The Diagnosis and Management of Cushing's Disease

University of Texas Southwestern Medical Center
Internal Medicine Grand Rounds
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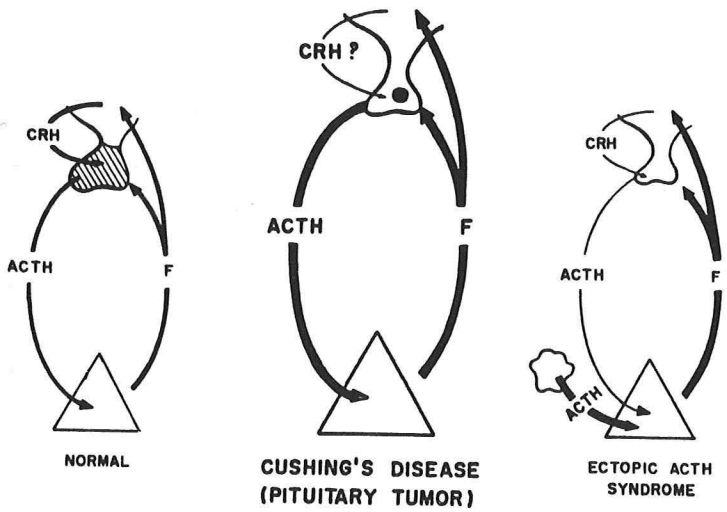

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Dr. McPhaul's interests have centered on the mechanism of actions of steroid hormones. In the laboratory, this focus has been to study how genetic alterations of the protein that mediates the actions of the male hormones (the androgen receptor) contribute to defects of male development and to the clinical behavior of human prostate cancer. His clinical interests have been in the diagnosis and management of diseases of the pituitary, including Cushing's disease.

The clinical picture of a patient with florid Cushing's syndrome is not difficult to recognize. The combination of recent weight gain, weakness, bruisability, and striae in a previously healthy patient would elicit the tentative diagnosis of Cushing's syndrome from most physicians. The problem is that the same clinical symptoms and signs are extremely common in the normal human population and are not at all specific for Cushing's syndrome.

Series Of Patients With Cushing's Syndrome And Or Disease

Series:	Plotz	Soffer	Ross	Urbanic	Ross
n =	33	50	50	46	70
	(1952)	(1961)	(1966)	(1981)	(1982)
Symptom					
Obesity	97	86	47	79	97
Hirsuitism	73	---	41	64	81
Hypertension	84	84	---	77	74
Striae	60	50	---	51	56
Plethora	89	78	---	---	94
Mental symptoms	67	40	20	48	62

Data derived from the series of patients with Cushing's syndrome described in references (49), (60), (51), and (52), and in a series of patients with Cushing's disease described in reference (71). In this latter series (71), striae were more common in younger patients, while muscle weakness and osteopenia were more frequently a component of the picture of older patients.

DISCRIMINANT INDICES OF CLINICAL FEATURES IN CUSHING'S SYNDROME

Clinical feature	Discriminant index in series	
	Present	Collected
Bruising	10·3	10·5
Myopathy	8·0	7·1
Hypertension	4·4	5·1
Plethora	3·0	3·6
Oedema	2·9	3·3
Hirsutism in women	2·8	2·7
Red striae	2·5	3·1
Menstrual irregularity	1·6	1·6
Truncal obesity	1·6	..
Headaches	1·3	1·1
Acne	0·9	..
Generalised obesity	0·8	..
Impaired glucose tolerance	0·7	0·7

In the series of Ross and Linch (52), the discriminant power of selected clinical features were estimated by comparing the frequency in a group of obese individuals without Cushing's syndrome. These comparisons suggested that a few signs had some discriminating value, particularly bruising, myopathy, hypertension, plethora, edema, hirsuiitism, striae.

States Characterized By An Excess Production Of Cortisol

The production of cortisol is a process that is tightly regulated in normal individuals. Several disease states have been described that are characterized by an abnormal high production of cortisol. The clinical condition that results - Cushing's syndrome - includes a host of diseases that can be traced to abnormalities at a number of levels within the hypothalamic -pituitary-adrenal (HPA) axis. The most common causes of Cushing's syndrome (other than that induced by the administration of potent corticosteroid medications by physicians) are tumors of the pituitary that secrete biologically active forms of ACTH. Less frequently, the pathogenesis of similar clinical pictures have been traced to autonomous production of cortisol by adrenal tumors, to the ectopic

secretion of biologically active ACTH by benign and malignant tumors, and (rarely) to the ectopic secretion of Corticotropin Releasing Hormone (CRH).

Classification of hypercortisolism

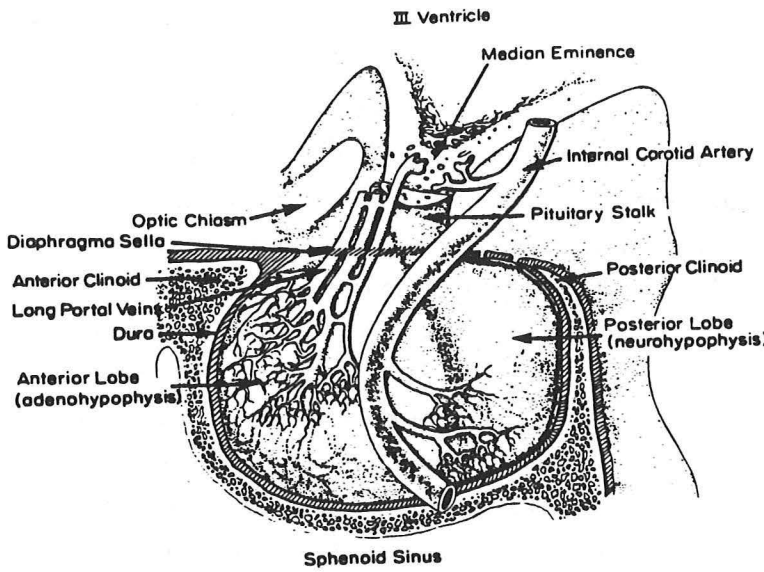
Physiologic states
Chronic stress
Pregnancy (last trimester)
Chronic strenuous exercise
Malnutrition
Pathophysiologic states
Cushing's syndrome
Endogenous
Exogenous
Psychiatric states
Melancholic depression
Obsessive-compulsive disorder
Chronic alcoholism
Panic disorder
Anorexia nervosa
Alcohol and narcotic withdrawal
Abdominal obesity
Glucocorticoid resistance

The precise incidence of these different entities varies somewhat from series to series. In all, however, the incidence of Cushing's disease predominates, accounting for some where in the neighborhood of 60-70% of all cases. It important to recognize that the vastly different therapies required for the correct treatment of different forms of Cushing's syndrome require that the correct diagnosis be rendered.

Components of the Adrenal Axis

The control of the adrenal glands begins with neurons located within specific regions of the hypothalamus (paraventricular nucleus). Cells arising in this and other areas of the brain project to the median eminence. The polypeptide hormone that is synthesized by these cells - corticotropin releasing

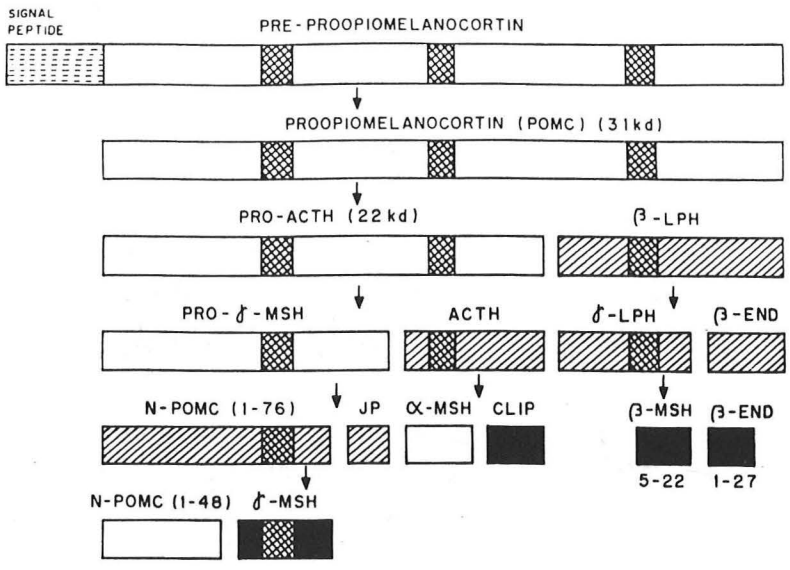
hormone (CRH) - is carried from synaptic terminals in the median eminence to the anterior pituitary by the long portal vessels.



Schematic representation of the human pituitary gland in relation to its surrounding structures. Refer to text for detailed description.

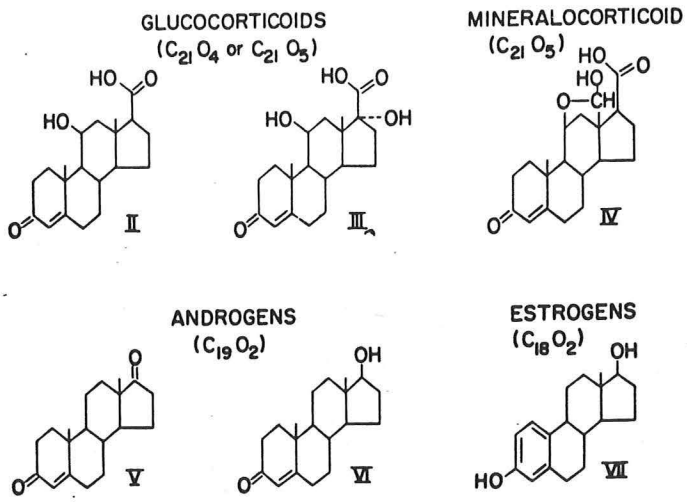
Corticocotropes, the cells of the anterior pituitary that synthesize and secrete adenocorticotropin or ACTH, are the targets of CRH action, and comprise approximately 10% of the cell population of the anterior pituitary. CRH binds to a specific CRH receptor molecule **Figure 3** expressed on the surface of these cells. This binding event stimulates the production of second messengers via G-protein-coupled pathways and results in the release of preformed ACTH, as well as stimulating the synthesis of the mRNA encoding the ACTH polypeptide.

The definition of the complexities of the biosynthesis and processing of ACTH represents one of the triumphs of modern endocrinology. Studies conducted over many years and using material from animals, humans and from cell lines demonstrated that the ACTH molecule is a single chain polypeptide hormone that is derived from a larger precursor by a series of processing events (2a). While many pituitary hormones are derived from larger precursors, few exhibit the diversity and complexity that has been described for ACTH. The 241 amino acid **P**ro**o**pio**m**elano**c**ortin (**POMC**) peptide contains five sets of repeated basic amino acid residues that represent potential sites at which proteolytic processing can take place. In fact, the exact POMC-derived peptides that are produced by a given cell depends on the specific processing enzymes (prohormone convertases [PC's]) that are expressed (2, 55, 56). In the corticotrope, only PC1 is present , leading to the production of four principal cleavage products. In other regions of the brain (e.g. the pars intermedia) additional PC's are expressed and additional cleavage products are secreted. Although biological activities have been described for many of the POMC peptides, a clear physiological role has not been defined for any except ACTH.



Posttranslational processing of proopiomelanocortin (POMC) in normal pituitary, pituitary and ectopic ACTH-secreting tumors. Hatched areas indicate peptides more abundant in the pituitary; cross-hatched areas indicate MSH sequences and black areas represent "abnormal" fragment markers of ectopic ACTH syndrome. (Adapted from D. Vieau *et al.* [46] with permission of the publishers, and from A. C. Hale *et al.* [45] with permission of the authors and publishers).

ACTH circulates through the peripheral blood to the cells of the adrenal glands where it binds to specific cell surface receptors (8). The binding of ACTH to these receptor molecules stimulates the production of cyclic AMP. As the result of this signal, the genes encoding the enzymes catalyzing the synthesis of steroid hormones are stimulated, as well as the mechanisms controlling the uptake and transport of cholesterol into the steroid synthesizing cells. During normal physiologic states, the adrenal glands of men and women produce approximately 20 mg of steroid hormone each day. These hormones fall into three general classes: the adrenal androgens, the mineralocorticoids, and the glucocorticoids. Each of these hormones possesses certain chemical properties that confer upon each the particular biological properties possessed. The amount of cortisol that is secreted daily varies somewhat depending on the technique employed to measure it. Careful studies of cortisol production rate using isotopic dilution indicated the at normal adults produce approximately 10 milligrams of cortisol daily (8).

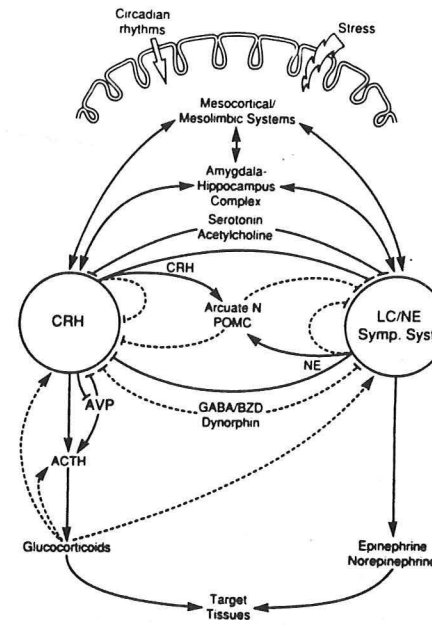


The effects of glucocorticoids are effected via the glucocorticoid receptor, a member of a very large family of ligand-regulated transcription factors (36). The glucocorticoid receptor, like many members of this family, contains discrete domains that mediate the binding of the cortisol ligand and the binding of the activated receptor to specific target DNA sequences within the genome of responsive cells. The binding of cortisol to the ligand binding domain results in the activation of the receptor, the acquisition of the capacity of the receptor to bind to its target DNA, and the modulation of responsive genes.

Control of the Adrenal Axis

The components described above are regulated in a fashion that permits physiologic changes to be reflected against a well-tuned the backdrop. The CRH secreting neurons display a diurnal rhythmnicity, with peak activity occurring in the early morning hours each day. This activity results in an early morning rise of ACTH levels and the production of cortisol that results in maximal levels at approximately 8-9 am each day. By afternoon, serum cortisol

levels normally fall to a fraction of their early morning levels. In addition to the influences responsible for the coordination of the normal diurnal variation, cortisol levels are greatly influenced by a number of states. Any stress if severe enough can give rise to a neural discharge that results tin the stimulation of the HPA axis.



A simplified, heuristic representation of the central and peripheral components of the stress system, their functional interrelations, and their relationships to other central nervous systems involved in the stress response. The corticotropin-releasing hormone (CRH) in the paraventricular nucleus and the centers of the arousal and autonomic systems in the brain stem represent major components of this system connected anatomically and functionally to each other (see text). LC/NE Symp. Syst., = locus ceruleus/norepinephrine-sympathetic system, POMC = proopiomelanocortin, AVP = arginine vasopressin, GABA = γ-aminobutyric acid, BZD = benzodiazepine, ACTH = corticotropin. (*Adapted from* Chrousos GP, Gold PW: The concepts of stress and stress system disorders. JAMA 267: 1244–1252, 1992; with permission.)

Etiology Of Cushing's Disease

For many years, the etiology of Cushing's disease was debated and abnormalities at both the hypothalamic and pituitary levels were considered as potential sites at which derangements could arise. Studies using markers of clonality (e.g. markers of X-chromosome inactivation) established that the pituitary tumors secreting ACTH are monoclonal in nature, eliminating mechanisms in which the abnormality is at the hypothalamic level (3). While little definitive progress has been made in elucidating the pathogenesis of ACTH-secreting pituitary tumors, findings in other pituitary tumor types -

particularly those secreting growth hormone (27) - suggest that genetic alterations that modify the capacity of cortisol to effect feedback at the level of pituitary and hypothalamus to suppress the production of ACTH and CRH will be involve.

Assays

The tests available to diagnose Cushing's syndrome and to identify the level in the HPA that is abnormal have undergone -- and are continuing to undergo -- considerable change.

Measurements / Estimates Of Cortisol Production

17 hydroxycorticosteroids

Much of the early literature measuring the production rates of cortisol employed indirect assessment of cortisol and its metabolites. One such type of assay was the Porter - Silber reaction which measured the colored products of the reaction between phenylhydrazine and corticosteroids containing 17,21-dihydroxy - 20 ketosteroids (59). This reaction measures approximately 50% of the extractable cortisol metabolites in the urine of normal subjects (18).

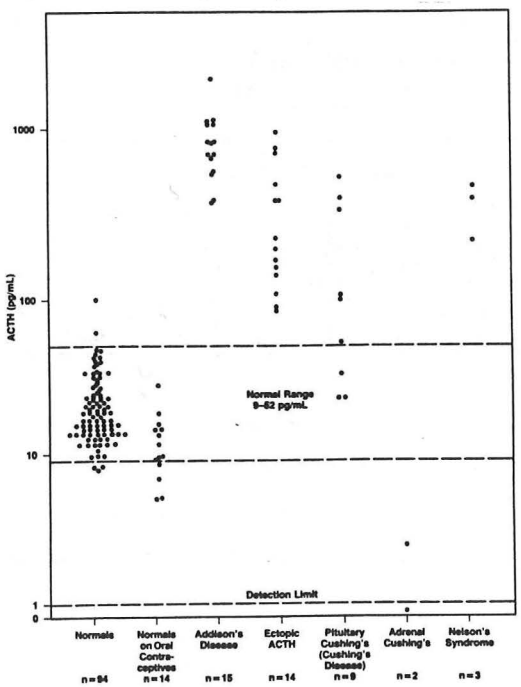
Urinary Free Cortisol

The Porter - Silber reaction measures only a proportion of cortisol metabolites, owing to the fact that the specificity of the reaction rests on a particular constellation of functional groups within the molecule. For this reason, a variety of techniques were developed to measure the quantity of cortisol excreted more precisely.

The first assays of this sort involved the extraction and concentration of urine, followed by the measurement of cortisol using an RIA (using specific antibodies directed at the cortisol molecule) or competitive binding assays. In more recent years, increasingly sensitive and specific assays have been developed to measure the amount of cortisol in specimens of urine using physically based methods. The precision of the more recently developed methods is remarkable. Many of the larger reference laboratories now routinely employ HPLC methodology to measure the levels of urinary free cortisol.

ACTH Assays

The initial assays that were commercially available did not display the sensitivity necessary to measure the low levels necessary to discriminate between 'normal' and suppressed levels of ACTH. The development of more sensitive RIAs and emergence of two antibody 'sandwich' assays, has made assays with the requisite sensitivity widely available.



Assays, the 1990's, and Managed Care

The preceding discussion was focused on the technical details of the assays that are commonly employed in the diagnostic evaluation ofd patients with possible Cushing's syndrome. A note of caution is in order with respect to the application of the commercially available tests. First, all tests are not created equal. An example of this 'non equivalence is shown below, a summary of laboratory values that were obtained in course of an evaluation of a patient subsequently shown to be normal. Note that the tests done by two laboratories were UFC measurements and report values for UFC that are quite similar. Second, is that the values reported by one laboratory are approximately twice those of the other laboratories. This is because the test that was performed by this laboratory was an RIA that performed on extracted urine. This dichotomy of available testing continues to characterize the tests measuring UFC available through many reference laboratories.

		Testing Laboratory/Methodology						
	Urine 24h T.V., mL	APL/FPIA	MML/HPLC			CNI/RIA	NHL/RIA	SKB/HPLC
Specimen Date		*[UFC], µg/24 h*						
			UFC	Cortisone, µg/24 h	Dexamethasone, µg/24 h			
7-16-95	1,950	128	71	222	n.d.	159.9	126.8	66
7-17-95	900	85	40	117	n.d.	119.7	99.9	38
7-18-95	2,725	51	17	89	21	57.2	38.2	6
7-19-95	1,200	24	6.5	42	20	25.2	25.2	<5
7-20-95	1,800	21	1.4	17	83	18.0	5.4	<5
7-21-95	1,825	18	n.d.	9.5	91	18.8	14.6	<5
Reference Interval		0-50	5-55	n.a.	n.a.	20-90	10-80	5-47

These considerations are particularly important when one considers the complex game of 'musical chairs' that may characterizes the patient-doctor-laboratory relationships in the current managed care environment. Knowing what assays have been employed and being aware of any changes that take

place in the reference laboratory(ies) that are used may be of major importance to the interpretation of lab results.

Diagnostic Evaluation

The tests performed in the evaluation of a patient with suspected Cushing's syndrome fall into two general groups: 1) those designed to establish the presence of excessive cortisol secretion and 2) those designed to determine the etiology of the cortisol overproduction.

Diagnostic Evaluation - Establishing That Cushing's Syndrome Is Present

Two principal tests are employed to establish the diagnosis of Cushing's syndrome: estimates of 24 hour cortisol production and tests to demonstrate the normal suppression of the HPA axis in response to supra-physiological glucocorticoid doses (or lack thereof).

Daily Urine-Free Cortisol Excretion*

Normal Controls	Obese Controls	Other Controls†	Cushing's Syndrome	Urine-Free Cortisol Upper Limit of Normal‡	Reference
69/70	—	—	2/14	100	21
8/8	—	—	0/11	50	40
—	—	—	4/21	80	8
14/14	20/24	—	0/19	50	9
38/38	5/5	—	0/9	181	25
12/12	4/4	21/28	0/11	20	36
—	—	—	0/11	80	37
13/13	—	—	0/10	98	41
23/23	28/28	53/53	0/14	108	22
10/10	—	—	1/20	89	11
22/22	9/10	—	2/9	50	12
34/34	—	29/32	0/39	95	39
—	15/15	—	0/24	108	31
18/18	18/18	—	5/21	75	32, 33
—	—	—	0/15	100	35
Total (%) 261/262 (99)	99/104 (95)	103/113 (91)	14/248 (5.6)		

*The data are expressed as *n/N* where *n* is the number of patients whose 24-hr urine-free cortisol excretion determinations are less than or equal to the specified upper limit of normal, and *N* is the total number of patients.

†Other controls include subjects suspected of having Cushing's syndrome and patients with chronic disorders. The following patients had elevations of the daily urine-free cortisol excretion:[41] 4/10 acutely-ill medical patients, 6/6 pregnant women, 4/15 well preoperative patients, and 8/11 postoperative patients.

‡Values listed are the upper limits of normal for 24-hr urine-free cortisol excretion determinations in μg/day.

The data presented above is a compilation of data from 15 series compiled by Lawrence Crapo (9) in which the utility of measurements of urinary free cortisol (UFC) was examined. As a group, the studies included 262 normal subjects, 104 obese individuals, and 248 patients with Cushing's syndrome. The application of these methods to these patients with Cushing's resulted in a false negative of 5.6%. Note that this type of test has a false negative rate of ~1% and ~5% when applied to normal and obese subjects, respectively. For comparison purposes, a summary of results from 14 series using 17 - hydroxycorticosteroids is shown.

The second screening test that is commonly employed for screening purposes is some form of dexamethasone suppression test. These tests have quite different designs, but are aimed to the same goal: to discriminate between individuals showing marginal or equivocal elevations of UFC. These tests are predicated on the premise that normal subjects will suppress and that patients with true Cushing's will not.

Single-Dose Overnight Dexamethasone Suppression Test*

Normal Controls	Obese Controls†	Other Controls†	Cushing's Syndrome	Cortisol Assay‡	Morning Cortisol Upper Limit of Normal (μg/100 ml)	Reference
119/120	19/19	—	0/9	PS	10	82
16/16	20/20	10/12	0/17	PS	5	83
44/44	33/40	—	1/10	DID	5	84
30/30	18/18	33/42	0/3	F	10	85
16/16	16/17	13/15	0/9	F	6	86
39/39	—	45/71	—	F	7	87
72/76	—	—	1/6	PS	10	2§
31/31	20/21	24/26	0/5	F	4.5	88
7/7	2/2	—	0/20	PS	4	30
50/50	7/15	—	1/24	F	7	31
37/37	16/21	33/40	0/5	CPB	3.5	89
—	—	—	0/13	F	3.8	13
—	—	88/114	0/33	F	6	90
Total (%) 461/466 (99)	151/173 (87)	246/320 (77)	3/154 (1.9)			

*The data are expressed as n/N where n is the number of subjects whose morning (8–9 a.m.) plasma cortisol levels are less than or equal to the indicated upper limit of normal following 1 mg (2 mg[85] and 1.5 mg[86]) of oral dexamethasone at 11 p.m. or 12 midnight, and N is the total number of patients in each designated category.

†Obese controls include subjects with generalized and central obesity. Other controls include hospitalized and nonhospitalized patients with nonacute illnesses, except in reference 12 where other controls include normal and obese subjects and subjects with nonacute illnesses.

‡Plasma cortisol assay methods: PS, Porter-Silber colorimetric reactions; DID, double-isotope derivative; F, fluorimetric reaction; CPB, competitive protein-binding. Details of these plasma cortisol assays are discussed in the text.

§Reference 2 is a review of the literature combined with a presentation of the authors' own data. In this and other tables only the authors' data is tabulated.

The overnight dexamethasone suppression test (45) employs the administration of 1 mg of dexamethasone at midnight followed by a measurement of serum cortisol at 8 AM. This test has several attractive features. First, only about 2% of Cushing's disease will suppress into the normal range. In addition, this test has a low rate of false positive results when applied to normal control subjects. As summarized by Crapo (9), this test has a relatively high false positive rate when applied to obese subjects. Furthermore, although each of the studies uses cutoffs that are internally consistent, comparison of the results of the studies to each other is hampered by the wide variations of the upper limit of normal that was defined for each study. Most authors use a cutoff of 5 mcg /dL to delimit those that show suppression (and are therefore normal) from those that do not (and that therefore have Cushing's

Low-Dose 2-mg Dexamethasone Suppression Tests*

	Non-Cushing's Controls	Cushing's Syndrome	Urine Corticosteroid†	24-hr Urine Upper Limit of Normal (mg/day)	Reference
	133/133	1/35	17-OHCS	4.0	24
	12/12	1/13	17-OHCS	4.0	9
	—	0/14	17-OHCS	4.0	28
	15/15	2/6	17-OHCS	4.0	2
	32/32	3/17	17-OHCS	4.0	12
	15/15	0/24	17-OHCS	4.0	31
Total (%)	207/207 (100)	7/109 (6.4)			
	—	0/14	17-KGS	3.0	37
	25/25	0/25	17-KGS	7.0	101
	10/18	0/24	17-KGS	5.0	39
	15/15	0/24	17-KGS	7.0	31
	4/4	0/14	17-KGS	3.4	32, 33
	—	0/15	17-KGS	3.7	13
Total (%)	54/62 (87)	0/116 (0)			
	18/19	2/21	UFC	0.025	39
	15/15	0/24	UFC	0.019	31
Total (%)	33/34 (97)	2/45 (4.4)			

*The data are expressed as *n/N* where *n* is the number of patients whose daily urine corticosteroid excretion determinations are less than or equal to the designated upper limit of normal on the second day (third day in references 32 and 33) of 2-mg/day oral dexamethasone administration, and *N* is the total number of patients.

†The corticosteroids are designated as follows: 17-OHCS, 17-hydroxycorticosteroids determined as Porter-Silber chromogens; 17-KGS, 17-ketogenic steroids; UFC, urine-free cortisol.

The low-dose dexamethasone suppression test is a somewhat more complicated protocol, but one that is designed to accomplish the same goals that is approached by the overnight dexamethasone test. The patients that suppress below a specific level are considered normal. Those that do not are considered to have Cushing's disease. This protocol was a part of the 'full' dexamethasone suppression test that was described by Liddle in 1960 (30), and is often performed in conjunction with the 'high-dose' dexamethasone suppression test (see below). This test does not have to be used that way and criteria used for its interpretation are equally valid when performed alone as when performed as a part of a 'full' dexamethasone suppression test.

Variations, Problems, And Pitfalls Diagnosis Of Cushing's Syndrome

Pseudo-Cushing's

Several situations pose particular difficulties in the diagnosis of Cushing's syndrome. In Cushing's syndrome, the excess cortisol feeds back to inhibit the production of CRH in the hypothalamus and suppresses ACTH secretion by the normal corticotrophs. In alcoholics and in some patients with depression, excess cortisol production appears to mediated centrally. That is, the CRH secreting neurons of the hypothalamus appear overactive, but in the context of a pituitary-adrenal axis that is otherwise restrained by the normal feedback mechanisms (20).

Artefactual Nonsuppression

Although suppression test are useful in screening for the presence of Cushing's syndrome, one should not be dissuaded from the diagnosis in

cases in which the diagnosis is strongly suggested on clinical grounds. Drugs interactions, such as has been observed with rifampicin (25), should be among the first explanations considered when the results of suppression tests are not congruent with the clinical picture.

Cyclic Cushing's

One of the most interesting forms of Cushing's syndrome is cyclic or periodic Cushing's syndrome (53,72). This entity is characterized by the intermittent production of excess cortisol and may be caused . Some individuals demonstrate increased production of cortisol at all time with periodic elevations. Such individuals pose minimal diagnostic difficulty, as although the results of routine screens (such as a 24 hour UFC) may vary, all will be abnormal. Considerably more problematic are those patients who have UFC measurements that well within the normal range between peaks of cortisol excretion. In the face of a strong clinical suspicion, repeated measurements of UFC may be required to document the periods of excess cortisol production.

Diagnostic Evaluation -- Establishing The Level Of Abnormality In The HPA Axis

By using a combination of the aforementioned screening tests, it should be possible to establish whether of not an individual has Cushing's syndrome or not. Once this is established, the focus of the testing is directed at determining the site within the HPA axis that is causing the abnormal production of cortisol. In essence, we must discriminate between the major etiologies listed below.

Relative Prevalence of Various Types of Cushing's Syndrome among 630 Patients Studied at Different Times.*

DIAGNOSIS	PERCENT OF PATIENTS
Corticotropin-dependent Cushing's syndrome	
Cushing's disease	68
Ectopic corticotropin syndrome	12
Ectopic CRH syndrome	<1
Corticotropin-independent Cushing's syndrome	
Adrenal adenoma	10
Adrenal carcinoma	8
Micronodular hyperplasia	1
Macronodular hyperplasia	<1
Pseudo–Cushing's syndrome	
Major depressive disorder	1
Alcoholism	<1

Identification Of Adrenal Lesions Causing Cushing's Syndrome

Much effort was expended to establish protocols capable of discriminating adrenal causes of Cushing's syndrome from ACTH-dependent forms of the syndrome. Even after the development of radioimmunoassays for ACTH, the sensitivity of many commercially available assays led them to be useful only in distinguishing subjects with greatly elevated levels of ACTH (i.e. ectopic ACTH production) and not in discriminating subjects with levels of ACTH in the 'normal' range from subjects with suppressed ACTH levels.

Currently, the tests available through most major reference laboratories have sensitivities of 1-5 pg/ml, a range that can clearly identify patients with suppressed levels of ACTH. Thus the demonstration of a suppressed ACTH level in a patient with documented Cushing's syndrome would suggest the presence of adrenal pathology and indicate the need for imaging studies of the adrenal glands.

The major premise that has guided the development of the dynamic tests that are currently employed is that diseases that result in autonomous function of the adrenal(s) will be characterized by a suppression of ACTH levels and will not exhibit the suppression expected for ACTH-dependent forms of Cushing's (particularly Cushing's disease). Tests focused on this point are important components of the evaluation of patients with Cushing's syndrome, owing to the lack of reliability of imaging studies of the pituitary and adrenal glands (see below). It is important to recognize that establishing the correct diagnosis - made by using biochemical criteria - is crucial to directing patients toward the proper therapy.

The tests that are most widely employed to distinguish between Cushing's disease and other causes are derivative of protocols reported by Grant Liddle in the 1960. In the 'high dose' component of his suppression test, t his investigator employed the oral administration of 2 mg dexamethasone every six hours (for forty eight hours, total of eight doses) and assessed the effects on cortisol excretion using measurements of 17 - hydroxycortico-steroids. The criteria that he established in his study of 35 patients with Cushing's syndrome (8 patients with adrenal tumors and 27 patients with pituitary tumors) are shown below and have been widely used for many years.

High-Dose 8-mg Dexamethasone Suppression Test*

	Cushings's Disease	Adrenal Tumor	Nodular Hyperplasia	Ectopic ACTH	Urine Corticosteroid†	Reference
	24/24	0/7	—	—	17-OHCS	24
	2/4	1/7	—	0/3	17-OHCS	28
	6/6	0/6	—	0/6	17-OHCS	29
	6/6	0/3	1/1	2/2	17-OHCS	2
	9/11	0/10	—	0/3	17-OHCS	31
	—	—	0/14	—	17-OHCS	105, 106
Total (%)	47/51 (92)	1/33 (3.0)	1/15 (6.7)	2/14 (14)		
	16/17	0/9	—	—	17-KGS	101
	18/24	1/2	2/3	2/2	17-KGS	39
	9/11	0/10	—	0/3	17-KGS	31
	8/11	0/3	—	—	17-KGS	13
	8/11	1/2	2/2	—	17-KGS	33
Total (%)	59/74 (80)	2/26 (7.7)	4/5 (80)	2/5 (40)		
	21/21	—	3/3	2/3	UFC	39
	8/11	0/10	—	0/3	UFC	31
	8/8	0/1	1/1	—	UFC	33
Total (%)	37/40 (93)	0/11 (0)	4/4 (100)	2/6 (33)		

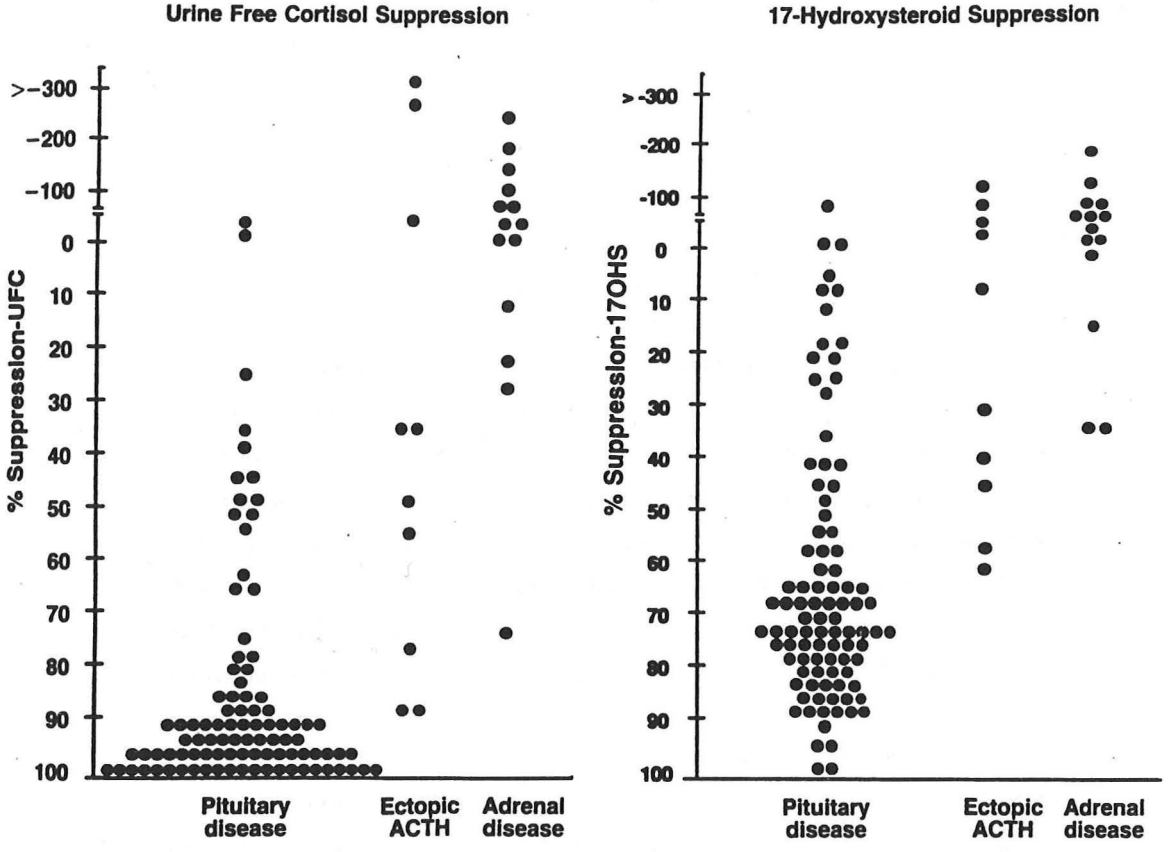
*The data are expressed as n/N where n is the number of patients with the indicated etiology of Cushing's syndrome whose 24-hr urine corticosteroid excretion determinations are at least 40% decreased from baseline on the second or third day of 8 mg/day oral dexamethasone administration, and N is the total number of patients.

†The urine corticosteroids are designated as in Table 8.

The studies performed by Dr. Liddle established that patients demonstrating a suppression of cortisol production following dexamethasone administration were likely to have a pituitary lesion as the etiology if their Cushing's syndrome (30). These initial studies were reinforced by a number of other investigators using similar methodologies.

Despite the utility of these protocols, the discrimination afforded by these tests were not perfect. For this reason, as methods became available to assay urinary cortisol directly, new investigations were conducted using these protocols to examine their utility using measurements of UFC instead of 17-HCS. The results of three relatively small studies are shown and demonstrate a marginally improved ability to discriminate between the different patient categories.

A surprising aspect about the progress of the testing for Cushing's is that a considerable effort has been made in developing new tests, particularly at the NIH, while some of the basic screening tests employed have not had much attention paid to them. A case in point is the application of measurements of the 24 hour urine free cortisol in the diagnosis of Cushing's disease. The assay has changed, with time, the tests have continued to improve, but the criteria for the interpretation of the results obtained using these newer methods is not at all clear. This aspect can be seen in the study of Flack and colleagues (17).



Suppression of urine free cortisol and 17-hydroxysteroid excretion during a standard high-dose dexamethasone suppression test in 118 patients with surgically confirmed causes of the Cushing syndrome. ACTH = adrenocorticotrophic hormone; 17-OHS = 17-hydroxysteroid; UFC = urine free cortisol.

Sensitivity and Specificity of Different High-Dose Dexamethasone Suppression Test Criteria for Pituitary Disease*

Criterion for Suppression	Sensitivity [95% CI]	Specificity [95% CI]	Misclassification of Patients with Ectopic ACTH Secretion
	n/n(%)		%
UFC > 50%	85/94 (90 [83 to 95])	19/24 (79 [58 to 93])	40
17-OHS > 50%	73/94 (78 [68 to 86])	22/24 (92 [73 to 99])	20
UFC > 80%	76/94 (81 [71 to 88])	22/24 (92 [73 to 99])	20
UFC > 90%	65/94 (69 [59 to 78])	24/24 (100 [86 to 100])	0
17-OHS > 64%	65/94 (69 [59 to 78])	24/24 (100 [86 to 100])	0
UFC > 90% or 17-OHS > 64%†	78/94 (83 [74 to 90])	24/24 (100 [86 to 100])	0

* ACTH = adrenocorticotrophic hormone; 17-OHS = 17-hydroxysteroid excretion; UFC = urine free cortisol.
† $P = 0.009$ for the diagnostic accuracy of the combined test compared with a urine free cortisol suppression of more than 90% or with a 17-hydroxysteroid suppression of more than 64%. $P = 0.016$ for the diagnostic accuracy of the combined test compared with a suppression of hydroxysteroid excretion of more than 50% (traditional criterion).

These authors addressed the need for criteria to interpret the results of dexamethasone suppression test using UFC as readout and made some pretty interesting observations. First, they found that the application of the same criteria used in the interpretation of HD dexamethasone suppression test using 17 HCS (>50% suppression on the second day) is applied to the results of UFC measurements, bad things happen. Although the sensitivity of these results is approximately 90%, the specificity is poor and 21% of patients with non-pituitary disease (including 40% of patients with ectopic ACTH production) are identified as being due to pituitary causes. Because of this finding, the authors reanalyzed their data using different combinations of criteria to analyze their results. These authors found that the use of the results of both the 17-HCS and UFC measurements permitted a dramatic improvement of the sensitivity and specificity of their tests than that that was afforded by either test alone.

It might seem somewhat perplexing that he combination of these criteria should afford such a dramatic improvement, and the authors afford several possibilities, principally that either individual variations among the subjects

permits the correct identification of some patients using one test , while others will be identified only using the other. the second possibility is that we are simply looking at the use of 'duplicate' measurements. That is, the results of one test are simply affording a type of check against errors (e.g. in the assays) inherent in the other test results. As the authors point out in their discussion, if this were to be true, ' measurements of a single steroid might have yielded a similar improvement in diagnostic accuracy'. To my knowledge, additional series that have examined the findings of this protocol in other groups of patients do not exist.

Variations on a theme -- the overnight high dose DST

In recent years, the principles established in the high dose dexamethasone suppression test have led to the development of alternate, more rapid testing protocols. One such protocol is the 8 milligram overnight dexamethasone suppression test. This test is attractive in that it does not require multiple urine collections required by the classic Liddle protocol. Interpretation of this test is based on the comparison of a baseline cortisol measurement (drawn at 8 AM) to a cortisol level drawn at 8 AM the morning following an oral 8 milligram dose of dexamethasone given at 11 PM the night before. The series reported by Tyrell and coworkers in 1986 showed an impressive sensitivity (92%) and a specificity of 100% (ref. 70). When other workers at the NIH (12) attempted to reproduce these findings, however, they were unable to achieve comparable sensitivity or specificity when the criteria originally described were employed. Even when readjusting the criteria to maintain 100% specificity (>68% suppression), the sensitivity was 71%. Of particular interest, however, was the fact that these same authors analyzed this same data - alone and in combination with the results of the standard high dose DST performed in the same patients (with measurements of 17 HCS and UFC. When combined with the results of the standard high dose DST, the use

of the results of all three tests led to a discrimination of Cushing's disease patients with a specificity of 100% and a sensitivity of 91%.

Sensitivity and specificity of different test criteria for the overnight 8-mg DST and the 6-day DST for pituitary disease

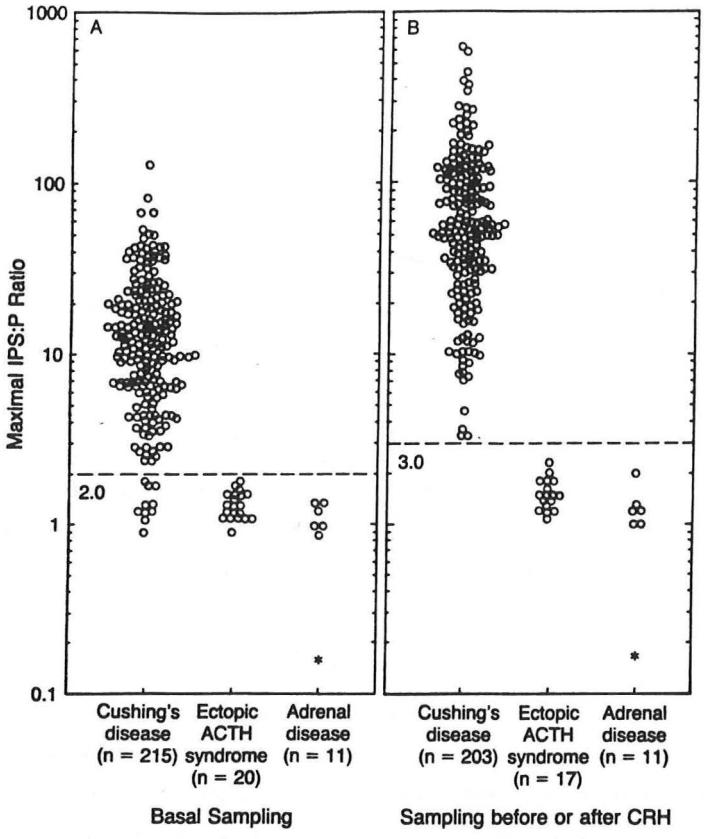
Criterion for suppression	Sensitivity	Specificity	Misclassification of patients with ectopic ACTH secretion (%)
8 mg DEX			
Serum cortisol[a] >50%	30/34 (88 [73–97])	4/7 (57 [18–90])	43
Serum cortisol[a] >80%	20/34 (59 [41–75])	7/7 (100 [59–100])	0
Serum cortisol[b] >68%	24/34 (71 [53–85])	7/7 (100 [59–100])	0
6-Day DEX			
17-OHS >50%	30/34 (88 [73–97])	5/7 (71 [29–96])	29
17-OHS >64%	25/34 (74 [56–87])	6/7 (86 [42–100])	14
17-OHS >69%	23/34 (68 [49–83])	7/7 (100 [59–100])	0
UFC >90%	22/34 (65 [46–80])	7/7 (100 [59–100])	0
Combined 6-day DEX			
UFC >90% or 17-OHS >64%	28/34 (82 [65–93])	6/7 (86 [42–100])	14
UFC >90% or 17-OHS >69%	27/34 (79 [62–91])	7/7 (100 [59–100])	0
O/N 8 mg DEX + combined 6-day DEX			
Serum cortisol >68% or UFC >90% or 17-OHS >69%	31/34 (91 [76–98])	7/7 (100 [59–100])	

17-OHS, 17-Hydroxysteroid excretion; UFC, urinary free cortisol. Values are the number/number, percentage, with the 95% CI in brackets.
[a] 0800 h post-DEX/0800 h pre-DEX.
[b] 0900 h post-DEX/0830 h pre-DEX.

Radiographic studies

The convoluted protocols employed to identify the etiology of Cushing's syndrome are necessary owing to two simple facts. First, is the occurrence of incidental lesions that are identifiable using currently available imaging techniques in the general population. The second is that the lesions causing Cushing's disease are often extremely small and many are below the limits of sensitivity of the instruments that are currently available. Although MRI is clearly superior to CT imaging of the pituitary, the frequency with which microadenomas of the pituitary can be visualized varies considerably, even in recent series (11,13,16)

Inferior Petrosal Sinus Catheterization and Sampling



One of the most powerful methods to be developed in the last decade is the simultaneous measurement of ACTH levels in the peripheral blood and in the veins draining the pituitary following the catheterization of the inferior petrosal sinuses. One of the largest series that has been reported is the series of Oldfield and coworkers (46). These workers described the catheterization of the inferior petrosal sinuses in over 250 patients 4/ 5 of which had Cushing's disease. Although not performed in all, a large proportion were studied at baseline as well as following stimulation with an injection of CRF.

Diagnostic Accuracy of Inferior Petrosal Sinus Sampling before (235 Patients) and after (220 Patients) CRH Administration in Distinguishing Cushing's Disease from Ectopic Adrenocorticotropin Syndrome.

Diagnosis	Before CRH (Basal Sampling)			After CRH Administration					
	1st samples	2nd samples*	1st or 2nd samples	2–3 min	5 min	10 min	15–20 min	30 min	Maximal ratio for any sampling time†
	patients with correct diagnosis/patients tested								
Ectopic adrenocorticotropin syndrome‡	20/20	16/16	20/20	15/15	14/14	17/17	2/2	3/3	17/17
Cushing's disease§	201/215	187/195	205/215	197/198	194/201	182/199	12/14	15/18	203/203
Accuracy (percent)	94	96	96	100	97	92	88	86	100

*Twenty-four patients, including two of the nine patients with Cushing's disease whose maximal basal IPS:P ratio was less than 2.0, did not have a second set of basal samples.

†All patients with Cushing's disease who received CRH had at least one set of samples in which the adrenocorticotropin IPS:P ratio was ≥3.3, whereas all patients with ectopic adrenocorticotropin syndrome who received CRH had maximal IPS:P ratios of ≤2.3.

‡IPS:P ratio below 2.0 during basal sampling and below 3.0 after CRH.

§IPS:P ratio of ≥2.0 during basal sampling and of ≥3.0 after CRH.

The authors found that they were able to successfully catheterize 281 out of 284 consecutive patients. The measurement of basal ACTH levels (peripherally and centrally) in this fashion allowed the discrimination of patients with Cushing's disease from those with other forms of Cushing's syndrome with an accuracy of 96%. Analysis of levels obtained at two and three minutes following CRH infusion permitted all of the Cushing's disease patients to be identified. These authors also reported that comparison of the two petrosal sinus ACTH levels permitted the prediction of the side of the adenoma in 70% of cases, information that could be quite valuable if one is dealing with a lesion that is not visible by MRI and may not even be visible at operation. The accuracy of predicting the location of the adenoma within the pituitary has been somewhat variable, ranging from 50%-70%. A recent report from Mamelak and coworkers (35) suggests that this may be due, at least in part, to asymmetry of venous drainage in a significant proportion of cases. These authors suggest that venous arteriography at the time of IPSS would help to identify those cases in which such asymmetric drainage occurs and in which lateralization would be expected to be less reliable.

Although now considered by most to be the 'gold standard' in testing in establishing the existence of Cushing's disease, several problems are inherent in the IPSS technique. First, unlike dexamethasone suppression tests, IPSS is an invasive procedure and significant complications, including permanent brain stem injuries, have been encountered (41). Performance of such procedures by groups not experienced in the execution of these methods might be expected to maximize the frequency of untoward events. Second, this technique is requires the coordination of a large number of personnel and equipment to perform this technique successfully. As such, it seems logical to reserve the IPSS test for use in the analysis of two major groups of patients in which the diagnosis is most uncertain: 1) those patients with clear-cut biochemical evidence of Cushing's disease, but negative or equivocal MRI studies of the pituitary, and 2) patients with a pituitary lesion visible by MRI, but in whom suppression and / or stimulation tests are equivocal (68).

Treatment Options

The treatment of Cushing's syndrome depends upon the etiology that is established during the course of the investigations. The identification of an adrenal adenoma would dictate the surgical removal or debulking of the lesion. In the case of adrenal adenomas, such surgeries are performed increasingly frequently using laparoscopic techniques (19). In like fashion, the detection of a bronchial carcinoid would permit a curative surgical resection to be performed.

Radiation therapy and bilateral adrenalectomy were the therapies that were first employed in the therapy of patients with Cushing's disease have largely been superseded by transsphenoidal resection of the adenoma (34, 39). As the bulk of such pituitary tumors are microadenomas, such transsphenoidal approaches by an experienced pituitary surgeon carries a high probability that a surgical cure will be achieved. In a large retrospective

European study, 76% were cured following TS resection (4), as assessed clinically and biochemically and 13% recurred with a median time to recurrence of 39 months. In this study and in others (67), an undetectable serum cortisol in the post-operative period was the best predictor of cure. The study of McCance et al included patients with measurable but low levels of cortisol who displayed long term remission (38). Some groups have suggested that early repeat surgery when hypercortisolism is detected following transsphenoidal resection (50).

The treatment of patients with residual or recurrent disease following and initial attempt at a curative resection is often less than satisfactory. Whether due to the inability to locate the tumor, cure rates in most series following a second attempted resection are no better that 50%. For this reason, it is often necessary to employ additional definitive therapies. These most often include radiotherapy and bilateral adrenalectomy.

Case no.	Age	Sex	Follow-up (years)	Mean plasma ACTH level (ng/l)			Outcome
				Diagnosis	Basal value at time of RT	Latest value	
RT as primary therapy							
6	29	M	7·8	85	300	71	On op'DDD
7	56	M	8·0	80	250	64	Died on metyrapone
8	56	F	8·3	108	161	50	Off treatment
9	47	M	8·5	72	146	39	Off treatment
10	31	F	8·8	44	76	26	Off treatment
11	36	F	9·5	42	71	42	Off treatment
12	61	F	11·2	102	184	21	Off treatment
13	42	F	11·4	80	104	45	On op'DDD
14	48	M	11·5	74	200	35	Off treatment
15	21	F	11·9	61	260	16	Adrenalectomy
16	24	F	13·5	68	234	10	Transsphenoidal
17	15	F	13·7	68	87	22	Adrenalectomy
19	27	M	14·2	230	189	172	Transsphenoidal
20	52	F	14·8	100	140	72	On op'DDD
21	40	F	15·5	115	153	26	Off treatment
RT after unsuccessful transsphenoidal surgery							
22	8	M	1·2	18	39	18	Off treatment
23	40	M	1·5	48	74	28	On metyrapone
24	54	M	1·5	40	40	104	On op'DDD
25	37	F	2·8	115	122	150	Adrenalectomy
26	48	F	3·0	46	40	18	Off treatment
27	47	F	3·1	118	91	35	Off treatment
30	46	F	11·1	57	83	11	On op'DDD
RT after transfrontal surgery for macroadenomas							
31	44	F	3·8	257	256	5990	Dead
32	58	M	7·5	163	161	1284	Dead
33	27	F	8·0	58	103	19	Adrenalectomy

Radiotherapy (RT) has been used both as a primary therapy and as an adjunctive therapy in patients following an unsuccessful transsphenoidal surgery. Doses and protocols have varied somewhat, long-term follow-up is available for a number of series. In the series of Howlett et al (21), of the 21 patients treated with RT 12 (57%), were off all therapy, the remainder requiring medication or adrenalectomy. The percentage of patients in which medical therapy could be withdrawn was similar for patients treated with radiation therapy as a primary therapy (7/15, 47%) and as those in which it was employed as a secondary therapy (5/9, 56%). As a group, it was possible to withdraw medical therapy in these patients a median of four years after radiation therapy. The patients treated by Littley et al were given a lower dose of radiation (20 Gy), had a lower incidence of remission (11/ 24, 47%), and a significant number who later relapsed (31).

A variety of drugs have been used to block the normal functional of the adrenals in an attempt to achieve a medical adrenalectomy, including aminooglutethimide, mitotane, and metyrapone. Although each has been employed, none of these medications has been well tolerated and only metyrapone has been tolerated well enough to permit its use as a medical therapy. The recognition that ketoconazole acted to block the synthesis of cortisol derived from cases reports in which adrenal crises developed in patients treated with higher doses of the drug. This observation has led to its use in various forms of Cushing's syndrome to effect a reversible inhibition of cortisol synthesis. While it has been found to be well tolerated and useful - even for long-term therapy of patients with Cushing's disease (32, 61), its utility in the treatment of patients with Cushing's syndrome caused by ectopic ACTH production is less clear. The major cautions to be used when administering ketoconazole for control of Cushing's disease is the hepatotoxicity that is has

been observed (14) and the potential for the induction of inadvertent adrenal insufficiency. This drug appear to have its most important application as a temporizing therapy, for example, in the control of cortisol secretion in patients who have received, but not yet responded to, radiation therapy.

Summary of published results of ketoconazole treatment in 82 patients with Cushing's disease [19–26]

Authors	*n*	Dose mg	Duration w, m, y	Normalization urine cortisol	Side effects
Loli *et al.*, 86	7	600–800	3 m	3/7	0/8
McCance *et al.*, 87	6	800	– 1 w	5/6	3/6 Liver toxicity
Diop *et al.*, 89	5	800–1200	8 m	1/5	0/5
Cerdas *et al.*, 89	7	600	1 w	7/7	1/7 Liver toxicity
					3/7 Oedema
Tabarin *et al.*, 91	4	400–1200	4 w–6 m	1/4, 4/4	0/4
Mortimer *et al.*, 91	8	800	2 w	8/8	2/8 Liver toxicity
Sonino *et al.*, 91	28	400–800	3 w–3 y	26/28	4/34 Liver toxicity
Engelhardt *et al.*, 89, 93	17	600	1 w–1 y	6/17	3/29 Liver toxicity
				57/82 (70%)	16/108 (15%)

Paradigm for the evaluation and treatment of Cushing's disease

1. Screening tests to establish the diagnosis of Cushing's syndrome:

   a) 24 hour urine for free cortisol and creatinine (HPLC method preferable)
   b) Overnight (1 milligram) dexamethasone suppression test

   if Cushing's syndrome present, then

2. Serum ACTH level

   a) in the 'normal range', then proceed to step 3.
   b) if suppressed, image adrenals

   if Cushing's' syndrome, and ACTH levels not suppressed

3. Perform high dose dexamethasone suppression test measuring 17 HCS, urine free cortisol, and serum cortisol following test. (for interpretation of the test, use combination criteria suggested by Flack et al.; if equivocal, consider repeat using overnight 8 mg test protocol).

   and

   Thin cut of the sella, plus and minus gadolinium

   a) if discrete lesion visualized, transsphenoidal adenomectomy

b) if no lesion visualized or equivocal lesion visualized, petrosal sinus sampling indicated

4) If petrosal sinus sampling performed,

a) if central gradient demonstrated, then pituitary source inferred and TS adenomectomy indicated

b) if no gradient is demonstrated, this suggests an ectopic source (selective venous sampling, chest CT, somatostatin scintigraphy[11])

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