. 28, 1373, 1968.

13. Wood, L.C., R. Richards, and S.H. Ingbar, New Eng. J. Med., 282, 650, 1970.

14. Lurie, A.O., J. Amer. Med. Ass., 211, 1851 1970.

15. De Moor, P., and O. Steeno, R. Raskin, and A. Hendrikx, Acta. Endocr. (Kbh.) 33,297,1960.

16. Stewart, C.P., F. Albert-Recht, and L.M. Osman, Clin. Chim. Acta, 6, 696, 1961.

17. Peterson, R.E., Recent Progress in Hormone Research, 15, 231, 1959.

18. Murphy, B.P., in Clinical Endocrinology II, Astwood, E.B., and C.E. Cassidy, (editors) Grune and Stratton, New York, 1968).

Table I

Comparison of CPBA and Fluorometric Methods
(Plasma Corticosteroid Concentration* in μg/100 ml.)

Insulin Tolerance Test

No.	Pool-Plasma	Name	Baseline 1	Baseline 2	Baseline 3	Minutes After Insulin Injection 10	20	30	45	60	90
1.	11.0 (16.7)	W.	5.0 (8.8)	6.0 (8.7)	6.0 (7.8)	12.5 (8.8)	10.5 (11.3)	17.0 (19.1)	26.5 (27.2)	25.8 (30.0)	28.0 (35.0)
2.	11.3 (12.4)	C.S.P.	9.0 (8.5)	8.0 (8.8)	9.0 (8.4)	8.0 (8.5)	11.0 (8.5)	14.0 (8.5)	22.2 (24.7)	22.0 (29.4)	26.0 (28.9)
3.	12.3 (13.4)	L.R.	15.0 (15.9)	12.5 (14.4)	11.0 (15.3)	- -	11.5 -	15.0 (17.9)	21.0 (26.5)	25.0 (33.8)	29.0 (37.9)
4.	12.3 (16.3)	C.J.	7.0 (9.7)	14.0 (8.5)	3.0 (7.2)	4.0 (13.8)	7.2 (8.0)	5.0 (8.2)	24.0 (23.5)	25.0 (28.1)	28.0 (31.6)
5.	13.0 (14.2)	M.P.F.C.	13.0 (20.2)	11.5 (21.2)	9.0 (16.2)	12.0 (16.2)	10.0 (16.2)	13.0 (22.1)	24.5 (34.3)	25.0 (34.3)	25.0 (41.4)
6.	12.0 (14.0)	D.B.P.	11.0 (9.5)	12.0 (21.7)	10.0 (19.8)	8.5 (23.9)	5.0 (19.8)	9.0 (18.5)	15.5 (29.3)	18.0 (34.2)	18.5 (32.6)
7.	14.0 (14.4)	M.J.	8.5 (8.8)	5.0 (8.4)	2.0 (8.1)	2.0 (10.0)	6.0 (9.7)	9.0 (13.8)	19.0 (23.5)	24.0 (25.0)	24.5 (35.3)
8.	14.0 (11.1)	D.B.	6.0 (8.0)	3.0 (6.8)	7.5 (6.6)	8.0 -	5.0 -	10.0 (12.1)	15.0 (18.5)	21.0 (25.6)	24.0 (42.1)
9.	11.3 (11.8)	L.R.	12.0 (12.8)	12.0 (14.0)	10.0 (12.2)	10.5 (11.5)	10.5 (11.5)	13.0 (14.3)	21.0 (26.5)	21.8 (30.6)	22.0 (28.6)
10.	14.2 (13.3)	W.P.V.T.	- (9.8)	- (9.4)	9.0 (9.0)	6.2 (9.5)	6.0 (9.5)	16.2 (15.5)	19.2 (25.2)	23.0 (28.6)	19.8 (24.3)
Mean	12.6 (13.8)		9.6 (11.2)	9.3 (12.2)	7.7 (11.0)	8.0 (12.8)	8.3 (11.8)	12.1 (15.0)	20.8 (25.9)	23.1 (30.0)	24.5 (33.8)
S.E.M. ±	0.4	±	1.2	1.4	1.0	1.2	0.9	1.3	1.3	0.8	1.2
	** (0.6)		(1.3)	(1.8)	(1.5)	(1.8)	(1.4)	(1.5)	(1.4)	(1.1)	(1.9)
"t" test	N.S.		N.S.	N.S.	N.S.	S.	S.	N.S.	S.	S.	S.

* The numbers within parentheses denote plasma corticosteroid levels by the fluorometric method.

** S= Significant ($P < 0.05$), N.S.= Non-significant ($P > 0.05$). The statistical significance refers to corresponding mean values by the two methods.