Diagnostic Performance of Basal Free Cortisol/18-Hydroxy-11-Deoxycorticosterone (18-OH-DOC) Ratio in Endogenous Depression: Comparison with the Dexamethasone Suppression Test

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We assessed the 8:00 AM ratio of free cortisol/18-hydroxy-11-deoxycorticosterone (18-OH-DOC) in 56 endogenous depressive inpatients and in 22 normal volunteers. A ratio higher than 40 was associated with a diagnostic sensitivity for endogenous depression of 75%, a specificity of 95.5%, and a diagnostic confidence of 97.7%. These diagnostic results were at least equivalent to the Dexamethasone Suppression Test (DST) using a cortisol cut-off limit of 5 μg/dl. This may thus represent a simpler procedure than the DST in the diagnostic analysis of endogenous depression.

Introduction

The Dexamethasone Suppression Test (DST) currently represents the most widely used "biological marker" of major or endogenous depression (Carroll et al. 1981). About 50% of major or endogenous depressive patients exhibit an abnormal "escape" of cortisol secretion following the administration of dexamethasone. However, the specificity of the DST, which was described as very high in the initial studies of Carroll et al. (1981), remains a matter of controversy (Hirschfeld et al. 1983).

We recently suggested that levels of free (unbound) cortisol, the biologically active fraction of cortisol, could also be used as a reliable index of nonsuppression, with the possibility of recourse to the DST in all conditions where a total cortisol assay yields invalid results due to changes in protein levels as encountered in pregnancy or after the intake of oral contraceptives (Charles et al. 1986). We also demonstrated that saliva cortisol, which is directly related to plasma free cortisol, could be quite conveniently used in the DST (Ansseau et al. 1984).

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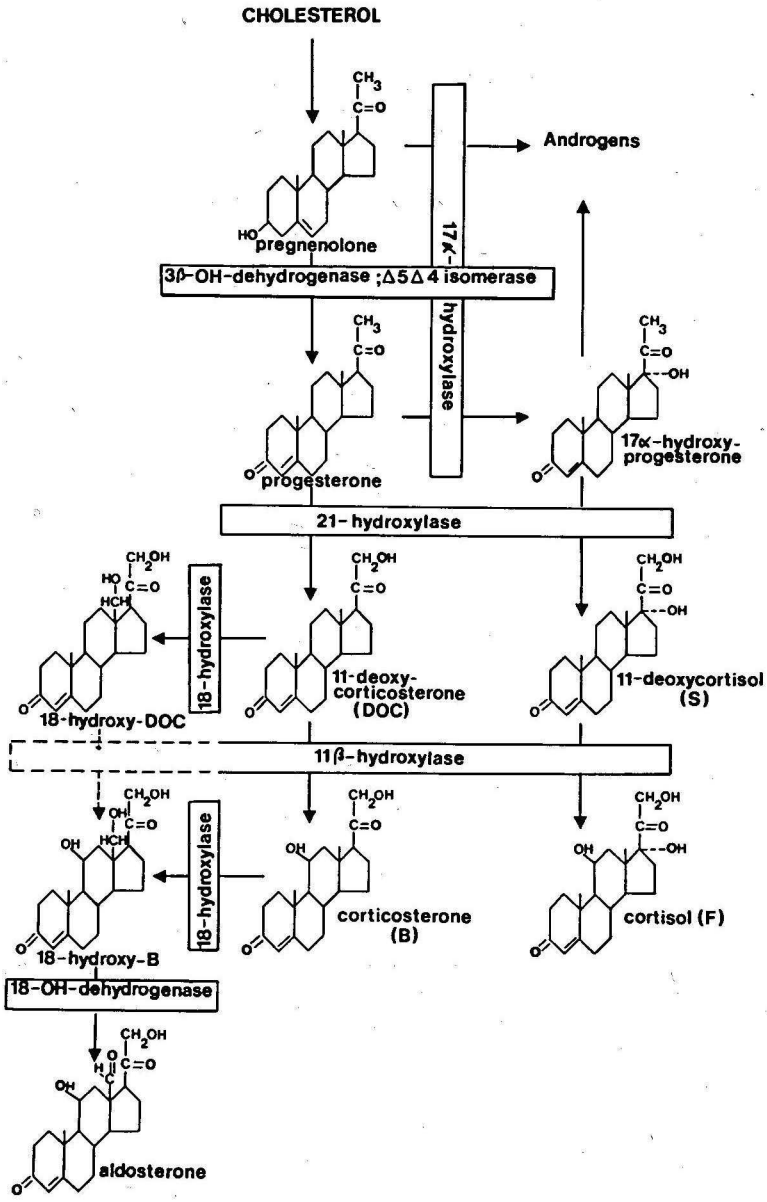

Figure 1. Main pathways of steroid biosynthesis. Aldosterone is mainly synthesized via corticosterone and 18-OH-corticosterone; the role of 18-OH-DOC as precursor is controversial (dotted lines).

We also tried to use 18-hydroxy-11-deoxycorticosterone (18-OH-DOC), another steroid not bound to plasma proteins, as a substitute for cortisol in the DST (Ansseau et al. 1985). However, the results of this study showed no relationship between cortisol and 18-OH-DOC levels following DST and a much lower diagnostic confidence for the 18-OH-DOC assay as compared with a cortisol assay. A possible explanation for these

negative findings can be found in the recent studies of Holsboer et al. (1982, 1983), which suggested that the activity of 11-beta-hydroxylase is significantly increased in endogenous depression. Holsboer measured the ratios of 11-beta-hydroxylated steroids to their corresponding non–11-beta-hydroxylated compounds as an indicator of enzyme activity, i.e., cortisol/11-deoxycortisol and corticosterone/11-deoxycorticosterone (DOC). We hypothesized that the discrepancy we observed between cortisol and 18-OH-DOC following dexamethasone could reflect higher levels of 11-beta-hydroxylated steroids as compared with non–11-beta-hydroxylated steroids. Indeed, 18-OH-DOC is a non–11-beta-hydroxylated steroid, in contrast to cortisol, which is 11-beta-hydroxylated (Figure 1). We therefore had two objectives in the present study: (1) to assess hypothalamo–pituitary–adrenal pathophysiological changes by means of the ratio of free cortisol to 18-OH-DOC in depressive patients, and (2) to perform a preliminary comparison of the diagnostic relevance of this ratio with the DST performed according to the methodology proposed by Carroll et al. (1981).

Methods

Subjects

The study involved 56 major depressive inpatients, endogenous subtype, representing consecutive admissions to the Biological Psychiatry and Psychopharmacology Unit of the University Hospital of Liège, Belgium. All diagnoses were made according to Research Diagnostic Criteria (RDC) (Spitzer et al. 1978) by two independent research psychiatrists without knowledge of laboratory results. The sample included 22 male and 34 female patients, with ages ranging from 22 to 71 years (mean age 46.5 ± 12.3 years). All patients had a score of at least 20 on the 21-item Hamilton Rating Scale for Depression (mean score ± SD, 29.8 ± 7.2). They were free of medical illness as evidenced by clinical examination, electrocardiogram (EKG), chest x-ray, electroencephalogram (EEG), and laboratory tests, and were tested after a drug-free period of at least 2 weeks.

The control group was composed of 22 normal volunteers (14 female and 8 male subjects), with ages ranging from 24 to 58 years (mean age 33.9 ± 8.5 years). All subjects were carefully screened for the absence of any personal psychopathological history of their own, or in first-degree relatives. Moreover, they were free of any medical illness and had not taken any drug during the previous month.

Before inclusion, all subjects were informed of the purpose of the study and gave their consent.

DST Procedure

The DST was performed according to the simplified procedure proposed by Carroll et al. (1981). A basal blood sample was collected at 8:00 AM on day 1, and oral dexamethasone (1 mg) was administered at 11:00 PM. A postdexamethasone sample was collected at 4:00 PM on day 2. The samples were immediately centrifuged and the plasma was stored at −20°C until analysis.

Assays

Plasma total cortisol concentrations were determined by radioimmunoassay (RIA) using ^{125}I-cortisol (Farmos Diagnostica, Finland) and anticortisol antiserum (made against the

Table 1. Percent Cross-Reaction of Various Steroids with Anti–18-OH-DOC Serum

Steroids	Percent cross-reaction
Progesterone	0.17
17-OH-progesterone	<0.001
11-Deoxycortisol	<0.001
Cortisone	<0.001
Testosterone	0.08
DOC	0.1
18-OH-DOC	100.0
Corticosterone	<0.001
Aldosterone	0.03
Cortisol	<0.001
18-OH-corticosterone	0.9
Prednisone	<0.001
Prednisolone	<0.001
Dexamethasone	<0.001

3-CMO-BSA conjugate), as previously described (Sulon et al. 1978), with intra- and interassay coefficients of variation of 4.3% and 8.3%, respectively. Free cortisol was measured by the equilibrium dialysis method, according to a previously described method (Demey-Ponsart et al. 1977), and 18-OH-DOC was measured by RIA (Sulon and Sparano, 1978). The antibody was highly specific for 18-OH-DOC, as assessed by cross-reactivity studies using other corticosteroids (Table 1). In particular, cortisol showed a cross-reaction below 0.001%. We tested cortisol levels up to 1 mg/dl, which did not interfere with 18-OH-DOC measurements. Intra- and interassay coefficients of variation were 3.9% and 10.0%, respectively, for steroid concentrations between 8 and 30 ng/dl, and the detection limit was 4 ng/dl. We previously demonstrated an excellent correlation between RIA and gas–liquid chromatography methods in plasma 18-OH-DOC determinations (Palem-Vliers and Sulon 1978).

Data Analysis

Mean steroid levels as well as steroid ratios were compared between endogenous depressives and normals using the Student's *t*-test. As steroid concentrations tended to be log-normally distributed, the data were analyzed by using a log transformation.

Steroid levels as well as steroid ratios were used to calculate diagnostic sensitivity, specificity, and confidence, according to the definitions of Vecchio (1966), with sensitivity referring to the proportion of endogenous depressives exhibiting abnormal results, specificity referring to the proportion of control subjects exhibiting normal results, and diagnostic confidence referring to the proportion of pathological results belonging to endogenous depressives.

Results

Pre- and postdexamethasone mean steroid levels and steroid ratios in endogenous depressives and normal controls are compared in Table 2. Before DST, endogenous depressive patients exhibited significantly higher levels of cortisol and free cortisol, as well

as higher ratios of free cortisol/18-OH-DOC than normal controls. After DST, endogenous depressives also presented significantly higher levels of total cortisol.

The distribution of individual cortisol levels following DST is displayed in Figure 2, and the distribution of the basal free cortisol/18-OH-DOC ratio is displayed in Figure 3. The diagnostic performance of various cut-off limits of total cortisol level following DST and of the basal free cortisol/18-OH-DOC ratio are presented in Tables 3 and 4. The best cortisol cut-off level seemed to be 5 μg/dl, yielding a sensitivity of 58.9%, a specificity of 90.9%, and a diagnostic confidence of 94.3%, whereas the best cut-off in the free cortisol/18-OH-DOC ratio appeared to be 40, yielding a sensitivity of 75%, a specificity of 95.5%, and a diagnostic confidence of 97.7%.

Using those two cut-offs, 53.6% of endogenous depressives exhibited two pathological results: 21.4% exhibited only an abnormal ratio and 5.4% only DST nonsuppression, whereas 19.6% yielded completely normal results. Among normal subjects, 86.4% had normal results on both parameters; 9.1% exhibited only DST nonsuppression, and 4.5% only abnormal steroid ratio. No normal subject presented both DST nonsuppression and abnormal steroid ratio.

Discussion

The results of this study show that the ratio of free cortisol/18-OH-DOC in a basal sample can be used with a diagnostic performance that is at least equivalent to the DST in the presence of endogenous depression. The main advantage of this procedure is the use of only one basal sample, without the need to administer an exogenous steroid.

The use of the ratio of free cortisol/18-OH-DOC yields better diagnostic power than the use of the level of a single steroid, either cortisol, free cortisol, or 18-OH-DOC. Indeed, even if significant differences existed between cortisol or free cortisol levels between depressives and normals in the basal sample, this difference yields poor diagnostic performance for individual results. For example, the best cut-off level of basal free cortisol (1000 ng/dl) gives a diagnostic sensitivity of only 62.5%, a specificity of 72.8%, and a confidence of 85.4%, as compared with 75%, 95.5%, and 97.7%, respectively, for the steroid ratio.

Our results confirm the hypothesis of Holsboer (1983) that multisteroid analysis can increase the diagnostic sensitivity of the DST. Holsboer et al. (1982, 1983) determined

Table 2. Comparison of Steroid Levels and Steroid Ratios before and after DST

	Endogenous depressives (n = 56)		Normal controls (n = 22)		t	p
8:00 AM before DST						
Total cortisol (μg/dl)	19.7	(6.9)[a]	14.0	(7.0)	10.9	<0.01
Free cortisol (ng/dl)	1443	(1067)	751	(417)	15.5	<0.001
18-OH-DOC (ng/dl)	26.2	(18.9)	28.9	(13.2)	0.8	NS
Free cortisol/18-OH-DOC	62.1	(31.9)	25.7	(9.6)	47.5	<0.0001
4:00 PM after DST						
Total cortisol (μg/dl)	7.8	(4.4)	2.6	(1.7)	19.0	<0.0001

[a]Standard deviation.

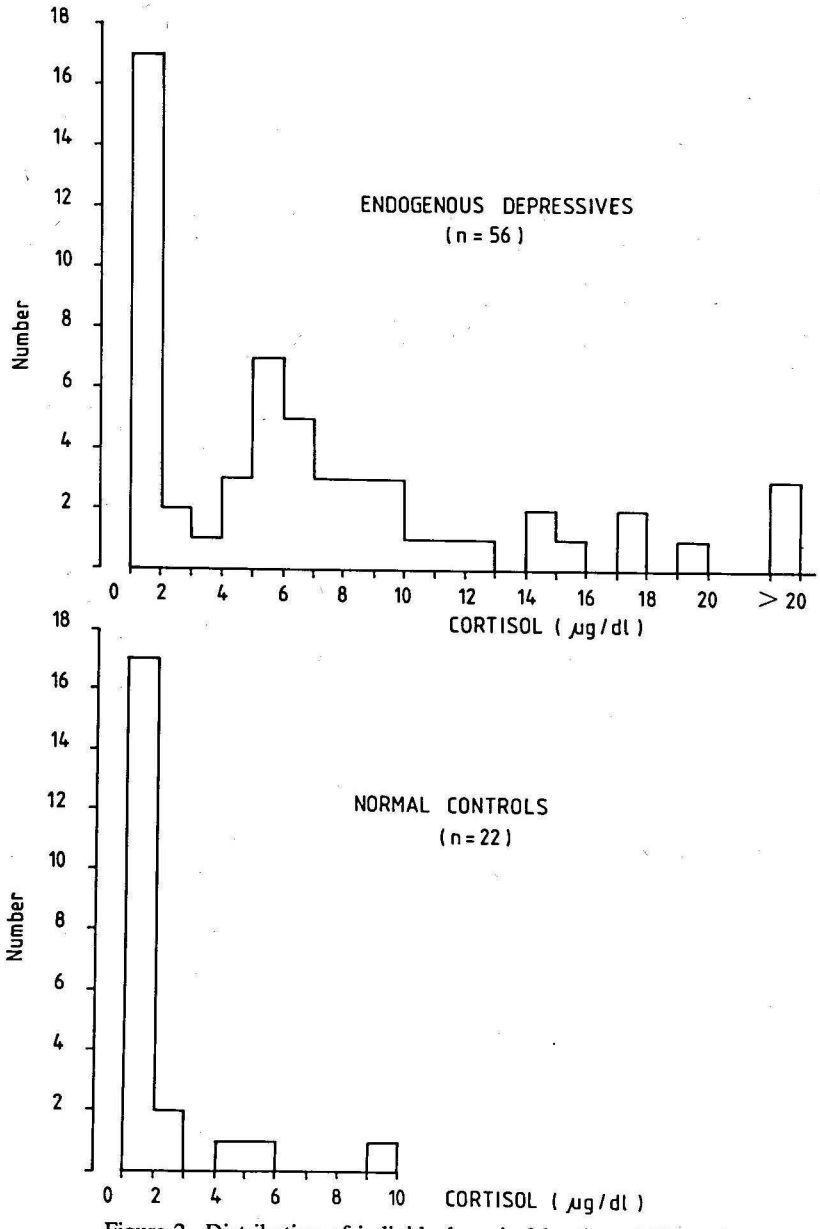

Figure 2. Distribution of individual cortisol levels at 4:00 PM following DST.

the concentrations of cortisol or corticosterone and their biosynthetic precursors 11-deoxycortisol or 11-deoxycorticosterone and found higher ratios in endogenous depressives than in normal controls, both before and after DST. However, the sample was only composed of six normal volunteers and six endogenous depressives, and the difference between the groups in the two baseline samples only reached statistical significance at 9:00 AM for the cortisol/11-deoxycortisol ratio and at 11:00 PM for the corticosterone/11-

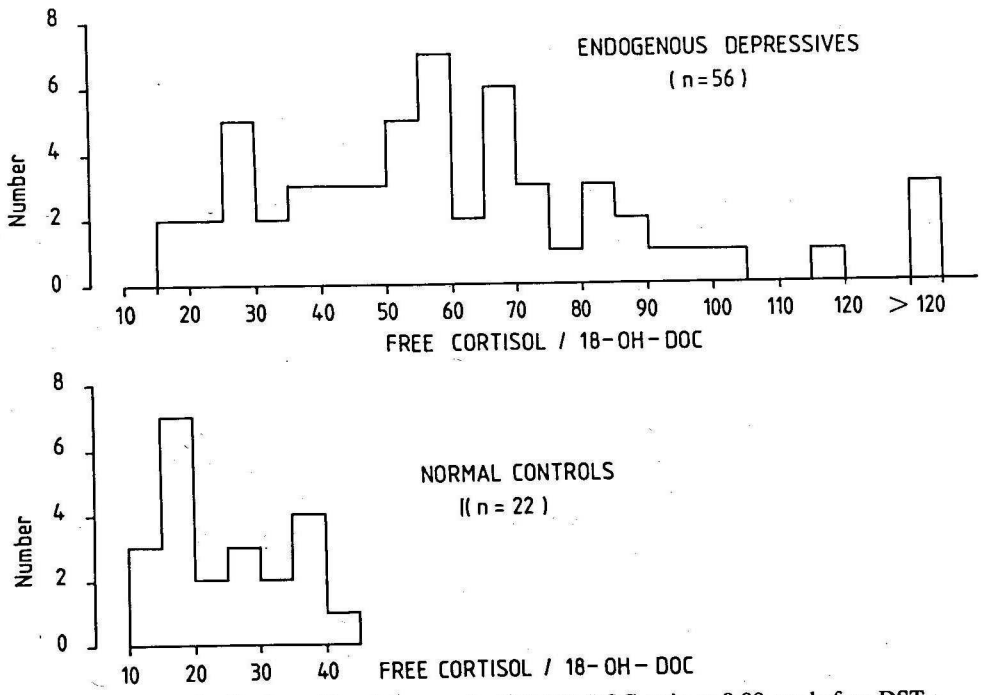

Figure 3. Distribution of basal free cortisol/18-OH-DOC ratio at 8:00 AM before DST.

Table 3. Diagnostic Performance of Cortisol Levels following DST for Endogenous Depression

Cut-off (μg/dl)	Sensitivity (%)	Specificity (%)	Diagnostic confidence (%)
3	66.1	86.4	92.5
4	64.3	86.4	92.3
5	58.9	90.9	94.3
6	46.4	95.5	96.3

Table 4. Diagnostic Performance of Basal Free Cortisol/18-OH-DOC Ratio for Endogenous Depression

Cut-off	Sensitivity (%)	Specificity (%)	Diagnostic confidence (%)
30	83.9	68.2	87.0
35	80.4	77.3	90.0
40	75.0	95.5	97.7
45	69.6	100.0	100.0
50	64.3	100.0	100.0
55	55.3	100.0	100.0
60	42.9	100.0	100.0

deoxycorticosterone ratio. Holsboer interpreted these results as indirect evidence for increased 11-beta-hydroxylase activity in endogenous depression. Our current results could support this hypothesis, as cortisol is a 11-beta-hydroxylated steroid, whereas 18-OH-DOC is a non–11-beta-hydroxylated steroid. However, it should be kept in mind that there is still uncertainty as to whether or not the comparison of the substrate to end-product from an individual pathway is a reflection of 11-beta-hydroxylase activity, to say nothing of trying to generalize this to steroids measured from two different pathways, such as cortisol and 18-OH-DOC.

Increased levels of 11-beta-hydroxylated steroids as compared to non–11-beta-hydroxylated steroids in endogenous depression could reflect higher adrenocorticotrophin hormone (ACTH) levels, as it has been demonstrated that 11-beta-hydroxylase activity is stimulated by ACTH (Gower and Cooke 1983). However, the main activity of ACTH at the level of steroid synthesis is a stimulation of the cleavage of cholesterol to form pregnenolone (reviewed in Riad-Fahmy et al. 1979). Moreover, although there is a reported increase in 11-beta-hydroxylase activity in response to ACTH in vitro, the same has not been shown in vivo. Studies on ACTH levels in depression have yielded controversial results. Several authors have shown higher ACTH levels in major depressive patients than in normal subjects (Kalin et al. 1982; Reus et al. 1982; Holsboer et al. 1984; Pfohl et al. 1985), whereas other studies failed to find such difference (Fang et al. 1981; Yerevanian and Woolf 1983). These discrepancies may be related to the complicated relationship between ACTH and cortisol: both ACTH and cortisol tend to be secreted in bursts, and studies that take only a single isolated blood sample may miss a major peak in plasma ACTH (Krieger and Allen 1985). The cortisol level at any given moment may not be as strongly related to the ACTH level at that instant as it is to the pattern of ACTH over the previous hour. We are currently assessing the relationships among ACTH, various steroids or steroid ratios, and endogenous depression.

The selection of 18-OH-DOC in our steroid ratio may seem somewhat questionable. The origin and role of this hormone are still debated. In humans, 18-OH-DOC has very little mineralocorticoid activity, and its secretion is not stimulated by the renin angiotensin system but by ACTH (May et al. 1979). Despite its chemical similarity with 18-OH-corticosterone, 18-OH-DOC is not a major precursor of aldosterone, as it is mainly found in the zona fasciculata, which lacks the final oxydoreductase necessary to aldosterone synthesis (Gower and Cooke 1983). Interestingly, 18-OH-DOC is not bound to specific plasma proteins. Therefore, its level is not influenced by corticosteroid binding globulin (CBG) level, which can vary in various conditions, particularly during pregnancy or with the intake of oral contraceptives.

To establish our ratio, we use free cortisol as the 11-beta-hydroxylated steroid. Free cortisol represents the biologically active fraction of cortisol. Cortisol, corticosterone, DOC, and 11-deoxycortisol compete for the same binding sites with comparable affinities. Thus, the unbound fraction of each of these hormones becomes difficult to predict without specific assay. Therefore, the selection of two unbound steroids could represent an advantage over the methodology of Holsboer (1983), who used total level (bound and unbound) of 11-beta-hydroxylated as well as non–11-beta-hydroxylated steroids.

In conclusion, the determination of the ratio of free cortisol/18-OH-DOC could present an interesting dimension in the biological analysis of endogenous depression. Indeed, with a methodology more simple than the DST, it yields at least a similar diagnostic performance. However, its actual diagnostic significance needs to be tested in a larger study including endogenous as well as nonendogenous depressive patients.

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References

Ansseau M, Sulon J, Doumont A, Cerfontaine JL, Legros JJ, Sodoyez JC, Demey-Ponsart E (1984): Use of saliva cortisol in the Dexamethasone Suppression Test. *Psychiatry Res* 13:203–211.

Ansseau M, Simon C, Sulon J, Demey-Ponsart E, von Frenckell R, Geenen V, Legros JJ, Van Cauwenberge H, Franck G (1985): 18-Hydroxy-11-deoxycorticosterone (18-OH-DOC) and Dexamethasone Suppression Test. Paper presented at the IVth World Congress of Biological Psychiatry, Philadelphia, September 1985.

Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Hasket RF, James NM, Kronfol Z, Lohr N, Steiner M, De Vigne JP, Young E (1981): A specific laboratory test for the diagnosis of melancholia. *Arch Gen Psychiatry* 38:15–22.

Charles G, Ansseau M, Sulon J, Demey-Ponsart E, Meunier J-C, Wilmotte J, Legros JJ (1986): Free cortisol and the Dexamethasone Suppression Test. *Biol Psychiatry* 21:549–552.

Demey-Ponsart E, Foidart JM, Hendrickx JC, Sodoyez JC (1977): Effect of serum dilution on binding of cortisol to thermolabile and thermostable serum proteins. *J Steroid Biochem* 8:1091–1095.

Fang VS, Tricou BJ, Robertson A, Meltzer HY (1981): Plasma ACTH and cortisol levels in depressed patients: Relation to dexamethasone suppression test. *Life Sci* 29:931–938.

Gower DB, Cooke GM (1983): Regulation of steroid transforming enzymes by endogenous steroids. *J Steroid Biochem* 19:1527–1556.

Hirschfeld RMA, Koslow SH, Kupfer DJ (1983): The clinical utility of the Dexamethasone Suppression Test in psychiatry: Summary of a National Institute of Mental Health workshop. *JAMA* 250:2172–2174.

Holsboer F (1983): The Dexamethasone Suppression Test in depressed patients: Clinical and biochemical aspects. *J Steroid Biochem* 19:251–257.

Holsboer F, Winter K, Dorr HG, Sippell WG (1982): Dexamethasone Suppression Test in female patients with endogenous depression: Determination of plasma corticosterone, 11-deoxycorticosterone, 11-deoxycortisol, cortisol and cortisone. *Psychoneuroendocrinology* 7:329–338.

Holsboer F, Doerr HG, Gerken A, Muller OA, Sippell WG (1984): Cortisol, 11-deoxycortisol and ACTH concentrations after dexamethasone in depressed patients and healthy volunteers. *Psychiatry Res* 11:15–23.

Kalin NH, Weiler SJ, Shelton SE (1982): Plasma ACTH and cortisol concentrations before and after dexamethasone. *Psychiatry Res* 7:87–92.

Krieger DT, Allen W (1975): Relationship of bioassayable and immunoassayable plasma ACTH and cortisol concentrations in normal subjects and in patients with Cushing's disease. *J Clin Endocrinol Metab* 40:675–687.

May CN, Lewis PS, Horth CE (1979): Radioimmunoassay of plasma 18 hydroxy-11-deoxycorticosterone and its response to ACTH. *Clin Endocrinol* 11:399–412.

Palem-Vliers M, Sulon J (1978): Estimation of 18-hydroxy-11-deoxycorticosterone in human plasma and urine including a comparison of various techniques. *J Steroid Biochem* 9:239–243.

Pfohl B, Sherman B, Schlechte J, Winokur G (1985): Differences in plasma ACTH and cortisol between depressed patients and normal controls. *Biol Psychiatry* 20:1055–1072.

Reus VI, Joseph MS, Dallman MF (1982): ACTH levels after the Dexamethasone Suppression Test in depression. *N Engl J Med* 306:283–284.

Riad-Fahmy D, Read G, Hugues A (1979): Corticosteroids. In Gray CH, James VHT (eds), *Hormones in Blood*, vol. 3. London: Academic Press, pp 179–262.

Roy AK, Ramirez LC, Ulick S (1976): Structure and mechanism of formation of the two forms of 18-hydroxy-11-deoxycorticosterone. *J Steroid Biochem* 7:81–87.

Spitzer R, Endicott J, Robins E (1978): Research Diagnostic Criteria: Rationale and reliability. *Arch Gen Psychiatry* 35:773–782.

Sulon J, Sparano F (1978): A non-chromatographic radioimmunoassay of 18-hydroxy-11-deoxy-corticosterone in human plasma. *J Steroid Biochem* 9:253–256.

Sulon J, Demey-Ponsart E, Bauduin E, Sodoyez JC (1978): Radioimmunoassay of corticosterone, cortisone and cortisol. Their application to human cord and maternal plasma. *J Steroid Biochem* 9:671–676.

Vecchio TJ (1966): Predictive value of a single diagnostic test in an unselected population. *N Engl J Med* 274:1171–1173.

Yerevanian BI, Woolf PD (1983): Plasma ACTH levels in primary depression: Relationship to the 24-hour Dexamethasone Suppression Test. *Psychiatry Res* 12:155–160.