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The Diagnosis and Differential Diagnosis of Cushing's Syndrome and Pseudo-Cushing's States

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I. Introduction

THREE quarters of a century have passed since Harvey Cushing's original descriptions of the clinical syndrome characteristic of glucocorticoid excess (1, 2). Interest in Cushing's syndrome, especially in recent years, continues to gain momentum from both a clinical and basic science viewpoint, as witnessed by the ever increasing literature on the subject. More than in any other area of clinical endocrinology, diagnosis, differential diagnosis, and management continue to challenge the physician and occasionally cause considerable controversy. This is reflected by our limited understanding, despite much study, of the biology of the possible causes. Recent advances, especially in molecular biology, have given some insight as to the basis of the biochemical tests that are in common clinical practice; yet in the majority of cases, diagnosis is an extremely pragmatic affair involving the utilization of diagnostic strategies that have been developed and validated over the last 30 yr or so. Cushing's syndrome has been the subject of numerous original papers and reviews. Due to the relative rarity of the condition, many years of experience are required before a given diagnostic approach can be formally validated, this often being in the form of retrospective analyses. The last few years have seen the introduction of several new approaches and the validation of existing ones in larger series. It thus seems a pertinent time to review our current understanding, address areas of debate and, at the risk of further controversy, to suggest some diagnostic approaches.

It is our belief that the clinical spectrum of Cushing's syndrome is shifting as this rare diagnosis is increasingly being considered by astute physicians in more common settings, such as the diabetic clinic. Indeed, recent work has suggested that up to 3–4% of individuals with poorly controlled diabetes mellitus with an obese phenotype may have Cushing's syndrome (3). Therefore, our ability to make the diagnosis of Cushing's syndrome is becoming an increasing challenge, since the pathological process of glucocorticoid excess is being considered at an earlier stage in its natural history. In this paper, we critically review the clinical features of the syndrome and the biochemical tests that confirm or refute clinical suspicion. There then follows a critique of the biochemical tests and imaging investigations employed in the differential diagnosis of Cushing's syndrome, with particular emphasis on more recent approaches, large-series validations and modifications of existing protocols. Finally, consideration is given to the vexed issue of the differentiation of Cushing's syndrome from pseudo-Cushing's states.

II. Definitions and Etiology

Endogenous Cushing's syndrome is a clinical state resulting from prolonged, inappropriate exposure to excessive endogenous secretion of cortisol and hence excess circulating free cortisol, characterized by loss of the normal feedback mechanism of the hypothalamo-pituitary-adrenal axis and the normal circadian rhythm of cortisol secretion (4). Other situations in which there is biochemical evidence of excess cortisol secretion, without the development of a 'Cushingoid

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state,' however subtle, such as in the setting of a long period in the intensive care unit, will not be considered here. The etiology of Cushing's syndrome may be excessive ACTH production from the pituitary gland, ectopic ACTH secretion by a nonpituitary tumor, or excessive autonomous secretion of cortisol from a hyperfunctioning adrenocortical tumor (5–9). Other than these broad 'ACTH-dependent' and 'ACTH-independent' categories, the syndrome may, in addition, be caused by ectopic CRH secretion (10–12), bilateral primary pigmented nodular adrenal hyperplasia and macronodular adrenal hyperplasia (13), the ectopic actions of gastric-inhibitory peptide or catecholamines (14–16), and other adrenal-dependent processes associated with adrenocortical hyperfunction such as McCune-Albright syndrome and Carney's complex (13, 17) (Tables 1 and 2). Pseudo-Cushing's states, which may have similar clinical presentations together with evidence of hypercortisolemia, may be caused by alcohol dependence (18–22) and depression (23–26). It may also be necessary to differentiate Cushing's syndrome from other clinical presentations with 'Cushingoid' features, such as cases of simple obesity in which some Cushingoid clinical features may be present (27–32). The incidence of pituitary-dependent Cushing's disease and adrenal adenomas in women is 3 to 4 times that of men: since this is the most common form of Cushing's syndrome, as a whole, therefore, women easily outnumber men (Table 1).

In current clinical practice it is increasingly likely that the diagnosis of Cushing's syndrome will be considered at an earlier stage in its history; as a result of improved scanning techniques, increasing numbers of adrenal masses are discovered incidentally during nonendocrine investigation. Many of these 'incidentalomas' demonstrate subtle autonomous secretion of cortisol (33), but the optimal investigation and management of these lesions remain controversial and will not be considered here (for reviews see Refs. 34 and 35).

III. Diagnostic Overview

The clinical signs of Cushing's syndrome are protean, providing the stimulus for further biochemical evaluation and imaging. The diagnosis of Cushing's syndrome must be established before any attempt at differential diagnosis, since the tests employed in the differential diagnosis may be misleading, or uninterpretable, unless there is biochemical confirmation of the hypercortisolemic state. This latter criterion is vital and applicable to all of the tests detailed below, since the reported sensitivity, specificity, and diagnostic accuracy are only valid during periods of active and sustained hypercortisolism. In some circumstances diagnosis may be relatively straightforward, while in others, and especially at times when the degree of hypercortisolism is only mild and variable, diagnosis and differential diagnosis may remain elusive.

TABLE 1. Etiology of Cushing's syndrome in 306 patients seen at St. Bartholomew's Hospital 1969–1997

Cause of Cushing's syndrome	Female	Male
ACTH-dependent:		
Cushing's disease	161	48
Ectopic ACTH syndrome	16	16
Unknown source of ACTH	13	3
ACTH-independent:		
Adrenal adenoma	19	5
Adrenal carcinoma	12	8
Nodular adrenal hyperplasia	1	4
Total	222	84

TABLE 2. Etiology of the ectopic ACTH syndrome causing clinical Cushing's syndrome in patients seen at St. Bartholomew's Hospital 1969–1997

Site of secretion	Female	Male
Bronchial carcinoid tumor	11	2
Small-cell lung cancer	1	5
Medullary thyroid carcinoma		3
Pancreatic carcinoid tumor	1	2
Thymic carcinoid tumor		1
Disseminated carcinoid tumor		1
Mesothelioma	1	
Pancreatic carcinoma		1
Colonic carcinoma	1	
Pheochromocytoma		1
Gall bladder carcinoma	1	
Total	16	16

IV. Clinical Features

Symptoms associated with hypercortisolemia include weight gain, lethargy, weakness, menstrual irregularities, loss of libido, depression, hirsutism, acne, purplish skin striae, and hyperpigmentation (6, 36–38). Associated problems such as diabetes mellitus or hypertension may also bring the patient to medical attention. The signs associated with Cushing's syndrome are extremely varied and differ in severity (Table 3). Signs that differentiate Cushing's syndrome from pseudo-Cushingoid states most reliably include the presence of proximal myopathy, easy bruising, and thinness and fragility of the skin (36, 37). In our experience signs such as buffalo hump, obesity, and hirsutism are poor discriminators. In the case of children, gain in weight associated with growth retardation are particularly prominent features that should alert clinical suspicion to the diagnosis (39–42).

For unknown reasons some ACTH-secreting tumors of any type causing Cushing's syndrome exhibit cyclical and intermittent secretion (47, 48). Thus, a history of cyclical depression may be present in the context of a Cushingoid appearance. Such cyclicity may extend over many months or even years (49) to complicate the diagnostic process, since, if patients are investigated as they 'cycle out,' confusing and misleading results of investigations may result in mismanagement. Confirmation of hypercortisolism is needed to allow reliable interpretation of the diagnostic tests; if absent at presentation, and if the diagnosis is strongly suspected, reevaluation at a later date may be required (*vide infra*).

The original descriptions of the 'overt' ectopic ACTH syndrome reported the effects of high levels of ACTH and cortisol, usually of rapid onset and most often due to ACTH secretion from small-cell lung cancers (50). Symptoms include profound weakness as a direct result of high circulating levels of cortisol and associated hypokalemia, while there is

TABLE 3. The frequency of clinical signs and symptoms of Cushing's syndrome in five series of adults (1952–1982) and two of children (1994, 1995)

Sign/symptom (%)	Plotz *et al.* 1952 (43) n = 33	Sprague *et al.* 1956 (44) n = 100	Soffer *et al.* 1961 (45) n = 50	Urbanic and George 1981 (46) n = 31	Ross and Linch 1982 (37) n = 70	Magiakou *et al.* 1994 (39) n = 59	Weber *et al.* 1995 (40) n = 12
Obesity or weight gain	97	84	86	79	97	90	93
Decreased linear growth						83	80
Hypertension	84	90	88	77	74	47	
Plethora	89	81	78		94		
Rounded face	89	92	92		88		
Hirsuitism	73	74	84	64	81	78	58
Thin skin				84			
Abnormal glucose tolerance	94		84	39	50		
Easy bruising	60	62	68	77	62	25	17
Weakness	83		58	90	56	45	50
Osteopenia or fracture	83		56	48	50		
ECG changes or atherosclerosis	66/89		34		55		
Menstrual changes	86	35	72	69	84	78	20
Decreased libido (men/women)	86		100/33	55	100		
Depression or emotional lability	67		40	48	62		25
Headache	58				47		50
Striae	60	64	50	51	56	61	
Edema	60		66	48	50		
Acne	82	64		35	21	47	58
'Buffalo hump'		67	34		54		
Female balding			51		13		20
Lipid abnormalities	39						
Decreased wound healing	42						
Delayed bone age						11	
Accelerated bone age						8	
Pigmentation						14	8

[Adapted with permission from L. Nieman and G. B. Cutler, Jr.: Cushing's syndrome. In: De Groot LJ (ed) Endocrinology. W. B. Saunders, Philadelphia, 1995.]

often little weight gain and absence of classically Cushingoid appearance. Pigmentation frequently appears as a result of the high circulating levels of ACTH. In contrast, ACTH-secreting carcinoid tumors, most frequently bronchial in origin, may present with clinical features indistinguishable from pituitary-dependent or primary adrenal disease; this clinical situation is now referred to as the 'occult' ectopic ACTH syndrome (9). Thus, the clinical history and examination may be extremely helpful in differentiating between pituitary and ectopic causes in cases of 'overt' ectopic ACTH syndrome, whereas they have poor discriminating power in the 'occult' ectopic ACTH syndrome.

V. Biochemical Diagnosis of Cushing's Syndrome

A. Cardinal features

The cardinal biochemical features of Cushing's syndrome are excess endogenous integrated secretion of cortisol, loss of the normal feedback of the hypothalamo-pituitary-adrenal axis (HPA), and disturbance of the normal circadian rhythm of cortisol secretion. The biochemical tests that are used in the diagnosis of Cushing's syndrome rely upon these parameters. In the investigation of Cushing's syndrome, initial biochemical tests of high *sensitivity* should be employed, although the diagnosis may later be refuted by tests of higher *specificity*. This is an acceptable trade off, since tests that are highly specific but relatively insensitive will inevitably miss individuals with mild disease. In the interpretation of published series, caution is required, since diagnostic criteria that provide discrimination between groups under study are inherently reliant on the assays on which they are based. Therefore, the responses for a given test require validation in the locally used assay before they may be reliably interpreted in a given patient. Supraregional and nationwide interassay quality control assurance provides a means of achieving this and is widely practiced.

B. Urinary free cortisol (UFC)

Collection of urine for estimation of cortisol and cortisol metabolites is a noninvasive procedure and is widely used as a screening test for the diagnosis of Cushing's syndrome. Under normal conditions, approximately 10% of serum cortisol is unbound and physiologically active. Free unbound cortisol passes through the kidneys, and although the majority is reabsorbed in the tubules, the remainder is excreted unaltered (4). Excess cortisol saturates circulating cortisol-binding globulin, resulting in an increase in the urine cortisol-UFC. UFC measurements have superseded the measurement of urinary 17-hydroxycorticosteroids (17-OHCS) or 17-oxogenic steroids, which are metabolites of cortisol and cortisone. In his review of 14 separate studies assessing the utility of 17-OHCS measurement for the diagnosis of Cushing's syndrome, Crapo (51) reported that the false negative rate was 11% of 315 individuals with Cushing's syndrome, while in 173 obese controls the false positive rate was 27%. Similarly poor results were obtained utilizing 17-ketogenic steroids (KGS) with a false negative rate in 24% of 235 patients with Cushing's syndrome (51). In contrast, 24-h UFC measurements by RIA should reflect the integrated cortisol secretion, with a raised level being consistent with Cushing's

syndrome. The upper normal range in most assays is between 220–330 nmol/24 h (80–120 μg/24 h). The majority of problems associated with this test relate to the adequacy of collection, although in some assays there may be cross-reactivity with exogenous glucocorticoids. Adequate written instructions will improve collections (52), but there was a false negative rate of 5.6% and false positive rate of 3.3% in the combined data on 479 obese, lean, and chronically ill individuals (51). Expressing the UFC over creatinine allows the adequacy of collection to be established and improves the specificity (53), although it should be noted that creatinine may vary with changes in lean body mass. More recently, UFC measurement was shown to have a diagnostic sensitivity and specificity of 100% and 98%, respectively, in the differentiation of 48 patients with Cushing's syndrome from 98 normal subjects and 95 obese individuals (54). However, although 24-h UFC measurement in 146 patients with Cushing's syndrome was shown to have a sensitivity of 95%, it was noted that 11% had at least one of four 24-h collections with values within the normal range (55). Furthermore, 'raised' 24-h UFC levels have been documented in 40% of 60 depressed inpatients (56) and in 50% of 45 women with the polycystic ovarian syndrome (57); by definition, almost complete overlap in levels is seen in the differentiation from various causes of pseudo-Cushing's states (58), emphasizing the potential for diagnostic confusion. The problem of cross-reactivity becomes a particular issue if the possibility of exogenous glucocorticoid administration exists (57). HPLC has recently been compared with RIA for the measurement of cortisol and cortisone in the assessment of endogenous Cushing's syndrome and Cushing's syndrome due to exogenous glucocorticoid ingestion (59). Using this method on single urine samples, 19 of 29 patients with histologically proven ACTH-dependent or ACTH-independent Cushing's syndrome had an increase in both cortisone and cortisol, while a further 8 of 29 had an increase in one or the other. Overall, 27 of 29 (93%) had HPLC measurements comparable to a competitive binding assay, with levels of cortisol, cortisone, or both that were higher than the normal range; in 6 patients with Cushing's syndrome due to exogenous glucocorticoids, the UFC and cortisone were suppressed, and prednisolone and prednisone were detected. Utilization of the competitive binding assay in this latter group resulted in every individual having a falsely elevated 'UFC' measurement. Since RIAs also suffer from problems of cross-reactivity, this approach may occasionally be useful in difficult cases where doubt exists as to the origin of glucocorticoid. Thus, if replicated in further studies, this approach may prove to be of value, as in this small number the sensitivity approaches that for four 24-h collections measured by RIA, but data on the values seen in pseudo-Cushing's states are needed for full evaluation. Nevertheless, similar discrimination might be made by a single 0900 h plasma cortisol, since this should be suppressed in conditions in which exogenous glucocorticoids have resulted in Cushing's syndrome, as long as the plasma cortisol RIA has little cross-reactivity for synthetic glucocorticoids and hydrocortisone is not being administered. Finally, a low dihydroepiandrosterone sulfate, because of suppressed plasma ACTH, may commonly be found in, and is a useful additional indicator of, exogenous glucocorticoid administration (57).

Overall, UFC estimations have a high sensitivity, but relatively low specificity; therefore, if several UFC collections are normal, Cushing's syndrome is highly unlikely.

C. Low-dose dexamethasone testing

Since the original description by Liddle in 1960 (60) of the 48-h 2 mg/day low-dose dexamethasone suppression test (LDDST), this diagnostic tool has remained an important part of the evaluation of suspected Cushing's syndrome. Administration of dexamethasone, which is not measured in most cortisol RIAs, results in suppression of the HPA axis in normal individuals and a fall in plasma and urinary cortisol. A variety of regimens exist for the dexamethasone administration, and a range of diagnostic 'cut-offs' that classify responses have been reported.

The overnight test involves the oral administration of 0.5–2.0 mg dexamethasone (most commonly 1 mg) at 2300 or 2400 h, after which a plasma cortisol sample is obtained at 0800 h or 0900 h the next morning (61). There appears to be no better discrimination with 1.5 mg or 2 mg of dexamethasone than with 1 mg administration (51). Because of its ease of administration as an outpatient, it has been widely advocated as a screening test. The reported cut-off values for suppression of serum cortisol in studies utilizing modern RIAs, but with relatively small numbers of individuals with Cushing's syndrome, range from 100–200 nmol/liter (3.6–7.2 μg/dl) (62, 63). Some patients with Cushing's syndrome, however, demonstrate unusual suppressibility to dexamethasone (64), and thus cut-offs at this level are likely to result in a significant number of false negative responses. Therefore, to enhance sensitivity, a recent extensive review assessing the utility of low-dose dexamethasone testing in the diagnosis of Cushing's syndrome suggested that suppression of the postdexamethasone plasma cortisol to 50 nmol/liter (1.8 μg/dl) or less effectively excludes Cushing's syndrome (65). At this level false positive rates will be higher, but the main value of the overnight test is that of ease of outpatient screening to exclude Cushing's syndrome, which may be an acceptable price to pay for enhanced sensitivity.

The original description of the 48-h 2 mg/day dexamethasone suppression test (60) reported the suppression of urinary 17-OHCS as an indicator of cortisol suppression. Serum cortisol RIAs provide a more simple measurement, with test sensitivities of 97–100% (58, 66, 67), comparable to the overnight test (51). In our own experience, testing in 150 individuals with proven Cushing's syndrome and measuring the 0900 h serum cortisol before and after the administration of 0.5 mg dexamethasone strictly every 6 h for 48 h and a cut-off value for suppression of 50 nmol/liter (1.8 μg/dl), resulted in a sensitivity of 98% (three patients with histologically proven Cushing's disease suppressed to less than 50 nmol/liter) (68). In a direct comparison of the responses of 39 patients with Cushing's syndrome compared with 19 with pseudo-Cushing's syndrome, a plasma cortisol concentration at 0800 of 38 nmol/liter (1.4 μg/dl), exactly 2 h after the last dose of dexamethasone that had been administered for 48 h as above, gave a specificity of 100% and a sensitivity of

90% for the diagnosis of Cushing's syndrome, while measurement of urinary steroids provided a sensitivity of only 50–60% (58). Increased sensitivity was achieved by analyzing the plasma cortisol after the administration of CRH (see *Section X*). More recently, and using the same criterion, these same authors have demonstrated the utility of this combined test in differentiating mild Cushing's disease (three demonstrated suppression of plasma cortisol on the LDDST limb of the test) from normal individuals (69). The 1-mg overnight test has a specificity of 87.5% (63), while the reported specificity for the 2 mg/day 48-h test is 97–100% (67). Thus, the standard Liddle 2 mg/day 48-h LDDST has the same sensitivity and higher specificity than overnight dexamethasone testing, but should be performed by measuring plasma cortisol rather than urinary steroids. It is our routine practice to use the Liddle 48-h, 2 mg/day dexamethasone suppression test in both inpatient and outpatient settings, since, with adequate written instructions, compliance is extremely high and the results are reproducible.

In all the variations of the oral dexamethasone tests, variable absorption and metabolism of dexamethasone will influence the result of the test (70, 71). Thus, in an effort to reduce false positive responses, simultaneous measurement of plasma cortisol and dexamethasone has been advocated for the overnight test to ensure adequate plasma dexamethasone concentrations of 5.6 nmol/liter (0.22 μg/dl) or greater and to confirm compliance (70). This does, however, require access to a costly dexamethasone assay, which is often unnecessary, although it may be particularly useful in cases of suspected malabsorption. To overcome this type of problem, the intravenous dexamethasone suppression test has been proposed (29). In this study, an infusion of dexamethasone at 1 mg/h between 1100 h and 1500 h caused a sustained suppression day of plasma cortisol to less than 83 nmol/liter (3 μg/dl) until at least 0900 h the next day in normal and obese subjects, while in patients with Cushing's syndrome the plasma cortisol was greater than 276 nmol/liter (10 μg/dl) at this 0900 h time point. Interestingly, distinction between Cushing's disease and either the ectopic ACTH syndrome or ACTH-independent Cushing's syndrome could be made on the basis of a 50% fall in plasma cortisol during the dexamethasone infusion. An alternative regimen involved intravenous administration of dexamethasone at a dose of 5 μg/kg/h for 5 h between 1000 h and 1500 h, which resulted in suppression of plasma cortisol to less than 38 nmol/liter (1.4 μg/liter) at 1900 h in 19 patients with simple obesity, while in 12 patients with Cushing's syndrome the plasma cortisol was 68 nmol/liter (2.5 μg/dl) or greater at this time point; at 0800 h the next day, the obese group had a sustained suppression of plasma cortisol, while those with Cushing's syndrome had values of 136 nmol/liter (5 μg/dl) or more (72). Such tests are clearly more complex to perform, but in certain circumstances their application may be useful.

Drugs such as phenytoin, phenobarbitone, carbamezepine, and rifampicin will induce hepatic enzymatic clearance of dexamethasone, thereby reducing the plasma dexamethasone concentration (73, 74) and resulting in false positive responses to dexamethasone testing. Estrogens increase the cortisol-binding globulin concentration in the circulation; since RIAs measure total cortisol, false positive rates are seen in 50% of women on the oral contraceptive pill (75). It is our routine practice, where possible, and particularly in mild cases, to stop such estrogen-containing drugs and delay investigation for 6 weeks to allow the cortisol-binding globulin to return to baseline. However, this may not be necessary in the case of transdermal estrogens.

D. Circadian rhythm assessment

In normal circumstances the level of serum cortisol begins to rise at 0300–0400 h and reaches a peak at 0700–0900 h, with levels then falling for the remainder of the day. Loss of the normal circadian rhythm in patients with Cushing's syndrome was first reported by Doe *et al.* in 1960 (76) and has been confirmed in several studies (77–80). In contrast, other reports have suggested that the rhythm may persist in certain patients but with levels that are set abnormally high (81–83). There is a large overlap in 0900 h serum cortisol values between patients with Cushing's syndrome and normal subjects (36, 84, 85); therefore, this sampling time affords poor discrimination. The overlap between patients with Cushing's syndrome and the normal range diminishes with clock time such that at time points in the range 1600–2100 h, 17% of patients with Cushing's syndrome have values within the normal range, falling to 3.4% at 2300 h (51). Urine cortisol samples have also been used for this purpose. Clearance of urine cortisol collected between 2200 h and 2300 h, and expressed as a ratio of nanograms/mg creatinine, ranged from 76 to 905 in 14 patients with Cushing's syndrome but only from 6 to 43 in 20 normal subjects, affording discrimination between groups (53). Similarly, a timed urine collection between 2000 h and 2400 h revealed a ratio (expressed as micromoles/mol creatinine) ranging from 27.5 to 855 in 20 patients with Cushing's syndrome, 1.1 to 9.4 in nonobese control subjects, and 9.4 to 27.8 in 34 obese subjects, thus giving an overlap with the Cushing's syndrome group in one individual with extreme obesity (86). In our own series a single sleeping plasma cortisol was greater than 50 nmol/liter (1.8 μg/dl) in 150 individuals with Cushing's syndrome (three of which had suppressed on a 2 mg/day 48-h LDDST), while in control subjects the sleeping midnight serum cortisol was less than 50 nmol/liter (1.8 μg/dl) in all (68) (Fig. 1). The test does, however, require inpatient admission for a period of 48 h or more to avoid false positive responses due to the stress of hospitalization, and the blood sample needs to be drawn within 5–10 min of waking the patient. To avoid false positive results due to anticipation, the patients should not be warned that the test is to be performed; if the patient is awake, the test is not readily interpretable. It is apparent from Fig. 1 that the test affords no discriminatory power between any of the causes of Cushing's syndrome. Moreover, this reported sensitivity is reliant, as stated above, on an active state of cortisol hypersecretion.

Similar blunting of the circadian rhythm is seen in many patients with depressive illness (87–89), especially in dexamethasone nonsuppressors, and there may be a complete absence of rhythm in the critically ill (90). A single sleeping midnight cortisol thus has 100% sensitivity for the diagnosis of Cushing's syndrome but, in view of the relatively small number of controls studied, it is not possible to comment on

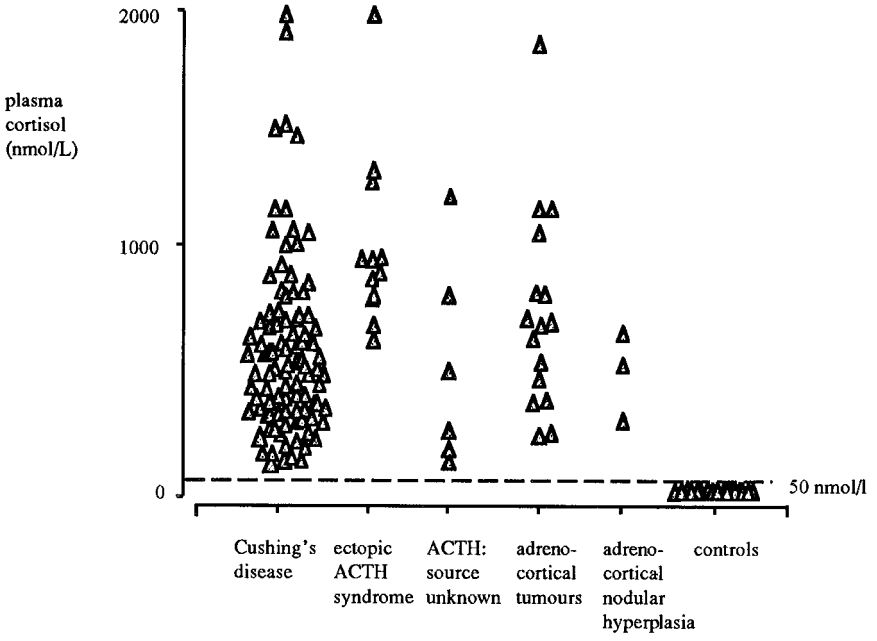

FIG. 1. Sleeping plasma midnight cortisol values in 150 patients with Cushing's syndrome and 20 normal volunteers. [Reproduced with permission from J. Newell-Price *et al*.: *Clin Endocrinol (Oxf)* 43:545–550, 1995 (68).]

its specificity. Furthermore, acute illness, the stress of hospitalization, heart failure, and infection may result in a false positive result (4). One report does, however, suggest that assessment of midnight cortisol values may allow discrimination between Cushing's syndrome and pseudo-Cushing's states since the level of plasma cortisol at midnight was greater than 207 nmol/liter (7.5 μg/dl) in 94% of 97 patients with Cushing's syndrome, while it was less than this in 31 individuals with pseudo-Cushing's states (91). This difference in cut-off point may reflect that those studied in this latter report were awake at the time of sampling. It is our experience that many patients with Cushing's syndrome will have *sleeping* midnight plasma cortisol values below this level (Fig. 1), and thus if this manner of sampling is employed, a cut-off level above 50 nmol/liter (1.8 μg/dl) is recommended as being consistent with Cushing's syndrome. In summary, a single undetectable sleeping midnight cortisol value effectively excludes active Cushing's syndrome, at least during inpatient investigation.

E. Cyclical Cushing's syndrome

As stated above, for unknown reasons some ACTH-secreting tumors of any type causing Cushing's syndrome exhibit cyclical and intermittent secretion (47, 48), and this may be reflected by a history of variable and intermittent depression with anxiety (92), an alteration in the level of prevailing glycemia, or indeed any of the plethora of symptomatology and signs outlined in Table 3. This may cause considerable diagnostic confusion, but careful documentation of the history is of paramount importance. Inpatient admission, sometimes on repeated occasions, with sampling for sleeping midnight plasma cortisol is one means of getting around this diagnostic conundrum, and proceeding to further investigation if documented hypercortisolemia is present. If inpatient admission cannot be justified, salivary cortisol estimations may be used to establish the diagnosis as an outpatient (93). Multiple and repeated 24-h UFC collections may also prove useful. Clearly, the diagnostic dimension of time is often needed to establish the diagnosis.

VI. ACTH-Dependent *vs.* ACTH-Independent Cushing's Syndrome

Only once the diagnosis of Cushing's syndrome is established may the differential diagnosis be entertained. The initial step is to establish whether ACTH is detectable in the plasma; if it is consistently undetectable, the diagnosis of ACTH-independent Cushing's syndrome may be made, and focus should turn to adrenal imaging. Usually this is a fairly easy discrimination to make: at our institution a plasma ACTH level of less than 10 pg/ml was seen in all patients with Cushing's syndrome due to a cortisol-secreting adrenal adenoma. It is, however, our experience that a few patients with pituitary-dependent Cushing's disease have the occasional undetectable plasma ACTH, as assessed by a conventional RIA with a lower limit of detection of 10 pg/ml (94). Thus, if measured by this means, we would recommend the collection of several plasma ACTH collections to avoid this potential problem, or the measurement of ACTH after CRH stimulation. Nevertheless, we have not seen any patient with ACTH-dependent Cushing's syndrome that has had more than one or two ACTH levels of less than 10 pg/ml. Immunoradiometric assays (IRMA) provide increased speed of assay, high reproducibility, and sensitivity for ACTH measurement. Using IRMA, ACTH levels consistently below 5 pg/ml are found in patients with cortisol-producing adrenal adenomas, autonomous bilateral adrenal hyperplasia, and Cushing's syndrome due to the administration of exogenous glucocorticoids (95). The use of such sensitive assays will provide very good discrimination, and when the levels are this low the diagnosis is clearly ACTH-independent; in contrast, when plasma ACTH is unequivocally measurable,

ACTH dependence exists. As such, they provide optimal screening at the critical decision limb as to whether the clinician is dealing with ACTH-dependent or ACTH-independent disease. A gray area does, however, exist and low-detectable ACTH levels need cautious interpretation and repeated measurement. IRMA measurements are more specific than RIA measurements, but it is this specificity that may, in theory, result in 'normal levels' being recorded in patients with the ectopic ACTH syndrome, since in this situation ACTH precursors may be present in large quantities, and these are not detected by ACTH IRMA (4, 96).

The high-dose dexamethasone suppression test (HDDST) was originally introduced to distinguish adrenal causes of Cushing's syndrome from Cushing's disease, and in the original report allowed an accurate discrimination in all those tested (60). The advent of reliable ACTH assays has facilitated the discrimination between adrenal causes and pituitary or ectopic ACTH secretion, although the HDDST remains useful in demonstrating functional autonomy (independent secretion of cortisol) of an adrenal adenoma or carcinoma disclosed on abdominal scanning. Some centers advocate the use of CRH testing (see below) to confirm a lack of ACTH response in this situation.

VII. Differential Diagnosis of ACTH-Dependent Cushing's Syndrome

A. Overview

In contrast to differentiating between ACTH-dependent and ACTH-independent etiologies, the differential diagnosis of ACTH-dependent Cushing's syndrome is far more taxing. Both corticotroph adenomas and tumors causing the ectopic ACTH syndrome are frequently small and thus difficult to visualize, and strenuous efforts at localization are required to allow correct management: these, in turn, rely heavily on biochemical testing to direct the imaging to the appropriate site. There is, however, no such thing as the single, simple, perfect, noninvasive test that will, in every case, allow the differentiation between Cushing's disease and the ectopic ACTH syndrome. Indeed, the existence of such a test would presuppose an invariant difference in the biology of these etiological entities that would allow a completely reliable classification based on the responses seen during biochemical testing, and this does not seem to be the case (97). The highest degree of accuracy is most likely to be obtained, therefore, by using a variety of tests that assess the spectrum of different physiological responses to a variety of agents.

In most centers, during the investigation of ACTH-dependent Cushing's syndrome the *a priori* probability that a patient has pituitary disease is usually between 85% and 90%. Therefore, statistically, the endocrinologist has a far better than even chance of getting the correct diagnosis with almost no investigation whatsoever, once the presence of detectable plasma ACTH has been established. It is widely held that pituitary surgery is the optimal management of Cushing's disease (98–110). One extreme approach might consist of proceeding directly to pituitary surgery after the sustained detection of plasma ACTH. Because of the inherent risks of the operation and the potential for hypopituitarism, especially in individuals of child-bearing age, this ultimate reductionist approach is unacceptable. Furthermore, such an approach would not improve the condition of a patient with ectopic ACTH secretion; indeed, delay in the correct localization and appropriate management of these tumors may result in metastatic disease (111). Biochemical testing is used in an attempt to improve upon the pretest likelihood and to direct the physician to the appropriate imaging and sampling modalities before formal management. Assuming that the default mode of treatment is pituitary surgery, the peripheral noninvasive tests in the literature are reported such that specificity is optimized (this inevitably being at the cost of reduced sensitivity) so that patients do not undergo inappropriate pituitary surgery. A major problem with all analyses, however, is the ascertainment of diagnosis. Is the 'gold standard' for pituitary disease a positive 'central' ACTH gradient on bilateral inferior petrosal sinus sampling (BIPSS), the neurosurgeon who 'sees' the tumor, the patient cured after a microadenomectomy but with negative histology, or the tumor immunostaining with anti-ACTH antibody? Depending on classification, the sensitivity and specificities for any test can be radically altered: no consensus currently exists, and it appears unlikely that one will emerge. This is further compounded by selection bias, intention to test/treat variables, and 'excluded cases.' We are left with recommendations ranging from 'do all the tests in all the patients' to suggestions not far from the one outlined above. Bearing these caveats in mind, we will review the tests currently employed in the differentiation of the causes of ACTH-dependent Cushing's syndrome. We must again emphasize that these tests are only interpretable in the context of sustained and current hypercortisolemia.

B. Basal testing

1. Plasma ACTH. Although the levels of plasma ACTH tend to be higher in the ectopic ACTH syndrome than in Cushing's disease, there is a large overlap between values, as assessed by RIA and IRMA (4, 85, 96), and therefore this affords poor discrimination between groups. The presence of POMC precursors, due to partial processing of this peptide and incomplete cleavage to ACTH, 'big ACTH' (112), is documented by a specific two-site IRMA in the ectopic ACTH syndrome, particularly when caused by small-cell lung carcinoma (113). Such 'overt' ectopic ACTH secretion is usually clinically obvious, unlike the 'occult' secretion due to carcinoid tumors, most often bronchial in origin. Recently, POMC precusors have also been documented in 12 patients with histologically proven ACTH-secreting carcinoid tumors, albeit at lower levels than seen in small-cell lung cancer, but higher than in any of the 27 patients with Cushing's disease caused by a pituitary microadenoma (114). In contrast, pituitary corticotroph macroadenomas may also exhibit poor processing of POMC (115, 116) causing diagnostic confusion. Furthermore, overlap has been documented in the levels of POMC present in 20 patients with the ectopic ACTH syndrome and 42 patients with pituitary-dependent Cushing's disease, with the values reflecting the aggressive nature of the tumors regardless of origin (117). Therefore, such analysis may prove to be helpful in discriminating Cushing's

disease from causes of 'occult' ectopic ACTH secretion, but the assays are not widely available and the data are currently conflicting.

2. Serum potassium. Serum potassium is usually low in the ectopic ACTH syndrome; therefore, this may be an extremely helpful discriminator, although up to 10% of patients with Cushing's disease exhibit hypokalemia (4, 85). The apparent reason for the hypokalemia is the saturation of 11β-hydroxysteroid dehydrogenase by excessive cortisol, which under normal physiological circumstances protects the mineralocorticoid receptor from the effects of cortisol (118). However, this generally reflects the prevailing levels of cortisol rather than the specific etiology. Thus, hypokalemia has high sensitivity for the ectopic ACTH syndrome, but a specificity that only approaches the pretest likelihood. In our experience we have seen only one patient with the ectopic ACTH syndrome (with a bronchial carcinoid) who did not have hypokalemia and associated alkalosis.

3. Ectopic cosecretion. In up to 70% of cases, occult ectopic tumors may express and cosecrete one or more additional peptides such as calcitonin, somatostatin, gastrin, pancreatic polypeptide, vasoactive intestinal peptide, glucagon, hCG-β, α-fetoprotein, α-subunit, neuron-specific enolase, GHRH, CRH, and carcinoembryonic antigen (119, 120). Thus, measurement of these specific peptides may sometimes be useful. The presence of an additional peptide provides stronger evidence for the ectopic ACTH syndrome, may also serve as a tumor marker during follow-up, and may occasionally be useful during venous sampling for localization (120).

C. Dynamic noninvasive testing

1. High dose dexamethasone testing. For more than 30 yr HDDST has remained one of the main biochemical tools used in the differential diagnosis of ACTH-dependent Cushing's syndrome. The basis of the test relies on the fact that, in most situations, the corticotroph tumor cells in Cushing's disease retain some responsiveness to the negative feedback effects of glucocorticoids while tumors ectopically secreting ACTH do not. The standard test was performed on 24-h collections of urine for the measurement of 17-OHCS or UFC, calculating the degree of suppression from day 1 to day 3 after the administration of oral dexamethasone at a dose of 2 mg every 6 h for 48 h. As with the LDDST, several suppression cut-offs that optimally define pituitary disease, in addition to combinations of measurements of different steroids, have been reported. Originally, suppression of urinary 17-OHCS by 50% or greater was reported as being consistent with Cushing's disease (60). Although this criterion has no intrinsic legitimacy, subsequent studies have confirmed the utility of this cut-off using more easily obtained plasma cortisol estimations, and calculating the suppression of values at 0800 h or 0900 h before and after 48 h of dexamethasone administration (85, 121–123). Recently, data from the National Institutes of Health indicate that measurement of UFC as an end point is as accurate a test as when 17-OHCS measurement is used, and that suppression of UFC by 90% or 17-OHCS by 64% in a given patient results in 100% specificity and 83% sensitivity for the diagnosis of pituitary disease (124). This series included 118 patients with surgically proven Cushing's syndrome: 94 patients with Cushing's disease, 14 with primary adrenal disease, and 10 with ectopic ACTH secretion. However, 2 yr later a further report from this center indicated that an increase in the level of suppression of 17-OHCS to 69% was required to maintain a specificity of 100%, albeit with a reduced sensitivity of 79%, and highlighted the utility of plasma cortisol measurement (123). Such results reflect the problems encountered in attempting to develop increasingly sophisticated cut-off criteria that maximize specificity to define disease etiology, since only one outlying responder is required to drastically alter the specificity of a given test; this inevitably results in a fall in sensitivity.

The 48-h HDDST is somewhat cumbersome; as an alternative, the 8-mg overnight dexamethasone suppression test has been developed, which involves the administration of a single 8-mg dose of dexamethasone orally at 2300 h with measurement of plasma cortisol at 0800 h before and after administration (125, 126). This test has a sensitivity ranging from 57% to 92% and a specificity ranging from 57% to 100% (123, 125–127). The time points and cut-offs that result in these reported figures vary. After the original description of the 8-mg high-dose overnight test, a 50% suppression of plasma cortisol at the second 0800 h sampling point resulted in 100% specificity and 92% sensitivity (125). More recently, a report from the NIH, including seven individuals with bronchial carcinoid tumors causing the ectopic ACTH syndrome, demonstrated 88% sensitivity and 57% specificity using these points and cut-off criteria (123). In the same study, 100% specificity and 71% sensitivity was achieved by a pre-dexamethasone sampling time of 0830 h and post-dexamethasone sampling time of 0900 h and increasing the suppression criterion to more than 68%. These minor time changes appear to have significant effects on the reported results, and further larger scale data are needed for confirmation of these revised criteria. Even so, it is apparent that they still do not appear to be as discriminatory as standard 48-h high-dose dexamethasone testing. This lowering of test sensitivity is an inevitable result of increasing experience and numbers, in addition to the increasing identification of small carcinoid tumors that may have previously gone undiagnosed.

Part of the failure of suppression during high-dose dexamethasone testing in patients with Cushing's disease may relate to inadequate levels of plasma dexamethasone due to inadequate absorption, increased clearance, or poor compliance (128). To circumvent these problems, the use of a 5-h intravenous infusion of dexamethasone at a rate of 1 mg/h has been advocated, with suppression of plasma cortisol by 50% or more being consistent with Cushing's disease (129). A slight modification of this test, with an infusion of dexamethasone for 7 h, demonstrated a fall in plasma cortisol of 190 nmol/liter or more in all of 90 patients with Cushing's disease, but only in 2 of 7 with the ectopic ACTH syndrome, giving a sensitivity of 100% and a specificity of 90% (130). Both false positive responders were ectopic secretors of CRH, and since this is a rare cause of Cushing's syndrome, more data on the responses seen in the ectopic ACTH syndrome are needed. Overall, the sensitivity of the HDDST ranges from 65% to 100%, and the specificity ranges from 60% to

100% (51, 85, 122–124, 131). Combining the results of the 48-h and overnight HDDST tests performed in every individual, and using the revised criteria for the 8-mg overnight test and conventional HDDST, resulted in a sensitivity of 92% and specificity of 100% (123). Needless to say, this raises the philosophical question of when further refinements should be abandoned, since the published criteria are becoming ever more complex and the benefit of test simplicity begins to be lost. It is hardly surprising that repeat testing, or combining the results of overnight and 48-h HDDST (123), improves results, but a logical extension of this argument is that multiple HDDST should get the correct answer if repeated a sufficient number of times. Clearly, active management of patients precludes this, and, as such, adopting the simplest approach (48-h standard HDDST using plasma cortisol samples) and combining the results with tests that act in a physiologically distinct manner would appear to be a more rational approach.

A more reductionist suggestion by Findling *et al*. (132) has been to call for the abandonment of the HDDST altogether, as it has been shown to provide little diagnostic advantage over clinical assessment in the differential diagnosis of ACTH-dependent Cushing's syndrome. The most recent study from these authors examined the *effectiveness* of the HDDST in clinical practice, whether conventional or overnight, and used simultaneous BIPSS as the gold standard for diagnosis of the origin of ACTH secretion (133). Rather than reporting extensive single-center experience, this thought-provoking work illustrated the results of dexamethasone suppression tests performed by physicians referring patients to the authors for BIPSS, and the rigor with which these tests had been performed was deliberately ignored in the study design. In their consecutive series of 112 patients referred for BIPSS, and using the standard response criterion of suppression of the postdexamethasone cortisol by greater than 50% as being consistent with Cushing's disease, the HDDST had a sensitivity of 81% and a specificity of 67% and as such is less accurate than the pretest likelihood of Cushing's disease. At no response level was it possible to achieve 100% specificity. Their analysis is uniquely based on the HDDST in practice and is, in effect, a meta-analysis, but clearly such data would be radically altered if some of the testing was being performed suboptimally for any number of the reasons that they highlight. Furthermore, the classification into pituitary or ectopic secretion of ACTH is based on the results of BIPSS as the 'gold standard,' rather than surgical confirmation, although this in itself is not a perfect test (see below). The HDDST has been shown to have better performance elsewhere, particularly when the standard 48-h HDDST is used (85, 121–123), and one conclusion might be that it should only be performed in centers with large experience. It is probably too early to call for the abandonment of the HDDST, but it certainly highlights the importance of the complete rigor required in all endocrinological assessment.

2. Metyrapone testing. Liddle and co-workers (134) introduced the 'long metyrapone test' to differentiate primary adrenal causes of Cushing's syndrome from other causes (134). This test is based upon the fact that metyrapone inhibits the synthesis of cortisol by inhibiting the cleavage of cholesterol to form pregnenolone (135) and, through the inhibition of 11β-hydroxylase, preventing the hydroxylation of 11-deoxycortisol to form cortisol (135). In patients with primary adrenal pathology, administration of metyrapone should not result in a rise in 17-OHCS excretion; in Cushing's disease, as a result of lowering of plasma cortisol and hence decreased negative feedback at both hypothalamic and pituitary levels, this should result in a compensatory increase in plasma ACTH. This will overcome the early step of metyrapone inhibition, producing a rise in urinary 17-OHCS secretion and an increase in plasma 11-deoxycortisol (135). Test protocols involve the collection of 24-h urine specimens for the estimation of 17-OHCS or 11-deoxycortisol excretion and/or the determination of plasma 11-deoxycortisol. Metyrapone at a dose of 750 mg is administered orally at 4-h intervals beginning at 0800 h for six doses, and urine and blood samples are collected on the day before, the day of, and the day after metyrapone administration. Crapo (51) has analyzed the data from 15 separate studies and shown that 101 of 110 (98%) patients with Cushing's disease demonstrated an increase in urinary 17-OHCS or 17-KGS, while only 8 of 49 patients with adrenal tumors showed such an increase. It should be noted, however, that 6 of 13 patients with ectopic ACTH secretion also had a rise in their urinary 17-OHCS or 17-KGS levels, affording very poor differentiation between ACTH-dependent groups. As is the case for the HDDST, the main use of the metyrapone test in more recent years has been in the differential diagnosis of ACTH-dependent Cushing's syndrome (136–142). The largest and most recent study again comes from the NIH (143). In this series, using surgical cure as the gold standard for pituitary disease, a rise in urinary 17-OHCS of more than 70% or a rise in plasma 11-deoxycortisol of more than 400-fold from baseline was seen in 71% of 170 patients with Cushing's disease, but not in any of the 15 patients with the ectopic ACTH syndrome; these data indicate a test sensitivity of 71% and specificity of 100% for the diagnosis of Cushing's disease, although one patient who was ultimately classified as having a unilateral hyperfunctioning adrenal nodule exhibited a rise in plasma 11-deoxycortisol of 730-fold (a rise, but probably not of this magnitude, would be predicted from enzymatic blockade). These authors were able to achieve a greater sensitivity, while maintaining 100% specificity, by combining the results of high-dose dexamethasone suppression with the results of the metyrapone test; a rise in urinary 17-OHCS of more than 70% or a rise in plasma 11-deoxycortisol of more than 400-fold on metyrapone testing, or on high-dose dexamethasone testing a suppression of urinary 17-OHCS by 69% or UFC by 90%, was seen in 88% of 170 patients with Cushing's disease but not in any of the patients with ectopic ACTH secretion (the independent results of the HDDST of this study have been discussed above). Such a result is comparable to the far more easily administered and interpreted CRH test (see below). This combined HDDST/metyrapone test is far more cumbersome than CRH testing and appears to be less accurate than combining the results of HDDST and CRH (4, 122, 144). Metyrapone and dexamethasone are, however, inexpensive and widely available; therefore, if CRH cannot be obtained, testing with metyrapone in this fashion may be a reasonable, although inferior, option.

To simplify testing with metyrapone, a shorter test was also developed to distinguish Cushing's disease from primary adrenal pathology (145–148). More recently, its use has been compared with the standard long metyrapone test in its ability to distinguish between 57 patients with Cushing's disease and 6 patients with the ectopic ACTH syndrome (149). Administration of metyrapone at a dose of approximately 30 mg/kg at 2400 h and analysis of 0900 h plasma values before and after metyrapone administration showed that suppression in plasma cortisol of more than 40%, or an increase of plasma 11 deoxycortisol by more than 220-fold, was seen in 37 of 57 patients with Cushing's disease (sensitivity, 65%), but not in any of the patients with the ectopic ACTH syndrome (specificity, 100%). The sensitivity, at a specificity of 100%, was improved to 84% by combining the results of the long and short test, but to achieve this the authors had to revise their previously documented criteria for the long metyrapone test (143). By itself, the short metyrapone test has very poor sensitivity and should be abandoned.

3. Testing with CRH. CRH was identified by Vale and coworkers in 1981 (150), and since this time it has been extensively used in the differential diagnosis of ACTH-dependent Cushing's syndrome. It had been hoped that this test would allow complete discrimination between pituitary and ectopic ACTH secretion; in the majority of patients with Cushing's disease the intravenous administration of CRH causes an excessive rise in plasma ACTH and cortisol, while in patients with the ectopic ACTH syndrome, such an effect is seen only rarely (24, 30, 94, 151–161). It seems likely that this disparity in response relates to the relatively greater expression of the CRH receptor in corticotroph adenomas compared with tumors ectopically secreting ACTH. It is important to note that the majority of reports documenting the use of this peptide in this context have used the ovine (oCRH) rather than the human-sequence peptide (hCRH).

Testing with CRH has been performed in the morning (122) and evening (158), and the most recent reports highlight the clinical utility of morning testing (156, 161). Since the circadian rhythm of cortisol secretion is lost in Cushing's syndrome, it is unnecessary to go to the added inconvenience of testing at 2000 h (127). The test is performed with the patient in a rested, fasted, and recumbent state. Most test protocols take samples for plasma ACTH and cortisol at basal samples between −15 and 0 min, and stimulated samples at 15, 30, 45, 60, 90, and 120 min after the intravenous administration of CRH, 1 μg/kg body weight, or a total dose of 100 μg. The test is well tolerated, with side effects consisting of mild short-lived mild facial flushing and a metallic taste in the mouth.

As a group, the responses to these peptides seen in patients with Cushing's disease and the ectopic ACTH syndrome differ in a quantitative rather than a qualitative fashion, and thus the absolute responses are of less value than percentage changes from basal values. In their meta-analysis of 10 studies reporting the use of peripheral CRH testing in 129 patients with Cushing's disease, 21 with the ectopic ACTH syndrome and 29 with primary adrenal disease, Kaye and Crapo (127) suggested diagnostic criteria consistent with Cushing's disease as being a rise from basal in peak plasma cortisol of $\geq$20%, or a rise in peak plasma ACTH of $\geq$50% after the administration of CRH. When these criteria are used for the plasma ACTH responses, the test has a sensitivity of 86% and a specificity of 95%, while plasma cortisol responses give an improved sensitivity of 91% and a similar specificity of 95%. Plasma cortisol samples are far more easily handled and analyzed and thus have advantages over and above the improved sensitivity compared with ACTH sampling. In contrast, in the largest reported series from the NIH, the responses to oCRH, administered at 0800 h, that best discriminated between pituitary and nonpituitary origins of ACTH secretion was a rise of 35% or more in the mean plasma ACTH concentrations at 15 and 30 min above the mean basal value at −5 and −1 min; this was seen in 93 of 100 patients with Cushing's disease, while a response less than this was observed in all 16 patients studied with the ectopic ACTH syndrome (13 of which had carcinoid tumors) giving a sensitivity of 93% and a specificity of 100% (156). Nevertheless, analysis of the responses utilizing the other basal time points employed (−15 and 0 min, or combinations thereof) revealed that some of the patients with the ectopic ACTH syndrome would have been misclassified. The plasma cortisol responses were less impressive, with a rise of 20% or more at the mean of the levels at 30 and 45 min giving a sensitivity of 91% and a specificity of 88%: no combinations of time points allowed the achievement of 100% specificity without sensitivity being severely compromised. The earlier time points used for ACTH reflect the time course in the response to CRH that tends to peak at 15–30 min. The authors themselves recommend more cautious cut-offs to guarantee specificity and also suggest the use of cortisol responses, unless the Hazelton RIA for ACTH, as used in their study, is employed. The number of patients studied in this latter single study (156) is comparable in size to the total number analyzed by Kaye and Crapo (127), and yet differing response criteria are recommended. This may be explained, in part, by differences in study protocols, since the meta-analysis included studies utilizing evening testing, and in part by different assays employed. The sensitivity of the response criteria set out by Nieman and co-workers (158) has also been validated by others (151).

The human sequence peptide has similar effects to the oCRH in normal individuals and patients with Cushing's disease (162–165). Although some reports indicate that testing with hCRH is less accurate than when using oCRH (152), we have found that the responses are qualitatively similar, albeit with a quantitatively lower response to hCRH, in patients with Cushing's disease, Cushing's syndrome due to adrenal adenoma, and in obese and lean volunteers (155). Published large series are needed that report the responses to hCRH that best discriminate between patients with the ectopic ACTH syndrome and Cushing's disease.

Combined analysis of all published series reveals that between 7% and 14% of all patients with Cushing's disease fail to respond to CRH if the best discriminating criteria are applied. This is somewhat disappointing compared with the initial hopes for the clinical utility of CRH as a discriminating agent. Although most of the Cushing's disease nonresponders exhibit suppression on a HDDST (122, 131), there

are rare cases in which an ACTH-secreting bronchial carcinoid tumor may suppress on a HDDST and exhibit responsiveness to CRH (166). Such cases are very uncommon, and the use of high-dose dexamethasone testing and CRH stimulation will, in the vast majority of cases, allow correct classification.

4. Testing with vasopressin. For many years it has been known that vasopressin also stimulates ACTH release and, in particular, that it potentiates the ACTH-releasing effects of CRH (167, 168). These actions are thought to occur via the specific corticotroph vasopressin receptor, the V_3 (also known as V_{1b}) receptor, which has recently been cloned (169, 170). The lysine or arginine vasopressin (AVP) test has been used in the differential diagnosis of ACTH-dependent Cushing's syndrome but has a false negative response in 27% of patients with Cushing's disease (171–178). Increases in urinary cortisol excretion have been observed in patients with Cushing's disease after the administration of 10 U of intraperitoneal AVP (176), while serum cortisol responses to this dose of lysine vasopressin (LVP) in patients with Cushing's disease have been noted to be less than that after 100 μg of CRH (179). Tabarin and co-workers (180) have shown that 18/21 patients with Cushing's disease responded to CRH while 17 showed similar responses to vasopressin. In this same study 2 of 7 patients with the ectopic ACTH syndrome responded to LVP while none showed a response to CRH. Thus, CRH appears to discriminate better than LVP between ectopic ACTH secretion and Cushing's disease. Furthermore, side effects consisting of abdominal pain, nausea, and flushing have precluded the routine clinical use of vasopressin for diagnostic testing, although it has been suggested that it may be better tolerated when used as a low-dose infusion, or as small bolus doses, in combination with CRH (181, 182). Overall, the LVP or AVP test appears to be inferior to CRH testing.

5. Testing with desmopressin. Desmopressin, a long-acting analog of vasopressin (183), has relative specificity for the renal V_2 receptor with little V_1-mediated pressor activity (184). While its specific V_{1b} receptor activity is uncertain, it has been shown previously to have no intrinsic *in vivo* ACTH-releasing characteristics when given as an infusion in man (185). Desmopressin has, however, been shown to cause a rise from baseline in peak plasma cortisol of more than 4 times the intraassay coefficient of variation (158) in 15 of 16 patients tested with Cushing's disease when given as an intravenous bolus dose of 5–10 μg, but not in one patient with an ACTH-secreting pheochromocytoma; a complete data set for ACTH responses was not reported (186). Since this peptide appears to be free of the V_1 receptor-mediated pressor side effects, it has been suggested that it may be used to aid the differential diagnosis of the causes of ACTH-dependent Cushing's syndrome. More recently, we have shown that a response, defined as a 20% rise in serum cortisol (156), after the administration of 10 μg desmopressin iv was seen in 14 of 17 patients with Cushing's disease and 1 of 5 patients with histologically proven 'occult' ACTH-secreting ectopic tumors (an ACTH-secreting medullary cell carcinoma of the thyroid) (187). Using ACTH response criteria of a 35% rise or more (156), 12 of 17 patients with Cushing's disease and 3 patients with the ectopic ACTH syndrome showed a response. These findings have been confirmed, using response criteria for CRH testing as defined by Kaye and Crapo (127), with responses being seen in 14 of 17 patients with Cushing's disease, while only 1 patient with the ectopic ACTH syndrome was studied, and no response was seen to either desmopressin or CRH (188, 189). Combining the data of all published series (Table 4) reveals that for the desmopressin test the cortisol responses have a sensitivity of 84% and specificity of 83%, while ACTH responses provide poorer discrimination with a sensitivity of 77% and specificity of 73%. Therefore, testing with desmopressin is inferior to testing with CRH in terms of sensitivity and specificity, although this peptide is cheaper and more easily available worldwide. Although the total numbers are small, the results are in keeping with those for LVP and AVP testing (151, 180). A possible explanation for the relatively poorer specificity of the desmopressin test is the more common expression of the V_{1b} (or V_3) receptor in ACTH-secreting nonpituitary tumors (190, 191). As such, it seems likely that testing with desmopressin alone will result in poorer discrimination than testing with CRH, although more studies are needed to confirm this impression (192). Nevertheless, some patients with Cushing's disease respond only to one peptide or the other (186–188, 192), and thus in certain circumstances testing with desmopressin may be useful.

6. Testing with peptide combinations. Since 7–14% of patients with Cushing's disease do not respond to CRH, the use of this peptide in combination with other peptides has been analyzed. Administration of 10 U AVP in combination with 1 μg/kg oCRH in patients with Cushing's disease resulted in a rise in plasma cortisol of 20% or more in 40 of 41 patients and a rise of 35% or more in plasma ACTH in all patients tested, a better response than that seen after administration of CRH alone (151). Thus, it has been suggested that this will be an improvement over the standard CRH test, although patients with the ectopic ACTH syndrome were not studied. Furthermore, vasoconstriction and an increase in blood pres-

TABLE 4. The desmopressin test in the differential diagnosis of Cushing's syndrome

Reference	Cortisol responses			ACTH responses		
	Cushing's disease	Ectopic ACTH	Adrenal	Cushing's disease	Ectopic ACTH	Adrenal
Malerbi *et al.* (186)	15/16	0/1	0/8	4/5[a]	0/1	0/2
Newell-Price *et al.* (187)	14/17	1/5	0/3	12/17	2/5	ND
Colombo *et al.* (188)	14/17	0/1	ND	14/17	0/1	ND
Totals	43/50	1/7	0/6	30/39	2/7	0/2
Sensitivity		84%			77%	
Specificity		83%			73%	

[a] ACTH data documented in five patients.

sure are important side effects of testing with AVP; therefore, caution is needed in patients with vascular disease. We have used desmopressin 10 μg iv and hCRH 100 μg iv in combination in 17 patients with Cushing's disease and 5 patients with the occult ectopic ACTH syndrome (age range 11–73 yr), all histologically confirmed, and compared the response of plasma cortisol and ACTH to those seen when testing with each peptide individually. An increase in plasma cortisol of 39% or more was seen in every patient with Cushing's disease, but 29% or less in each of the 5 patients with the ectopic ACTH syndrome, thus giving 100% sensitivity and specificity in this small series (187). Moreover, there are no associated adverse effects of the test, including no significant increases in blood pressure. The discrimination does, however, remain quantitative in nature, and it remains to be seen whether the coadministration of desmopressin and hCRH is an improvement over the standard CRH test.

7. Recent developments: testing with hexarelin. Administration of the synthetic peptide hexarelin, a member of the GH-releasing peptides (GHRPs) family, has recently been shown to have far greater ACTH- and cortisol-releasing effects than hCRH in 10 patients with Cushing's disease (193). The responses seen were remarkable in terms of the absolute levels of stimulated plasma cortisol, and even more impressively plasma ACTH, both of which were far higher than those seen even when testing with oCRH or hCRH in patients with Cushing's disease. In contrast, no ACTH rise was seen in two patients with the ectopic ACTH syndrome, while the cortisol response in one overlapped those of the Cushing's disease group. No ACTH or cortisol response was seen in 5 patients with cortisol-secreting adrenal adenomas. These results are very promising, and if replicated in larger studies, this may prove to be an extremely useful tool: even so, one cortisol responder in the ectopic ACTH group suggests that GHRP receptors may have also been present in these tumors, and indeed GHRP receptor expression in ACTH-secreting nonpituitary tumors has recently been demonstrated (194, 195). It seems likely, therefore, that with further experience more responders in the ectopic ACTH syndrome group will be reported.

D. Invasive testing

1. Inferior petrosal sinus sampling for ACTH. In the discrimination between pituitary and ectopic sources of ACTH, none of the noninvasive tests discussed above have been validated as providing 100% diagnostic accuracy in large series or meta-analyses. Therefore, alternative strategies have been developed. Although in many circumstances peripheral biochemical tests will provide evidence of pituitary disease, the presence of a pituitary lesion on imaging, especially if less than 4 mm in diameter, does not necessarily confirm functionality, since 10% of the general population harbor pituitary incidentalomas (196). Moreover, in at least 40–50% of cases of Cushing's disease, no abnormality will be disclosed on pituitary imaging (96, 197, 198). Thus, venous sampling from the inferior petrosal sinuses and/or cavernous sinuses is now widely practiced 1) to confirm or refute a central (pituitary) source of ACTH, especially when pituitary imaging is negative, and 2) to attempt to lateralize the site of a pituitary tumor to guide neurosurgical approaches.

The technique was originally described by Corrigan and co-workers (199) and involves the placement of venous sampling catheters in the inferior petrosal sinuses that drain the pituitary venous effluent (200, 201). A significant gradient between the pituitary (central) and peripheral values of plasma ACTH, obtained by simultaneous sampling, is indicative of Cushing's disease. Although in early reports the procedure was performed with sequential catheterization of each of the petrosal sinuses (199, 202, 203), it was soon realized that simultaneous bilateral inferior petrosal sampling was required as the drainage of the pituitary tends to have dominant and nondominant drainage to the inferior petrosal sinuses, and therefore unilateral sampling may miss a central source (204–207). The basal ratio of the values of plasma ACTH obtained from central and peripheral samples that have been taken as indicative of Cushing's disease have been reported as greater than 1.4 or 1.5 (206, 208–210), or greater than 2.0 (4, 39, 40, 132, 211–218). Since patients with the ectopic ACTH syndrome have been documented to have maximal basal ratios of 1.7 or 2.0 (211, 219), it would seem prudent to take this more conservative ratio of ≥2.0 (4). Although a baseline ratio of more than 2.0 is consistent with Cushing's disease, ACTH secretion is intermittent, and a significant minority of patients with Cushing's disease have a ratio less than this on the basal samples. For this reason, after basal samples are obtained, a stimulating agent, most commonly CRH, is usually administered to increase the sensitivity of the test. Both hCRH and oCRH have been used for this purpose with great success; after administration of 100 μg CRH iv, peripheral and simultaneous bilateral inferior petrosal sinus plasma ACTH samples are obtained at 3, 5, 10, and 15 min (in varying reports). In most studies a peak stimulated central-to-peripheral ratio of 3.0 or more, which usually occurs between 3 to 5 min post-CRH, is indicative of Cushing's disease (40, 132, 211, 215–217, 219–221). Recently, metyrapone pretreatment has been used with success to enhance the central-to-peripheral gradient (222), suggesting it may have a role when CRH is not available. Interestingly, the criterion that was validated in the largest published series from the NIH (211) required revision downward to maintain '100% sensitivity' in their childhood series, since although 42 of 43 patients with Cushing's disease had stimulated values of 3.0 or more, 1 patient with Cushing's disease had a stimulated ratio of 2.5, while all 6 with the ectopic ACTH syndrome had basal and stimulated ratios less than or equal to 2.2 (39). If this revised criterion were applied to their larger previous series (211), it would result in false positives (ectopic secretors misdiagnosed as pituitary tumors), and thus a stimulated response of 3.0 or more seems more appropriate. Applying the criteria of a basal ratio of ≥2.0 or a CRH-stimulated ratio of ≥3.0 to published series that have compared the results seen in Cushing's disease and the ectopic ACTH syndrome (Table 5) reveals an overall sensitivity of 96% and specificity of 100% (although false positive responses have been documented; *vide infra*). Certain points of this analysis are worth drawing out. First, the remarkable report from the NIH (211) reported a specificity and sensitivity of 100% for the CRH-stimulated procedure in the dis-

TABLE 5. Petrosal sinus sampling: centralization studies

Study	oCRH or hCRH	Successful catheterization of both petrosal sinuses	Cushing's disease basal ratio ≥2.0	Basal ratio ≥2.0 or stimulated ≥3.0[a]	Ectopic ACTH basal ratio ≤2.0	Basal ratio ≤2.0 or stimulated ≤3.0
Findling *et al.* (202)		8/9	5/5	5/5	3/3	
Landolt *et al.* (220)	hCRH	7/8	6/6	6/6	3/3	1/1
Snow *et al.* (214)	oCRH	9/10	33/35	33/35	1/1	
Shulte *et al.* (230)		15/16	8/9	8/9	1/1	
McCance *et al.* (210)		12/13	8/10[b]	8/10		
Findling *et al.* (132)	oCRH	27/29	18/20	20/20[c]	9/9	9/9[c]
Taberin *et al.* (231)	oCRH	25/27	16/20	20/20	5/5	5/5
Oldfield *et al.* (211)	oCRH	278/281	205/215	215/215[d]	20/20	20/20[d]
Boscaro *et al.* (223)	oCRH	22/22		17/22		4/4
Colao *et al.* (212)	hCRH	29/29	18/25	24/25	4/4	4/4
Magiakou *et al.* (39)	oCRH	50/50	41/43	42/43[e]	3/3	3/3[f]
Zarilli *et al.* (232)	hCRH	22/26	12/21	19/21	4/4	4/4
Cuneo *et al.* (222)	[g]	14/18	3/3	10/10		3/3
Boolell *et al.* (233)		16/20	16/16	16/16		
Vignati *et al.* (234)	oCRH	9/9		9/9		
Mampalam *et al.* (235)				33/35		
McNally *et al.* (213)	hCRH	8/8	4/4	6/8		
de Herder *et al.* (216)	hCRH	17/20	13/17	15/17		
Doppman *et al.* (236)	oCRH	15/15	13/15	15/15		
Weber *et al.* (40)	hCRH	4/4	4/4	4/4		
Lopez *et al.* (217)	NR	30/32	22/24	23/24		
Total		617/646 (96%)		548/569 (96%)		
			Sensitivity 96% Specificity 100%			

Horizontal line divides studies that do and do not include cases of the ectopic ACTH syndrome.
[a] Unless otherwise stated.
[b] Ratio >1.5.
[c] Stimulated ratio of >2.0, 18 patients received CRH.
[d] 203 patients with Cushing's disease, and 17 with the ectopic ACTH syndrome received CRH.
[e] Stimulated ratio >2.5.
[f] CRH not administered to children with ectopic ACTH syndrome.
[g] Metyrapone pretreatment.

crimination between 246 surgically confirmed patients with Cushing's disease and 20 with the ectopic ACTH syndrome. However, the authors *excluded* 32 patients from their analysis, including 3 with pituitary macroadenomas, since they were unable to formally classify them. Clearly, inclusion of these would have influenced the results. Following classification of these patients according to the results of their HDDSTs and CRH tests, the result of inferior petrosal sinus sampling agreed in 24 and disagreed in 8, giving an overall diagnostic accuracy of 95%. A follow-up report of these 32 individuals, particularly the 8 with discordant results, would be extremely instructive. Second, CRH stimulation is an important part of the test that significantly contributes to its sensitivity. Third, the test is successfully performed with bilateral simultaneous sampling from the inferior petrosal sinuses in 96% of combined series (Table 5); it remains, however, an invasive technique requiring a high degree of skill and familiarity and is thus best performed in centers with suitable experience. False negative results occur in 4% (Table 5) (209, 210, 213, 214, 216, 217, 223, 224), while false positive results are extremely uncommon; they may result from cyclical secretion of ACTH, or theoretically from treatment with cortisol-lowering agents, which results in the desuppression of the normal corticotrophs, which might then respond to CRH (225). Since this technique does not reliably distinguish normal individuals, or those with pseudo-Cushingoid states, from Cushing's disease (226), it is essential to confirm the presence of hypercortisolism before performing the test. This is of particular relevance when considering complications. Although the test is well tolerated, *i.e.*, most patients experience slight discomfort in the ear while the catheters are being placed, adverse effects, when they do occur, may be catastrophic and have included brain stem vascular damage (227–229). Such rare complications appear to relate to catheter design and might be avoided by the immediate cessation of the procedure, and catheter withdrawal, at the onset of the slightest neurological symptom (229). Heparinization of patients is recommended (229), and in our experience of more than 120 procedures we have had only one serious complication, *i.e.*, one patient suffered a nonfatal pulmonary embolus.

While the use of simultaneous BIPSS is an extremely powerful technique for establishing the central origin of ACTH secretion, its use for localization of pituitary microadenomas is more controversial. An intersinus ratio of 1.4 or greater has been suggested as being consistent with the ipsilateral localization of a microadenoma (206). Using this ratio, a combined analysis of reports documenting its use in the lateralization of coticotroph microadenomas (Table 6) reveals that the diagnostic accuracy of inferior petrosal sinus sampling is 78% (range 50–100%), using findings at pituitary surgery as the 'gold standard.' CRH stimulation does not significantly improve the accuracy of the localization in patients with Cushing's disease. If, however, the ACTH level in the non-

TABLE 6. Petrosal sinus sampling: lateralization studies using pituitary surgery as the 'Gold Standard'

Study	Ratio >1.4 pre-CRH stimulation	Post-CRH stimulation	Overall diagnostic accuracy	Reversal of intersinus gradient
Manni *et al.* (204)	3/3	NP	100% (3/3)	
Oldfield *et al.* (206)	10/10	NP	100% (3/3)	
Landolt *et al.* (220)	4/6	6/6	100% (6/6)	
Snow *et al.* (214)	6/10	NP	60% (6/10)	
Shulte *et al.* (208)	10/10	9/9	100% (10/10 or 9/9)	
McCance *et al.* (210)	4/8		50% (4/8)	
Boodell *et al.* (233)	13/16	NP	81% (13/16)	
Taberin *et al.* (231)	10/17	10/17	59% (10/17)	0
Oldfield *et al.* (211)	71/104	75/105	71% (75/105)	10
Boscaro *et al.* (223)	NP	8/13	62% (8/13)	
Colao *et al.* (212)	17/23	19/23	83% (19/23)	
McNally *et al.* (213)	5/5	4/5	80% (4/5)	0
Landolt *et al.* (215)	18/38	29/38	76% (29/38)	7
de Herder *et al.* (216)	7/11	8/11	73% (8/11)	3
Magiakou *et al.* (39)	67%	76%	76%	
Doppman *et al.* (236)	9/15	11/15	73% (11/15)	
Weber *et al.* (40)	4/4	4/4	100% (4/4)	
Zarilli *et al.* (232)			79% (11/14)	
Lopez *et al.* (217)	7/12	7/12	58% (7/12)	
Diagnostic accuracy			78%	

NP, Not performed.

dominant inferior petrosal sinus is less than 3 times the peripheral ACTH level, the accuracy improves to 83% (211). Occasionally, a reversal of lateralizing gradient is seen from the pre- to the post-CRH values, and in this case the test cannot be relied upon for lateralization (237). Although the lateralizing result may direct the surgeon to begin an initial examination of the pituitary gland on the side ipsilateral to the catheter gradient, a full exploration is required if 22% (0–50%) of tumors are not to be missed. Recommendations for ipsilateral hemihypophysectomy, in the absence of a clear tumor being visualized at operation, on the basis of the lateralizing data from inferior petrosal sinus sampling are hard to substantiate, since in 20–50% of cases the tumor may be contralateral, although anecdotally this approach has been successful (238). In an effort to improve the lateralizing ability, it has been suggested that the concentration of ACTH should be corrected for the influence of nonpituitary blood draining into the inferior petrosal sinus by analysis of the concentrations of other anterior pituitary hormones (213, 239). Further validation is required of this approach in larger series.

Normal individuals demonstrate unilateral gradients, despite anatomically symmetrical inferior petrosal sinuses (226). This may be caused by the normal pituitary having a 'dominant' side (240). In patients with Cushing's disease, however, the normal corticotrophs should be fully suppressed, and thus the existence of a dominant side to the pituitary should not affect the results. Recently, asymmetric drainage has been demonstrated by gentle cavernous sinus venography before bilateral venous sampling from the inferior petrosal and cavernous sinuses (241). In 9 of 23 (39%) patients with Cushing's disease, the drainage was asymmetric, with 6 of the 9 demonstrating drainage of both cavernous sinuses to the right petrosal sinus and no drainage to the left. Inferior petrosal sinus sampling and cavernous sampling correctly lateralized the tumor in all 12 with symmetric drainage, but in those with asymmetric drainage it was correct in basal and CRH-stimulated samples in only 3 and 4 of 9, respectively. As a whole the lateralization was correct in 70%, which is similar to other published series. These data may explain some of the mis-lateralization that occurs when performing BIPSS, and would suggest that the results for lateralization cannot be relied upon in patients with Cushing's disease who have asymmetric drainage.

2. Cavernous sinus venous sampling. Teramoto and co-workers (242) have suggested that sampling directly from the cavernous sinuses, rather than the inferior petrosal sinuses, may improve diagnostic accuracy and obviate the need for the administration of CRH, an approach that has been used by others (243). However, Doppman and co-workers (236) have recently compared this technique in the same 15 patients with Cushing's disease to that of basal and stimulated BIPSS, and found a false negative rate of 20% during cavernous sampling. Because of the added expense of the catheters required for cavernous sinus sampling, and potentially inferior results, this approach cannot be recommended at present.

3. Other approaches. Intuitively, selective venous sampling from a region that harbors a tumor ectopically secreting ACTH should be a rational and effective means of tumor localization. In practice, however, such an approach is usually unnecessary, although in certain instances it may be helpful (120). At other times misleading results may be obtained. Sampling from the thymic veins has been shown to have false positive results that resulted in inappropriate and ineffective thymectomy (244).

The use of selective preoperative bronchiolar lavage for the localization of ACTH-secreting bronchial carcinoid tumors has been reported with conflicting data. Although some report its diagnostic utility (132), in a series of seven patients, including six with proven ACTH-secreting bronchial carcinoid tumors, no ACTH was detected (245).

VIII. Other Causes of Cushing's Syndrome

In addition to the ACTH-dependent and ACTH-independent causes of Cushing's syndrome, there are some rare causes that traditionally do not easily fall into one classification. Adrenal macronodular hyperplasia is an unusual and poorly understood entity in which the adrenal glands are typically hyperplastic in the regions between nodules and may represent a transition between ACTH dependence and ACTH independence. Plasma ACTH levels may be low or undetectable, and an excessive cortisol response may be seen on CRH testing; there may also be less than 50% suppression on a HDDST causing confusing results (4, 13). Food-dependent Cushing's syndrome is a very rare cause of ACTH-independent Cushing's syndrome, which seems to be due to the presence of gastric inhibitory polypeptide receptors in the adrenal cortex that may cause massive macronodular adrenal hyperplasia (14); another cause of ACTH-independent Cushing's syndrome is the ectopic presence of β-adrenoreceptors responding inappropriately to circulating catecholamines (16). Primary pigmented nodular adrenal hyperplasia (PPNAD) tends to appear in the second decade of life, and in common with macronodular hyperplasia, it may present with low or undetectable levels of plasma ACTH and failure to suppress cortisol on a HDDST (13). It has previously been thought to be due to stimulating adrenal immunoglobulins (246, 247), and histopathologically is recognized by deeply pigmented nodules with intervening adrenal involution, distinguishing it from macronodular hyperplasia, while microscopically the cells have lipofuscin-laden cytoplasm (248). Although this may be a sporadic condition, it may also be associated with the autosomal dominantly inherited Carney complex, consisting of mesenchymal tumors, in particular atrial myxomas, pigmented skin lesions, and endocrine disorders including PPNAD peripheral nerve lesions (249). Since the gene for this complex has now been mapped to chromosome 2 (250), the hypothesis that circulating antibodies are the cause of sporadic PPNAD is highly questionable. Finally, constitutive activation of Gsα in the adrenal may occur in McCune-Albright syndrome, resulting in autonomous hypercortisolemia.

IX. Imaging

A. Pituitary

In ACTH-dependent Cushing's syndrome, pituitary imaging is used to identify and localize the position of a pituitary microadenoma, and hence to guide initial surgical exploration. Computed tomography (CT) imaging most commonly reveals a hypodense lesion that usually fails to enhance with contrast administration (251). CT scanning does, however, have a poor sensitivity of 47% and a specificity of 74% for the identification of pituitary microadenomas in a review of nine studies including 278 patients with Cushing's disease (127). Although initially very promising, gadolinium-diethylenetriamine pentaacetic acid enhanced magnetic resonance imaging (MRI) offers only a modest improvement over CT scanning, with a sensitivity ranging from 50–60% (127, 197, 198, 252–255). The majority of corticotroph microadenomas have a hypointense signal on MRI, which fails to enhance with gadolinium. However, since in approximately 5% of pituitary microadenomas the tumor will take up the gadolinium contrast medium giving an isointense signal on MRI, precontrast images are essential (96). Furthermore, since incidental pituitary adenomas have been reported in up to 27% of postmortem examinations (256), and in 10% of the 30- to 40-yr age group on MRI (196), any imaging result must be interpreted in the context of the biochemical evaluations discussed, as false positive results are possible. In some series, if a clear tumor is identified on MRI, the chances that the position disclosed on imaging will correlate with the surgical findings are in the region of 75–98% (216, 257); therefore, it has been suggested that it is similar or superior to BIPSS for localization (216). In contrast, one large series reported that in only 52% of 41 cases did the MRI correlate with the surgical findings (252). Thus, a significant number of pituitary microadenomas are not visualized on MRI and preoperative localization is not always possible by this technique. Furthermore, BIPSS is far from completely reliable for this purpose (discussed above). In an effort to improve localization, the use of intraoperative ultrasonography has recently been proposed (258, 259). In the most recent of these reports (259), 4 of 18 corticotroph microadenomas were clearly identified by preoperative MRI in a position that correlated with the surgical findings, while two further adenomas identified preoperatively were found at the time of surgery to be at a different site. In contrast, 13 of 18 were disclosed on intraoperative ultrasound as hyperechoic masses; this technique, which is very operator-dependent, had no complications. In this study no details were given of the results of BIPSS, but it appears from this small series that intraoperative ultrasound may prove useful in the localization of pituitary microadenomas and further highlights the limitations of MRI for this purpose.

B. Adrenal

Adrenal imaging plays an important role in the diagnostic workup. In many circumstances a cortisol-secreting adrenal tumor will be obvious. The distinction between adrenal adenoma and carcinoma is based on the evidence of vascular invasion or metastases, but tumors greater than 6 cm in diameter on scanning should be regarded as malignant (4). In a review of 13 studies Fig *et al.* (260) reported that 14 patients with adrenal carcinoma and 70 patients with adrenal adenoma causing Cushing's syndrome were all correctly identified by computed tomography (CT) of the adrenal glands. Unfortunately, imaging is not always so clear-cut, and some degree of nodularity of the glands may be apparent (13). Since a unilateral cortisol-secreting adrenal tumor will result in suppression of ACTH secretion, the remaining ipsilateral and contralateral adrenal gland should be atrophic in appearance, and if any degree of hypertrophy is present, the possibility of asymmetric macronodular hyperplasia should be considered (261). Careful scrutiny of abdominal imaging is required to avoid the inappropriate unilateral excision of bilateral disease masquerading as an adrenal adenoma. Rarely, ACTH-independent massive macronodular adrenal hyperplasia (weighing 69–149 g) may be present on

imaging with complete replacement of both adrenal glands, lack of a central to peripheral ACTH gradient on BIPSS, and an absence of an adenoma on MRI of the pituitary gland; in such cases bilateral adrenalectomy is indicated (96). However, macronodular hyperplasia may exist in Cushing's disease, although usually with not so dramatic appearances, and in all cases of ACTH-dependent Cushing's syndrome the adrenal glands may be bilaterally or unilaterally hyperplastic (261), with or without nodularity, although this may not be present in one third of cases (96). In such cases, further detailed and careful biochemical evaluation is crucial (*vide supra*).

C. Ectopic secretion

Small-cell lung cancer and bronchial carcinoid tumors are the most common source of ectopic ACTH secretion. Although the former is usually obvious, the latter may prove extremely difficult to localize (4, 9, 96). High-resolution CT scans may reveal small bronchial carcinoid lesions inapparent on plain radiography (Fig. 2) (7, 132, 262, 263), and since bronchial carcinoid tumors are usually 1 cm or less overlapping cuts of 1 cm or less should be employed (4)(Fig. 2). Small bronchial carcinoid tumors may, however, be confused with pulmonary vascular shadows. We have found that imaging the thorax in both supine and prone positions is a simple and extremely effective means of resolving this diagnostic difficulty, since vascular shadows change and tumors do not. MRI seems to be an improvement over CT for this purpose and results in improved discrimination: scanning in 10 patients with surgically proven bronchial carcinoid tumors demonstrated high signal intensity on T2-weighted and short-inversion-time inversion recovery images in all, while CT scanning was equivocal in two (264). Thymic carcinoid tumors causing Cushing's syndrome are generally larger than 2 cm and readily visualized by CT (244). Although the most common site for an ectopic source of ACTH secretion is in the chest, many varied sites may ultimately be localized (Table 2). Therefore, it may be necessary to perform extensive CT scanning of the abdomen to disclose, in particular, pancreatic islet cell tumors, intestinal carcinoid tumors, and pheochromocytomas (262). Islet cell tumors causing Cushing's syndrome are frequently large and have usually metastasized by the time of diagnosis and imaging (265), which presumably relates to the relatively late secretion of ACTH in the natural history of these tumors.

A lesion disclosed on abdominal and chest imaging in the search for the source of ectopic ACTH or CRH secretion does not necessarily prove functionality. Furthermore, despite extensive imaging, many tumors remain occult (4, 9, 262). Many carcinoid tumors, small-cell lung cancers, and medullary cell carcinomas of the thyroid express high numbers of high-affinity somatastatin receptors (266). It has therefore been proposed that the use of radiolabeled octreotide may be of use to confirm functionality (267) or disclose occult lesions not visualized by other means (268). In this latter series, although 8 of 10 patients with ectopic ACTH syndrome were disclosed on somatostatin receptor scintigraphy, all were apparent on conventional imaging; therefore, the use of somatostatin receptor scintigraphy has been challenged since it did not reveal any lesions that were not disclosed by conventional imaging modalities (238). It remains to be seen whether improvements in this technique will allow disclosure of truly occult lesions.

X. Differentiation from Pseudo-Cushing's States

The differentiation between mild Cushing's syndrome and pseudo-Cushing's syndrome can prove extremely difficult

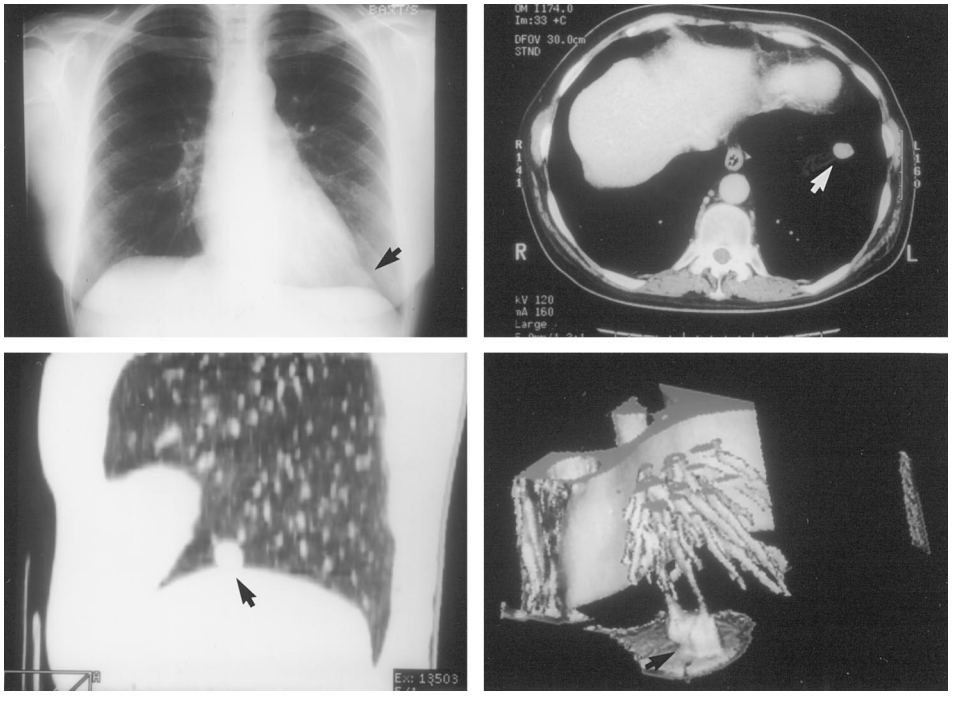

FIG. 2. Imaging of the thorax in the ectopic ACTH syndrome. A, Plain chest radiograph demonstrating suspicious lesion behind the left heart border (*arrow*). B and C, Axial and sagittal CT images demonstrating a bronchial carcinoid tumor (*arrow*) abutting the diaphragm. D, Three-dimensional reconstruction illustrating adherence of the tumor to the diaphragm (*arrow*), which was confirmed at surgery.

and poses a considerable challenge to the physician. A pseudo-Cushing's state may be defined as some or all of the clinical features that resemble true Cushing's syndrome together with some evidence of hypercortisolism, but resolution of the underlying primary condition results in the disappearance of the Cushing's-like state. Such findings may appear particularly in patients with depression and alcohol-induced pseudo-Cushing's syndrome. The recently recognized heritable generalized glucocorticoid resistance, due to mutations in the ligand binding domain of the glucocorticoid receptor (269–272), may also be a source of confusion. Since there is diminished feedback by glucocorticoids, the level of ACTH, and hence cortisol levels, is high. While some individuals may be asymptomatic, others may present with varying degrees of hypertension, with or without hypokalemia and weakness (272), through the action of salt-retaining steroids and the saturation of 11β-hydroxysteroid dehydrogenase by cortisol (118). Hyperandrogenism may exist because of the high ACTH drive, and in women this may cause particular diagnostic difficulty since the clinical features including acne, hirsutism, oligomenorrhea and amenorrhea, are often those seen in Cushing's syndrome. However, the classic end-organ effects of glucocorticoid excess, including thinning of the skin, proximal myopathy, easy bruising, and early onset osteoporosis, are not usually present, and these features, as well as a family history, are therefore useful in the differentiation of these conditions. Furthermore, although resistance to dexamethasone will be observed, there is usually preservation of the normal circadian rhythm of cortisol secretion, albeit at a higher set point, and therefore circadian rhythm studies may be of use for purposes of differentiation.

The depression associated with Cushing's syndrome has been reported as most typically being agitated in nature (92). Thus, such a history may aid the clinician, but is by no means invariable. Previous photographs may help to illustrate the progression of signs in patients with Cushing's syndrome, while lack of such progression, especially over many years, is more in keeping with a pseudo-Cushing's state. If the Cushingoid features and biochemistry are mild, and doubt exists as to the exact diagnosis, one approach is to treat the depression, with close clinical follow-up to establish whether or not the Cushingoid features resolve.

In both Cushing's syndrome and pseudo-Cushing's states there is prevailing hypercortisolemia, and hence there may be almost complete overlap between groups on basal 24-h UFC collections (58). When the results seen during investigation of individuals with Cushing's syndrome and pseudo-Cushing's states are compared directly, a value of UFC above 100 nmol/liter on the second day of a 48-h 2 mg/day LDDST gave a specificity of 100% and a sensitivity of 56% for the diagnosis of Cushing's syndrome, while a 48-h plasma cortisol of 38 nmol/liter or more gave a specificity of 100% and a sensitivity of 90%. In contrast, although patients with depression usually demonstrate a blunted response to the administration of CRH, there is a large overlap with the responses seen in patients with Cushing's disease, and thus testing with this peptide does not provide good discrimination (30, 122, 157, 273). In an effort to further improve diagnostic accuracy, it has recently been suggested that improved discrimination between Cushing's syndrome and pseudo-Cushing's states may be achieved by using a combined test with the administration of CRH after the 48-h, 2 mg/day LDDST, with a response to CRH being seen in individuals with Cushing's syndrome but not in those with pseudo-Cushing's states and a mild degree of hypercortisolism (58). In this retrospective study there was complete discrimination between patients with Cushing's syndrome and pseudo-Cushing's states, and it has thus been recommended for this purpose (274). Although the basal UFCs showed almost total overlap between Cushing's syndrome and pseudo-Cushing's groups (see above), emphasizing the similar biochemical pictures seen in these groups, with postinjection of CRH 100 μg iv, a plasma cortisol value at 15 min of greater than 38 nmol/liter (1.4 μg/dl) was seen in all patients with Cushing's syndrome, but in none with a pseudo-Cushing's state, giving it a sensitivity and specificity of 100%. It is interesting to note that the Cushing's syndrome group comprised 35 patients with Cushing's disease, 2 with the ectopic ACTH syndrome and 2 with primary adrenal pathology: there was complete overlap of plasma cortisol values at 15 min after CRH stimulation, and thus this test cannot be used for the differential diagnosis of ACTH-dependent Cushing's syndrome. Furthermore, many plasma cortisol RIAs will have poor precision at this level of cortisol, and care is needed in application of this test. A prospective follow-up report on a further 98 patients resulted in a specificity of 96% and sensitivity of 98% since 2 patients were misclassified (275). It thus seems likely that, although imperfect, this may prove to be a useful test in differentiating Cushing's syndrome from mild secondary hypercortisolism. Slightly inferior discrimination has been reported for a combined 1 mg overnight dexamethasone suppression test followed by a 10-IU LVP stimulation test during testing of 34 patients with Cushing's syndrome, 18 normal controls, 4 depressed subjects, and 5 with a 'Cushingoid' appearance, with an 88.9% sensitivity and 100% specificity for Cushing's syndrome (276).

In depressed patients, although there is often loss of suppression on a LDDST and a loss of the normal circadian rhythm of cortisol, there is usually a cortisol response to adequate insulin-induced hypoglycemia, while such a response is seen in only 18% of patients with Cushing's syndrome (51, 87, 88, 277). In certain patients with pseudo-Cushing's syndrome associated with depression, the insulin tolerance test may be useful, although overlap clearly exists. Because of the insulin resistance induced by elevated serum cortisol, the use of 0.3 U/kg iv of soluble insulin for the insulin tolerance test, if used, is recommended for this purpose (278).

The use of the opiate agonist loperamide has also been suggested for the purpose of discriminating between Cushing's syndrome and pseudo-Cushing's states. The test involves the oral administration of 16 mg loperamide at 0800 h, with plasma cortisol measured 3.5 h later. Loperamide causes the inhibition of CRH (279) and thus a suppression of plasma ACTH and cortisol in normal individuals but not in Cushing's syndrome (280–283). When available data from these reports are combined, a total of 49 patients with Cushing's syndrome (42 with Cushing's disease, 2 with the ectopic ACTH syndrome, and 5 with hyperfunctioning adrenal tu-

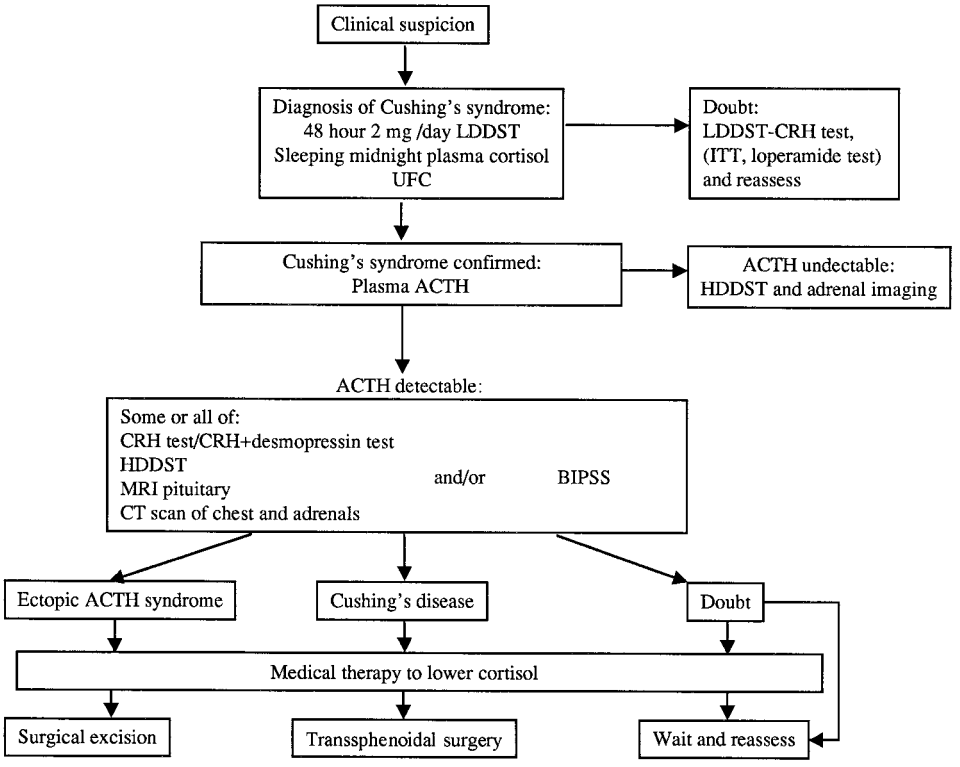

FIG. 3. A diagnostic approach to the diagnosis and differential diagnosis of Cushing's syndrome.

mors) showed no suppression below 138 nmol/liter (5 μg/dl) in plasma cortisol, while 128 of 138 normal individuals, obese subjects, and individuals with pseudo-Cushing's syndrome (including depression), suppressed below this level. Therefore, this gives the test a sensitivity of 100%, and a specificity of 93%, and as such it is comparable with the 1 mg overnight dexamethasone suppression test. It may prove particularly useful in the differentiation of Cushing's syndrome from depressed individuals, since suppression on loperamide was documented in certain of these patients in whom there had been no suppression on dexamethasone testing. At present, the numbers of individuals with Cushing's syndrome tested in this way remain small and more data are needed, especially on the responses seen in depressed individuals. The opiate antagonist naloxone has also been used for this purpose, with administration resulting in diminished stimulation in plasma ACTH and cortisol in patients with Cushing's syndrome (284). The numbers in these studies are small, and further data are needed for formal evaluation of this approach. [Naloxone has also been used in BIPSS testing in small numbers of patients with Cushing's disease (285).]

Recently, the use of desmopressin has been reported in a comparison between the effects seen on plasma ACTH and cortisol in women with Cushing's disease, depressed women, and normal female controls (286). After administration of desmopressin 10 μg iv, 14 of 14 patients with Cushing's disease exhibited a rise in plasma cortisol of 36% (4 times the intra-assay coefficient of variation), while such rises were seen in 2 of 20 normal subjects and 4 of 11 patients with depression. No systematic ACTH responses to this peptide were observed in normals or depressed patients, whereas a rise was seen in all the patients with Cushing's disease; as such, the responses resemble those seen in this context after CRH administration (30, 122, 157). It should be noted, however, that some patients with Cushing's disease fail to respond to desmopressin (186, 188, 192). Thus, it seems possible that differentiation between Cushing's disease and depression, of high specificity but lower sensitivity for Cushing's disease, may be made using this peptide on the ACTH, but not cortisol, responses.

Patients with alcohol-induced pseudo-Cushing's syndrome may cause diagnostic difficulty, with biochemical evidence of hypercortisolemia, resistance to dexamethasone, and loss of the normal circadian rhythm of cortisol secretion (18–22). A detectable blood alcohol level will be of great use for discrimination from Cushing's syndrome. Admission of the patient to an acute investigation ward may allow closer observation, and in patients with alcohol-induced pseudo-Cushing's syndrome, the sleeping midnight plasma cortisol value has been shown to become undetectable within 5 days, effectively excluding Cushing's syndrome (18).

XI. Conclusions

From the above discussion it is clear that many approaches are used in the diagnosis and differential diagnosis of Cushing's syndrome, with some being more valid than others. Ideally, the minimum number of investigations should be employed that allow accurate diagnosis and further management, and if at all possible these should be noninvasive. Since no single test is perfect, combinations of tests are employed to build up an overall picture, as even invasive in-

vestigations such as BIPSS fail to yield 100% diagnostic accuracy. Since Cushing's syndrome has a high morbidity and mortality, and the accuracy of diagnosis is paramount, we would argue that more, rather than fewer, tests should be employed in any given patient, as they may not fit the statistical sensitivities and specificities detailed above. Moreover, we regard the investigation as urgent, and to ensure complete diagnostic rigor we routinely admit patients to our acute investigation ward for their initial and subsequent diagnostic work-up. We believe that such an approach, although more expensive in the short term, stands a greater chance of success in each individual patient.

Biochemical confirmation of Cushing's syndrome is best achieved through the use of the 48-h, 2 mg/day LDDST, sleeping midnight plasma cortisol, and UFC measurements. Our routine practice uses the LDDST and midnight plasma cortisol assessment. In cases of doubt, and subtle hypercortisolemia, the LDDST-CRH test or the loperamide test may be useful.

Once Cushing's syndrome is confirmed biochemically, measurement of ACTH is required by either a sensitive RIA or IRMA. If ACTH is undetectable, attention may then be turned to the adrenal. If ACTH is clearly detectable, many physicians may opt to proceed directly to BIPSS and pituitary imaging, with subsequent imaging being determined by the results of these investigations. An alternative strategy is to perform BIPSS only in cases in which the results of the HDDST (the 8 mg/day, 48-h test is superior to the 8-mg overnight test), CRH test, or CRH *plus* desmopressin test are equivocal, especially if a pituitary lesion is visible on imaging, and CT or MRI of the chest is normal. Confirmation of a central source of ACTH on BIPSS is extremely reassuring, particularly in cases where early reoperation is necessary with a view to total hypophysectomy, considering the long-term morbidity of hypopituitarism and the recently established adverse effects of adult GH deficiency. Therefore, it is our policy to rely most heavily on the results of BIPSS, which is performed in most patients with ACTH-dependent Cushing's syndrome.

All functional and physical modalities for the preoperative lateralization of a pituitary microadenoma are, unfortunately, disappointing. It is clear that complete examination by an experienced surgeon of the entire pituitary gland may be necessary if the tumor is not immediately encountered, and intraoperative ultrasound may prove in the future to be a valuable aid in this respect. In the absence of a clearly visible pituitary lesion, hemihypophysectomy on the basis of BIPSS data may not yield a surgical cure. Figure 3 illustrates a possible diagnostic approach that encompasses a direct route via BIPSS but also emphasizes peripheral testing.

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