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SHORT COMMUNICATION

A COMPARISON OF SPECTROFLUOROMETRIC AND COMPETITIVE PROTEIN-BINDING METHODS FOR THE MEASUREMENT OF PLASMA CORTICOSTEROIDS[1]

By Raj K. Sarin, Gerald M. Connell, and John A. Linfoot

Donner Laboratory and Donner Pavilion, University of California, Berkeley, California 94720

[1] Supported in part by the United States Atomic Energy Commission.

RUNNING TITLE: MEASUREMENT OF PLASMA CORTICOSTEROIDS

PROOF SHOULD BE MAILED TO: JOHN A. LINFOOT, M.D.
DONNER PAVILION
COWELL MEMORIAL HOSPITAL
BERKELEY, CALIFORNIA 94720

A Comparison of Spectrofluorometric and Competitive Protein Binding Methods for the Measurement of Plasma Corticosteroids

Measurement of plasma corticosteroid concentration is a determination that now is available in most clinical laboratories. Methods which specifically measure cortisol, the most abundant and biologically active corticosteroid 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 1/2 4) are simple and quick, but may suffer from the problem of non-specific fluorescence, which results in falsely elevated values (5, 6). Murphy (7, 8) has described a relatively more specific method for the measurement of plasma cortisol that 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 more valid technique for a simple and rapid corticosteroid determination.

It has been reported that insulin-induced hypoglycemia results in a marked elevation of plasma corticosteroid concentration (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 was 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 intravenous injection of regular insulin (0.1 unit per kg body weight). Three baseline blood samples were drawn before insulin injection and further specimens were taken at 10, 20, 30, 45, 60, and 90 minutes after insulin administration. All samples were frozen until analyzed within a maximum period of six months. Plasma was analyzed in duplicate for corticosteroid concentrations by the two methods. The spectrofluorometric assay of Mattingly (1) was employed, and the CPBA technique of Murphy (8) was used with florisil as the adsorbent and H^3-corticosterone as the label. A 2.5% solution of dog plasma was used as the source of corticosteroid-binding globulin. In addition, plasma from a number of healthy individuals was pooled. Duplicate aliquots of this pooled plasma were analyzed for corticosteroid content by the CPBA and spectrofluorometric methods in conjunction with each assay. Analyses of this pooled plasma on ten different occasions gave coefficients of variation of 9.8% and 13.0% for the respective CPBA and spectrofluorometric methods. 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 corticosteroid data obtained during the ITT strengthened this observation. At nine different time periods when plasma was analyzed, the mean corticosteroid 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 ($p < 0.05$) when analyzed by t test. 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. 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, ($r = 0.15$, $p > 0.10$). When values greater than 20 µg% (by CPBA) were paired with their respective fluorometric values, a correlation coefficient of 0.61 ($p < 0.01$) was obtained. For the entire array of data, CPBA analysis yields data which are 23.1% lower in numerical value than those 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. 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 (13, 14) and cholesterol (15) recently have been shown to cause abnormally high values by fluorometric [illegible]

[illegible] steroid values which are elevated by [illegible]

normal range, total accuracy is not clinically important. Furthermore, this would often prompt a more thorough analysis by other means. In situations in which values are low, an interfering 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, 16, 17). 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 (18), 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-desoxycortisol, 17-hydroxyprogesterone, and, to a much lesser extent by cortisone, progesterone, and testosterone (19). The CPBA provides a more useful and perhaps more accurate estimate of plasma corticosteroid values, particularly when the levels fall outside the usually accepted normal range ($6 \underset{N}{-} 20$ 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.

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Received May 5, 1970

Table 1

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

Insulin Tolerance Test

No.	Pool Plasma	Name Subject	Baseline 1	Baseline 2	Baseline 3	Minutes After Insulin Injection 10	20	30	45	60	90
1.	11.0	W.	5.0	6.0	6.0	12.5	10.5	17.0	26.5	25.8	28.0
	(16.7)		(8.8)	(8.7)	(7.8)	(8.8)	(11.3)	(19.1)	(27.2)	(30.0)	(35.0)
2.	11.3	C.S.P.	9.0	8.0	9.0	8.0	11.0	14.0	22.2	22.0	26.0
	(12.4)		(8.5)	(8.8)	(8.4)	(8.5)	(8.5)	(8.5)	(24.7)	(29.4)	(28.9)
3.	12.3	L.R.	15.0	12.5	11.0	-	11.5	15.0	21.0	25.0	29.0
	(13.4)		(15.9)	(14.4)	(15.3)	-	-	(17.9)	(26.5)	(33.8)	(37.9)
4.	12.3	C.J.	7.0	14.0	3.0	4.0	7.2	5.0	24.0	25.0	28.0
	(15.3)		(9.7)	(8.5)	(7.2)	(13.8)	(8.0)	(8.2)	(23.5)	(28.1)	(31.6)
5.	13.4	[illegible]	13.0	11.5	9.0	12.0	10.0	13.0	24.5	25.0	25.0
	[illegible]		[illegible]	(21.2)	(16.2)	(16.2)	(16.2)	(22.1)	(34.3)	(34.3)	(41.4)
6.	[illegible]		[illegible]	12.0	10.0	8.5	5.0	9.0	15.5	18.0	18.5
	[illegible]		[illegible]	(21.7)	(19.8)	(23.9)	(19.8)	(18.5)	(29.3)	(34.2)	(32.6)
7.	[illegible]		[illegible]	5.0	2.0	2.0	6.0	9.0	19.0	24.0	24.5
	(14.[illegible]		(8.8)	(8.4)	(8.1)	(10.0)	(9.7)	(13.8)	(23.5)	(25.0)	(35.3)
8.	[illegible]		6.0	3.0	7.5	8.0	5.0	10.0	15.0	21.0	24.0
	[illegible]		(8.0)	(6.8)	(6.6)	-	-	(12.1)	(18.5)	(25.6)	(42.1)
9.	[illegible]		12.0	12.0	10.0	10.5	10.5	13.0	21.0	21.8	22.0
	(11.[illegible]		(12.6)	(14.0)	(12.2)	(11.5)	(11.5)	(14.3)	(26.5)	(30.6)	(28.6)
10.	14.[illegible]	[illegible]	-	-	9.0	6.2	6.0	16.2	19.2	23.0	19.8
	(13.[illegible]		(9.8)	(9.4)	(9.0)	(9.5)	(9.5)	(15.5)	(25.2)	(28.6)	(24.3)
Mean	12.[illegible]		9.6	9.3	7.7	8.0	8.3	12.1	20.8	23.1	24.5
	(13.[illegible]		(11.2)	(12.2)	(11.0)	(12.8)	(11.8)	(15.0)	(25.9)	(30.0)	(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
	[illegible]		(1.3)	(1.8)	(1.5)	(1.8)	(1.4)	(1.5)	(1.4)	(1.1)	(1.9)
Statistical [illegible]	[illegible]		p<0.3	p<0.2	p<0.1	p<0.05***	p<0.05***	p<0.2	p<0.01***	p<0.01***	p<0.01***

* Values in parentheses denote plasma corticosteroid levels by the fluorometric method.
[illegible] refers to p values of t-test determinations after comparing the mean values of the [illegible] indicated by *** indicate a significant difference.