THE PITUITARY

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CHAPTER

16

Cushing's disease

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PATHOPHYSIOLOGY

Cushing's syndrome refers to the manifestations of chronic glucocorticoid excess and may result from various causes (Table 16.1). In Cushing's disease pituitary adrenocorticotropic hormone (ACTH) oversecretion induces bilateral adrenocortical hyperplasia and excess production of cortisol, adrenal androgens and 11-deoxycorticosterone, which together provoke the clinical and biologic features of the disease.

EPIDEMIOLOGY

Cushing's disease is the most frequent cause of spontaneous Cushing's syndrome in adults. In most series its prevalence is approximately 70% with a definite female preponderance, the female/male ratio ranging between 3:1 and 10:1 [1–4]. In our series of 809 adult patients with spontaneous Cushing's syndromes, Cushing's disease accounts for 68% of the cases, and the female/male ratio is 2.8 (Table 16.2). Distribution of the age at diagnosis shows a peak in adult females in the 25–45-year range (Figure 16.1).

In children, the causes of Cushing's syndrome have a different distribution. Primary adrenocortical tumors are more frequent and Cushing's disease accounts for about 50% of the cases. Children with this condition are usually older than 9 with an equal sex ratio [5–9]. Cushing's disease accounts for 50% of Cushing's syndrome and is almost always caused by a pituitary microadenoma. The commonest age of presentation of pediatric Cushing's disease is during adolescence, and there is a strong predominance of males in prepubertal patients. Ectopic ACTH syndrome is extremely rare, occurring much less frequently than in adults. Unilateral adrenal tumors are an important cause of pediatric Cushing's syndrome (about 40%) and are almost always adrenal carcinoma in children, with rarely a pure hypercortisolism, but usually associated virilization. Primary bilateral adrenocortical hyperplasia is a rare but important cause of pediatric Cushing's syndrome, usually associated with the Carney complex, and typically occurs in adolescence or early adulthood. A total of 398 cases of pediatric Cushing's syndrome are reported in the literature, with 182 Cushing's disease, 11 ectopic ACTH syndrome, 164 adrenocortical tumors, 16 McCune Albright syndrome and 11 primary bilateral adrenocortical hyperplasia. The peak incidence was 14.1 years in Cushing's disease, 10.1 years in ectopic ACTH syndrome, 4.5 years in adrenocortical tumors, 1.2 in McCune-Albright syndrome and 13 in primary bilateral adrenocortical hyperplasia [8].

Cushing's disease is rare; its true incidence, which varies with age and sex, is difficult to evaluate. Incidence data are available on pituitary [10,11] and adrenocortical [12,13] tumors. The prevalence of corticotroph tumors in the former [14] and that of Cushing's syndrome in the latter [15–17] provide an indirect means whereby the incidence of Cushing's disease may be roughly estimated to be in the range of 1–10 new cases per million per year. European population-based studies reported an incidence of newly diagnosed Cushing's disease of 0.7 to 2.4 cases per 1 million inhabitants per year [18,19]. In Vizcaya (Spain), the prevalence of known cases of Cushing's disease at the end of 1992 was

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TABLE 16.1 Causes of Cushing's Syndrome

SPONTANEOUS
ACTH-dependent
Pituitary ACTH oversecretion
Cushing's disease
Primary corticotroph
Anterior pituitary adenoma
Anterior pituitary mixed adenoma
Anterior pituitary cancer
Ectopic corticotroph adenoma
Multiple endocrine neoplasia type 1
Intermediate lobe pituitary adenoma?
Primary hypothalamic dysfunction
CRH-producing tumor
Hypothalamic CRH-producing tumor
Ectopic CRH-producing tumor
Nonpituitary ACTH oversection
Ectopic ACTH syndrome
Endocrine tumors
Mononuclear cells
Cortisol hyperreactive syndrome?
ACTH-independent (Adrenal Cushing's Syndrome)
Unilateral adrenocortical tumors
Adrenocortical adenoma
Adrenocortical carcinoma
Bilateral adrenocortical disorders
Primary pigmented nodular adrenal disease
ACTH-independent macronodular adrenal hyperplasia (AI-MAH)
Gonadal tumors
IATROGENIC
Exogenous Glucocorticoids
Exogenous Cortrosyn

CRH, corticotropin-releasing hormone.

39.1 per million inhabitants [18]. According to the Nationwide Inpatient Sample database, the largest all-payer inpatient care database in the US which contains data from approximately 8 million discharges annually from 1004 hospitals located in 37 states, there were an estimated 3525 cases of transsphenoidal resection of Cushing's disease between 1993 and 2002 [20].

Recent data suggest that Cushing's syndrome is more common than had previously been thought. In addition

TABLE 16.2 Etiology of 809 Spontaneous Cushing's Syndromes in Adults

	Number of Patients (%)	Female/Male Ratio
Cushing's disease	550 (68)	2.8
Primary adrenocortical tumor	199 (25)	4.2
Benign adrenocortical adenoma	111 (14)	0.5
Adrenocortical carcinoma	88 (11)	3.6
Ectopic ACTH syndrome	58 (7)	1.4
Primary adrenocortical nodular dysplasia	2 (0.2)	–

to the classical overt Cushing's syndrome, mild forms of Cushing's syndrome (named subclinical Cushing's syndrome or occult Cushing's syndrome) have been identified in patients with type 2 diabetes [21–24], hypertension [25], osteoporosis [26], and subjects with an adrenal incidentaloma. The reported prevalence of Cushing's syndrome is between 2% and 9.4 % in overweight type 2 diabetic patients and reaches 10.8% in patients with T-scores of 2.5 or less and vertebral fractures, although a final diagnosis could not to be confirmed in all patients. For instance, definitive mild Cushing's syndrome was identified in four patients (2%) among 200 overweight, type-2 diabetic patients with poor metabolic control (HbA1C > 8%), with three Cushing's disease and one surgically proven adrenal adenoma. Definitive diagnosis remains to be established in seven additional patients (3.5%) [21].

CHRONIC ACTH AND PROOPIOMELANOCORTIN (POMC) PEPTIDE OVERSECRETION BY THE PITUITARY

Normal Synthesis and Secretion of ACTH

Mechanisms of ACTH Biosynthesis

The mechanisms of ACTH biosynthesis have been fully elucidated in the last 30 years; a high-molecular-weight ACTH-precursor molecule was identified and characterized in the ACTH-producing AtT-20/D16-v mouse tumor cell line [27]. Recombinant DNA methods unravelled the primary structure of this precursor [28] – called POMC – in many species including humans [29–31]. This is fully described in Chapter 2.

The overall mechanism of POMC gene expression in man is shown schematically in Figure 16.2; a single POMC gene per haploid genome is present on the distal region (2p23–25) of the short arm of chromosome 2 [32,33]; it consists of three exons, the coding regions

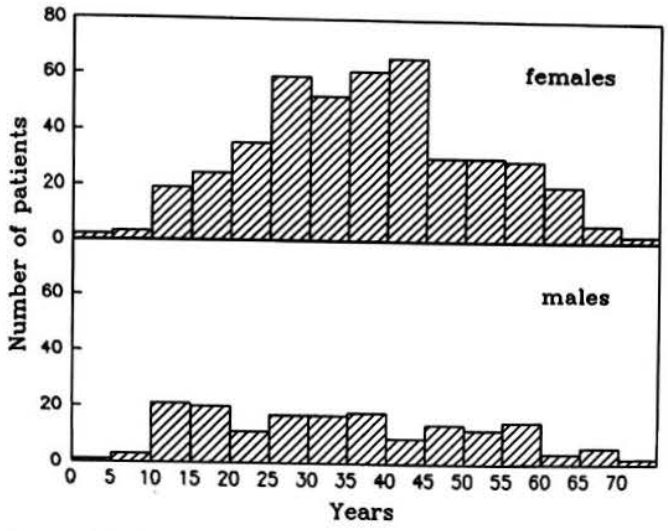

FIGURE 16.1 Patient age at the time of diagnosis of Cushing's disease.

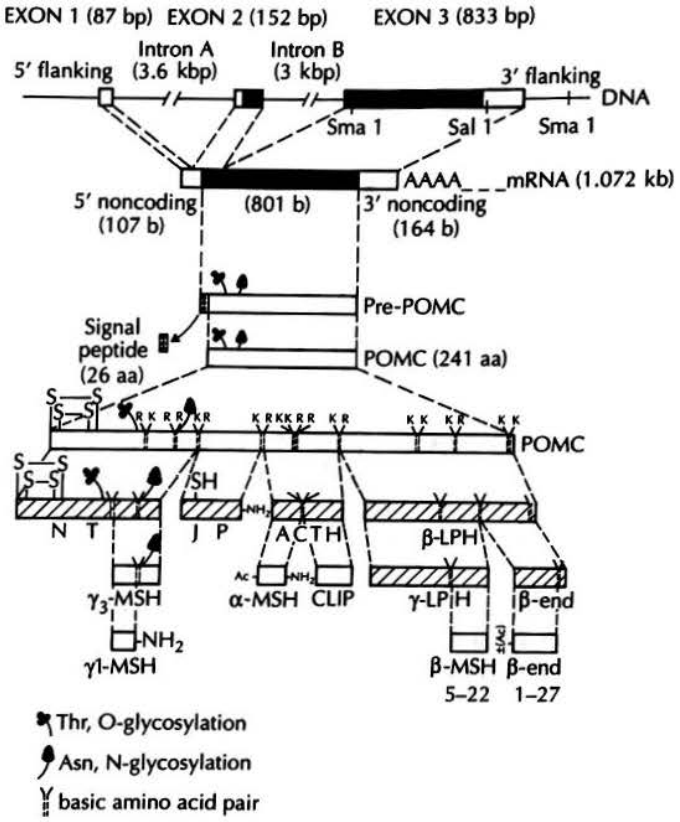

FIGURE 16.2 Schematic view of human proopiomelanocortin (POMC) gene expression. Black bars denote the protein-coding regions of the DNA and messenger RNA (mRNA). Hatched bars denote the peptide fragments found in the human anterior pituitary.

(in black) being present only on exons 2 and 3. After the splicing of the primary transcript a mature messenger RNA (mRNA) of 1072 nucleotides (nt) is generated and a poly (A)+ tail of about 200 nt is added. A pre-POMC molecule is first translated starting with a 26-amino acid signal peptide necessary for the translocation of the nascent protein through the membrane of the rough endoplasmic reticulum. Within the Golgi apparatus and the secretory granule the POMC molecule undergoes a series of proteolytic cleavages and chemical transformations which together result in the maturation or processing of the precursor [34]: proteolysis occurs at pairs of basic amino acids. Among the nine potential cleavage sites of the human POMC only four are utilized in the anterior pituitary, generating the N-terminal fragment [35,36], the joining peptide [37–39], ACTH [40–42], β-lipotropin (β-LPH), and a small amount of γ-LPH and β-endorphin (β-end) [42,43]. Other chemical transformations include glycosylation of the N-terminal fragment [44], C-terminal amidation of the joining peptide [38,39,45,46], and partial phosphorylation of ACTH on Ser_{31} [47,48]. An alternate mode of nonprimate POMC processing takes place in the intermediate lobe of the pituitary, releasing smaller peptides such as β-melanocyte-stimulating hormone (β-MSH), corticotrophin-like intermediate lobe peptide (CLIP) and α-MSH [49,50]. It does not normally occur in the human pituitary where the intermediate lobe is only fully present in the fetus [51].

POMC gene expression also occurs in many normal nonpituitary tissues [52,53]; it does so at a very low level and predominantly through an alternate mode of gene transcription [54–56], generating negligible amounts of POMC peptide [57–59]. It is assumed that the highly predominant, if not the sole, source of circulating ACTH and POMC peptides in humans, under normal circumstances, is the anterior pituitary corticotroph cell.

The coordinate proteolysis of POMC and the equimolar secretion of the various POMC peptides has two implications: any of the non-ACTH POMC peptides can be assayed in blood as an alternate marker of the overall pituitary corticotroph activity; a specific pattern of POMC peptides is associated with the pituitary corticotroph, and any qualitative abnormality suggests a pathologic nonpituitary source [60–62]. Yet, in highly aggressive, poorly differentiated pituitary corticotroph adenomas, decreased expression of convertases indicates that intact POMC may be secreted; these adenomas are therefore often "silent" [61,63].

Regulation of ACTH Secretion

The normal circadian rhythm of plasma cortisol is directly driven by pituitary corticotroph activity [64–66]. Its pattern is derived from variations in the number and amplitude of episodic ACTH bursts [67,68]. Pituitary corticotroph activity increases in the second half of the night, around 2–4 a.m., peaks on waking and gradually falls during the morning [69]. Various physical and psychologic stresses can interrupt this normal rhythm, at any time, with an acute rise in

ACTH. Both the normal circadian rhythm and the stress-induced changes are central nervous system (CNS) mediated under the primary – although not exclusive – control of hypothalamic corticotropin-releasing hormone (CRH) [70].

CRH [71] acts directly on the corticotroph cell through specific receptors that activate the adenylyl cyclase and increase intracellular cyclic adenosine monophosphate (cAMP) formation [72,73]. Arginine vasopressin (AVP), through its own specific V_1 type receptors, also acts on the corticotroph cell to activate phospholipase C, leading to increased phosphoinositide turnover, Ca^{2+} release and protein kinase C activation [74]. The action of AVP potentiates that of CRH [75] by further increasing cAMP formation [76–78]; cross-talk between the two transducing systems provides the synergistic action that promotes the maximal ACTH response by increasing both POMC gene transcription and secretory granule exocytosis [79–81]. This phenomenon, thoroughly studied in vitro on animal models, is also observed in humans; the simultaneous administration of CRH and AVP (or its analogue lysine vasopressin, LVP) induces a maximal ACTH rise, higher than that obtained by either secretagogue alone or their sum [82–84]. The specific AVP receptor of the corticotroph cell was recently cloned; this V3 (or V1b) receptor is closely analogous to the V1a receptor [85,86]. Interestingly, the AVP analogue, DDAVP or desmopressin, also has definite affinity for the V3 receptor, explaining that it is a powerful stimulator of ACTH secretion in a vast majority (ca. 85%) of patients with Cushing's disease [87].

Glucocorticoids exert a negative feedback on pituitary ACTH [88]. In patients with primary adrenal deficiency, basal and stimulated ACTH are increased. On the other hand, excess glucocorticoid administration or secretion by a primary adrenocortical tumor inhibits basal and stimulated ACTH. Prolonged glucocorticoid suppression of the hypothalamic–corticotroph axis characteristically induces long-lasting unresponsiveness, which may extend for months or years after the source of excess glucocorticoid has been withdrawn. Glucocorticoids inhibit hypothalamic CRH production [89] and also act directly at the corticotroph cell, as demonstrated in various animal models. They inhibit basal and stimulated ACTH release [90,91], as well as POMC gene transcription in a dose-dependent manner [92]. Interestingly this inhibition is not complete and a small proportion of POMC transcription is not suppressed, even by very high amounts of glucocorticoids [80,93].

A proposed neuro–immuno–endocrine loop is emerging which suggests that corticotroph function not only acts on immunocompetent cells – through cortisol production – but is itself the target of various immunomodulators [94]. Data obtained in the rat show that interleukin-1 and -6 both exert a stimulatory action on ACTH release at the hypothalamic and pituitary levels [95–97]. It is suggested that they participate in the physiologic ACTH rise in acute infectious stress, as they experimentally mediate that which occurs after bacterial lipopolysaccharide injections. Both cytokines are normally present in the rat anterior pituitary, apparently in a subpopulation of thyroid-stimulating hormone (TSH) cells and in folliculostellate cells for interleukin-1 and interleukin-6, respectively, thus raising the possibility that they act as local paracrine factors [98,99]. The role of the gp130-related cytokines, particularly LIF (leukemia inhibitory factor), has been convincingly established in the regulation of POMC expression and corticotroph cell development [100]. Further studies are obviously needed to establish the exact significance of these data, the effects of other regulatory peptides found in the pituitary [101] and their possible implication on the physiology and pathophysiology of ACTH release in humans [102,103].

Similarly PPAR gamma [104] and retinoic acid [105] play a role in POMC gene regulation, at least in animal models.

Oversecretion of ACTH in Cushing's Disease

Cushing's Hypothesis

The proposition that the pituitary was responsible for the clinical features of Cushing's disease was convincingly expressed for the first time in Harvey Cushing's classic monograph of 1932; the basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism) [106]. Cushing was recognizing that...

> ... striking clinical effects might be produced by minute, symptomatically predictable (pituitary) adenomas. So it is the degree of secretory activity of an adenoma which may be out of all proportion to its dimension, that evokes the recognizable symptom-complex in all hypersecretory states...

he was still wondering however:

> ... if the polyglandular features of the disorder are partly due, as premised, to a secondary hyperplasia of adrenal cortex ...

Much uncertainty remained at that time on the fine pathophysiologic mechanism of this disorder, yet the crucial clinical and pathologic observations had been made and the pertinent questions had been asked. Today it is recognized that chronic oversecretion of cortisol, androgens and 11-deoxycorticosterone by hyperplastic adrenocortical glands is directly responsible for the clinical features of Cushing's disease, a phenomenon which is primarily driven by pituitary ACTH oversecretion.

Demonstrating ACTH Oversecretion

When plasma ACTH became measurable by bioassay [107] it was found to be normal or slightly elevated in patients with Cushing's disease [108–110]. ACTH radioimmunoassay [64] came as an illuminating tool for the fine exploration of these patients.

A majority of patients with Cushing's disease have normal plasma ACTH values in the morning, although as a group their mean ACTH value is significantly higher than that of normal subjects [109,111–113]. However, even a normal ACTH value is inappropriately high or not normally restrained in view of the hypercortisolic state; repeated ACTH measurements over 24 hours show that patients with Cushing's disease have high evening values with a lack of the normal circadian rhythm [65,66,114]. Continuous sampling with a persistaltic pump has not been performed to study 24-hour integrated plasma ACTH, as has been done for cortisol [115,116]. The fragility of the molecule in blood probably precluded this approach, which might be performed by measuring other, more stable, POMC peptides such as β- and γ-LPHs [117].

ACTH Secretion is Dysregulated, not Autonomous

Besides being increased, corticotroph activity has acquired altered regulatory mechanisms that are the hallmark of Cushing's disease. Plasma ACTH – and cortisol – classically have lost their normal circadian periodicity; yet episodic fluctuations occur and in some cases a significant circadian variation may still be present (Figure 16.3) [66,118–120]. They are unresponsive to stress [121,122]; they have become partially resistant to the suppressive action of glucocorticoids [123]; they are – inappropriately – sensitive to the stimulatory action of CRH and/or AVP in spite of the hypercortisolic state [111,124,125].

These observations are of utmost importance. Not only do they provide the basis for pathophysiologic understanding of ACTH oversecretion, but they also support the rationale of the diagnostic procedures [126].

The Source and Mechanism of ACTH Oversecretion in Cushing's Disease

The Classic Anterior Pituitary Corticotroph Adenoma

That Cushing's disease is a primary pituitary disorder caused by a corticotroph adenoma is based on the frequency with which such adenomas are found at surgery and the histologic, biochemical and clinical evidences for a suppressed hypothalamic CRH.

PREVALENCE

Since the late 1970s many groups have reported the high frequency of pituitary microadenomas found at surgery in patients who were systematically subjected to sellar exploration by the transsphenoidal route, whether or not a pituitary tumor had been suspected by prior X-ray, computed tomography (CT) scanning, or, more recently, magnetic resonance imaging (MRI). As a rule such tumors are found in more than 80% of the cases [127–135]. Although small, and "silent," corticotroph tumors are sometimes found at autopsy of nonCushing's patients, the prevalence of such adenomas is definitively higher in patients with Cushing's disease [136].

HISTOLOGY

The basophilic adenomas of Cushing's disease have variable sizes; a large majority of them are microadenomas arbitrarily defined as being less than 10 mm with a mean of approximately 5 mm (Figure 16.4) [14,130,137]. Most are localized to a primary right- or

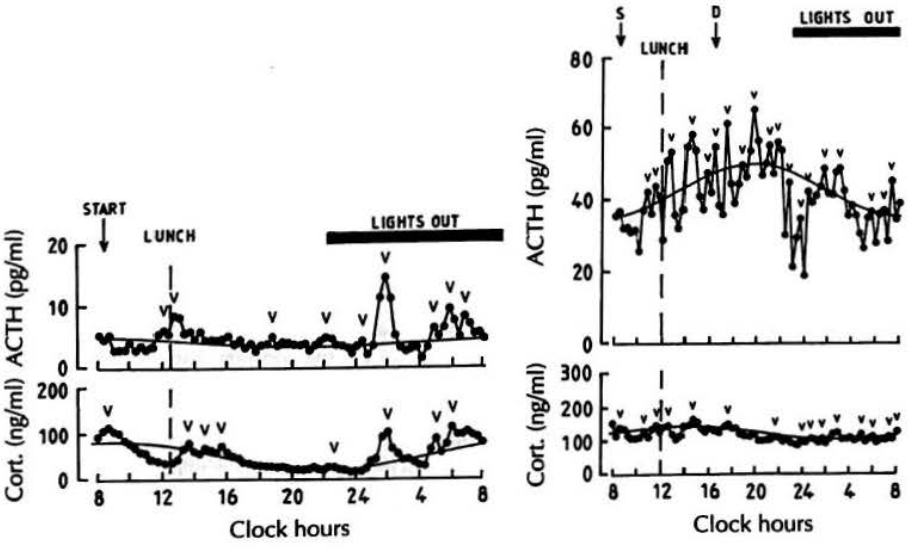

FIGURE 16.3 Twenty-four-hour profile of cortisol (Cort.) and adrenocorticotrophic hormone (ACTH) in a normal woman (left panel) and a woman with Cushing's disease (right panel). *From Liu et al. (54).*

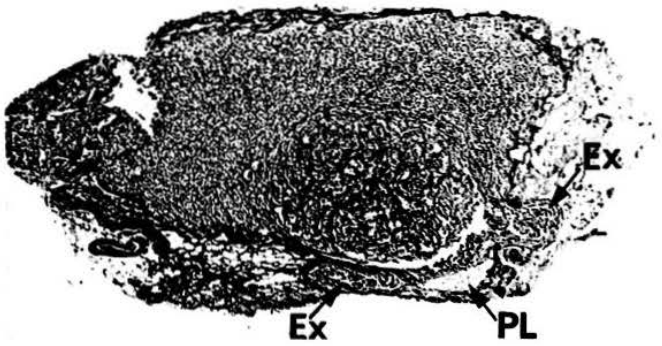

FIGURE 16.4 Pituitary gland from a necropsy in a patient with Cushing's disease (horizontal section x 10). A prominent microadenoma is located within the anterior lobe, in the vicinity of the posterior lobe (only a small portion of which is recognizable on this section: PL). Two invasive extensions (Ex) of the tumor are progressing within the neighboring tissues of the sella turcica. *Courtesy of L. Olivier.*

left-sided position within the gland, but a significant proportion (ca. 15%) are situated centrally [130]. Some are found outside the pituitary fossa and develop from the uppermost part of the pituitary gland (pars tuberalis), above the diaphragma, out of reach of the neurosurgeon via the transsphenoidal route, and several cases of Cushing's disease have been reported with an ectopic corticotroph adenoma [138–140] in the mucosa of the sphenoidal sinus [141], and even in an ovarian teratoma [142].

The classic basophilic adenoma is not encapsulated and is composed of compact cords of more or less homogeneous cells. The granule content is responsible for its basophilic and PAS-staining properties; the latter is now explained since non-ACTH POMC peptides (the N-terminal fragment) are glycosylated [44]. Electron microscopy shows secretory granules which are highly variable in size (from 100 to 700 nm) and in amount (Figure 16.5) [143]. Occasionally the paucity of the granule content explains why some adenomas appear chromophobe at the light microscope. Within the same tumor a variable pattern of granule load and size may be observed. In some adenomas [144] tumor cells show characteristic features of Crooke's cell [145], as depicted in the normal corticotroph of patients treated with corticosteroids: a ring-shaped homogeneous dense hyaline area constitutes an amorphous zone that repels the granules to the margin of the cell and close to the nucleus; ultrastructural studies show that it is made of filaments [146].

Immunocytochemistry has recently provided the ultimate means to recognize corticotroph cells by specific immunodetection of their content [143,148]. For a given antibody the signal is generally correlated to the cell granule load (Figure 16.6A, B). The sensitivity of the method sometimes allows the detection of an immune signal in what appeared to be a chromophobe adenoma [143]. Many adenomas will, unsurprisingly, react with different antisera directed against different POMC fragments, though some will respond only to a given antiserum. Although this type of observation may point to some peculiar mode of POMC processing in a particular tumor which would not generate a generally accessible epitope to the antibody, as has been described for example in endorphin adenomas [148], it should be kept in mind that different antisera may show variable sensitivities. More recently the specific recognizition of POMC RNA by in situ hybridization has been achieved in human corticotroph adenomas (Figure 16.6C).

The periadenomatous tissue shows a variable density of corticotroph cells, with frequent and typical Crooke's cells [146]. The coexistence of corticotroph hyperplasia and adenoma has been reported [149–152].

SUPPRESSED HYPOTHALAMIC CRH

A crucial clue to the pathophysiologic mechanism of ACTH oversecretion in Cushing's disease is that pituitary corticotroph adenomas are associated with a series of histologic, biochemical and clinical arguments that hypothalamic CRH is chronically suppressed: (1) histologically, examination of the periadenomatous tissue does not show – in the vast majority of the cases –

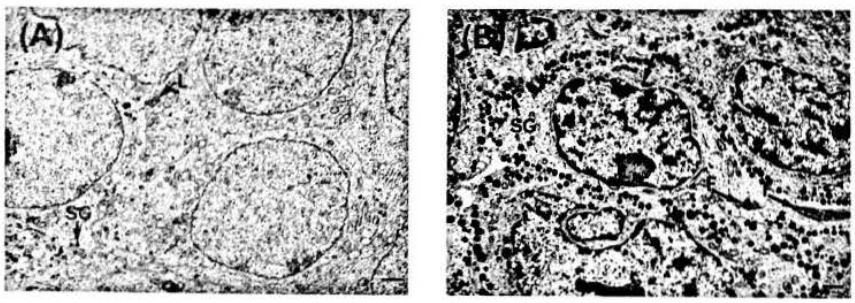

FIGURE 16.5 Ultrastructural study of two surgically removed microadenomas, exhibiting completely different cytological features (same magnification, bars = 1 μm). (A) This tumor is homogeneously constituted of poorly granulated cells (SG, secretory granules; L, lysosomal formations) with a large clear nucleus and a narrow ring of cytoplasm. (B) On the contrary, the second tumor is composed of granulated cells. The secretory granules (SG) vary in size, and are generally distributed along the plasma membrane. The tumor cells harbor a dense nucleus with a prominent nucleolus, and more or less developed bundles of filaments (F). *Courtesy of E. Vila-Porcile.*

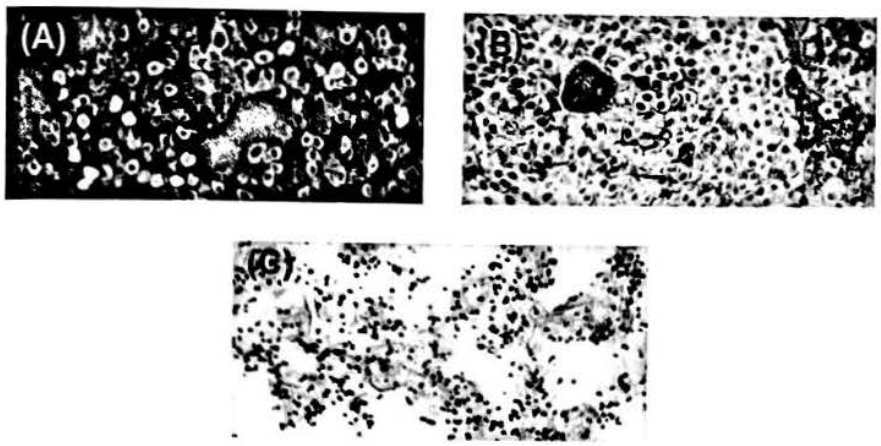

FIGURE 16.6 Cytologic study of surgically removed corticotroph microadenomas. (A) Immunofluorescence with an anti-$ACTH_{25-39}$ antibody; in this tumor, immunoreactive cells are scattered among unlabeled cells. Immunoreaction varies from cell to cell, and only concerns the cytoplasm, the nuclei thus appearing as dark dots (x350). (B) Immunofluorescence with an anti-β-endorphin antibody; in this second tumor, all the cells are heavily immunoreactive, and are densely clustered around a capillary (large dark area). The bright immunofluorescent labeling is homogeneous and is restricted to the cytoplasm (x350). *Courtesy of L. Olivier.* (C) In situ hybridization of human corticotroph tumor cells with a ^{32}P-labeled proopiomelanocortin (POMC) DNA probe. Diffuse hybridization signal indicated by the black silver grains localize high concentrations of POMC mRNA in the tumor cells. *Courtesy of P. L. Texier.*

specific evidence of corticotroph cell hyperplasia [153–155]; (2) biochemically, measurement of POMC peptides by various radioimmunoassays (RIAs) reveals low concentrations in periadenomatous tissue in comparison with the adenoma and also with normal human pituitaries [156,157]; and (3) clinically, suppressed hypothalamic CRH is supported by the lack of response to stress (insulin-induced hypoglycemia) in Cushing's disease in contrast to other situations of ACTH hypersecretion which are thought to be CRH-dependent (e.g., depression) [121,122]. It is supported also by clinical evaluation after selective pituitary surgery in case of both success and failure. In the former case, successful removal of the adenoma results in a state of selective pituitary corticotroph deficiency that spontaneously resumes its activity over months or years; all parameters of normality will be restored including perfect conservation of circadian rhythm [129,158–163]. Even the cases of failure are interesting; in such patients it was found that 24-hour urinary cortisol excretion and plasma ACTH were unchanged from preoperative values despite removal of a significant, generally half, portion of the anterior pituitary. This was taken as an indication that an adenoma was present but missed since, if the disease were due to diffuse corticotroph hyperplasia, it would be expected that partial hypophysectomy would have induced at least a partial decrease in ACTH and cortisol production [129].

POMC Gene Expression is Qualitatively Unaltered

Numerous studies performed in vitro show that in the vast majority of corticotroph adenomas the products of POMC gene transcription and of POMC processing are identical with those in the normal human anterior pituitary.

The gene transcription shows no gross abnormality and the POMC transcripts in pituitary tumors are similar to those in the normal pituitary [60,164–166]; a 1200-nt POMC mRNA is the highly predominant, if not sole, transcript (Figure 16.7). A small percentage (<5%) of transcripts result from an alternate mode of RNA splicing adding 30 nt at the 5′ end of the second exon. It has no implication on the open reading frame, which is not modified. Fewer than 1% of transcripts result from the use of an upstream promoter at −369 nt [167].

The N-terminal fragment [168], the joining peptide [38], authentic ACTH1–39 [40,41,61,62], β-LPH and variable amounts of γ-LPH and β-endorphin [62,169, 170] are the normal end-products of POMC processing

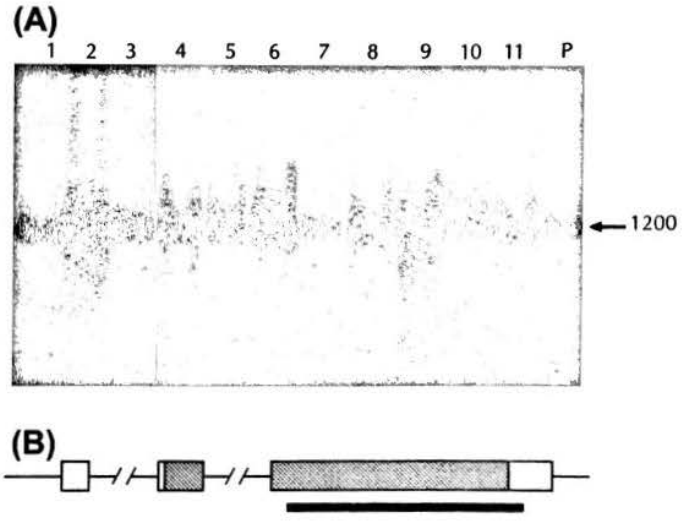

FIGURE 16.7 Northern blot analysis of RNAs from human pituitary corticotroph adenomas. (A) Lanes 1–11, 1 μg total RNA from pituitary tumors; lane P, 2 μg total RNA from a normal human pituitary. (B) In black, the pHOX3 probe used for hybridization corresponding to most of the coding region of exon 3.

found both in tumor extracts and in culture media. A somewhat higher proportion of β-endorphin over β-LPH − and γ-LPH over β-LPH − has been reported [60,158], yet the recruitment of proteolytic sites that are not normally activated in the normal pituitary is not observed and peptides like CLIP and α-MSH are neither produced nor released. This general finding supports the use of highly specific immunoradiometric assays (IRMAs) for plasma ACTH detection as a valid and significant means to evaluate patients with Cushing's disease [171]. In rare instances qualitative alterations of POMC gene expression have been described, in silent corticotroph adenomas and in pituitary cancers [172–174]; as already mentioned, poorly differentiated corticotroph adenomas may preferentially secrete intact POMC [63].

Thus, tumor POMC peptides, including ACTH, usually show no peculiar or unexpected molecular forms, in contrast with what is often found when POMC expression occurs in a nonpituitary tumor (Figure 16.8). Any of them can be used alternatively for clinical investigation, all their plasma values being highly correlated [175].

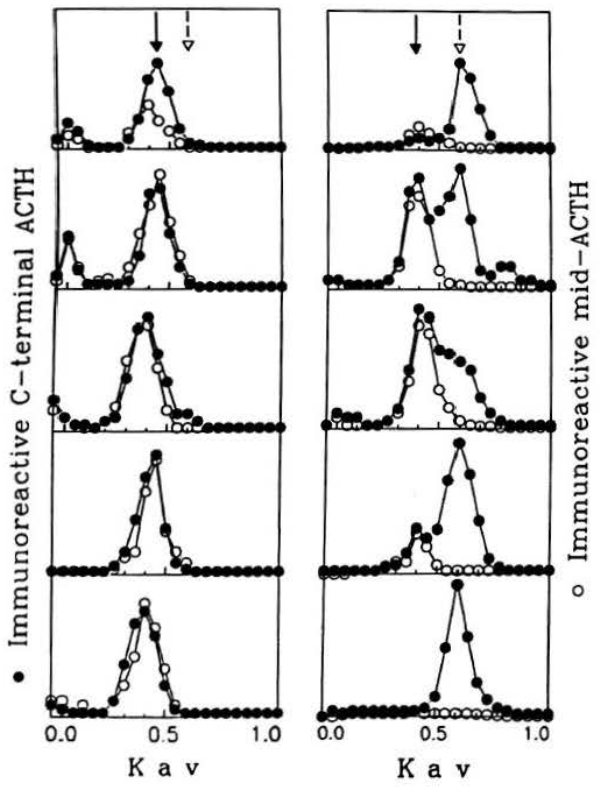

FIGURE 16.8 Immunodetection of adrenocorticotropic hormone (ACTH)-like peptides after Sephadex G-50 gel exclusion chromatography of tissue extracts. Left panel: only $ACTH_{1-39}$ is detected (↓) in one normal pituitary and four corticotroph adenomas. Right panel: variable amounts of corticotrophin-like intermediate lobe peptides (CLIP) (|) are present in five nonpituitary tumors responsible for the ectopic ACTH syndrome.

POMC Gene Expression is not Normally Restrained

THE DETERMINANTS OF ACTH OVERSECRETION

In a system normally regulated by a negative feedback loop, two determinants which are not exclusive of each other may theoretically provoke and maintain unrestrained hormone production: (1) the set-point defect at the cell level; and (2) the tumoral mass at the tissue level. These pathophysiologic mechanisms have been thoroughly studied in vivo and in vitro in various models such as primary hyperparathyroidism where the two determinants cooperate [176]. In the case of human corticotroph tumors, in vitro studies offer obvious difficulties: the latter tumors are much rarer, and direct comparison between the tumoral and the normal corticotroph cell is seldom achieved, yet a number of experimental and human studies provide insight for a pathophysiologic explanation of the phenomenon.

The Set-point Defect or Partial Resistance to Glucocorticoid

The hallmark of ACTH oversecretion in Cushing's disease is its partial resistance to the normal suppressive effect of glucocorticoids [123,177]. The dose–response curve between administered dexamethasone and plasma ACTH or endogenous cortisol production is shifted to the right (Figure 16.9). Because ACTH secretion by the pituitary tumor is not normally restrained, ACTH is overproduced, with subsequent chronic hypercortisolism. Since peripheral tissues have retained their normal sensitivity to the action of cortisol [178,179] they appropriately develop the features of Cushing's disease.

In vitro studies have confirmed that pituitary corticotroph adenomas are not autonomous and have indeed retained some sensitivity to the suppressive effect of glucocorticoids which invariably decrease basal and/or stimulated ACTH release [180–186]. A direct comparison between the responses of normal and tumoral cells in vitro is lacking most of the time. A single study measured the effects of two doses of dexamethasone (1 and 10 μg/dl) on both ACTH release and POMC mRNA content in cultured cells obtained either from corticotroph adenomas or from their (presumably normal) periadenomatous tissues. Whereas dexamethasone efficiently reduced both parameters in the periadenomatous cells, its suppressive effect was reduced in the tumoral cells [164].

Schematically, normal secretion of ACTH results from a fine equilibrium within the corticotroph cell between two opposite regulators with stimulatory (cAMP and protein kinase C pathways driven by CRH and AVP) and inhibitory (glucocorticoid pathway) actions. A subtle imbalance between the two regulators should lead to ACTH dysregulation and, in the case

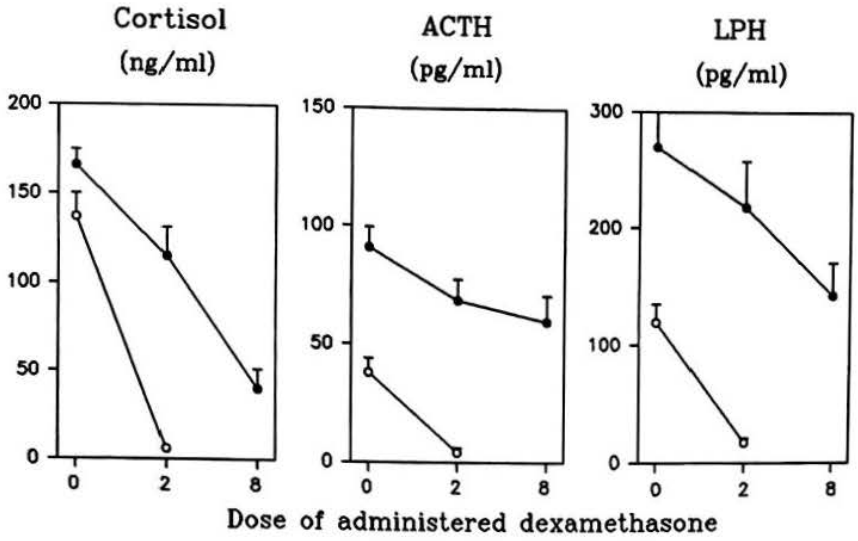

FIGURE 16.9 Variations (mean ± SEM) of plasma cortisol, adrenocortcotropic hormone (ACTH) and lipotropin (LPH) in response to increasing daily doses of dexamethasone administered for 2 days in 15 normal subjects (○) and in 16 patients with Cushing's disease (•).

overproduction, to an apparent state of resistance to glucocorticoids. Thus variable, and probably numerous, mechanisms may provoke a set-point defect. Other pathways may also play a role [100,104,105].

A gross abnormality in the nature of the glucocorticoid receptor in the tumoral corticotroph cell has not yet been demonstrated [187], although loss of heterozygozity at the glucocorticoid receptor gene locus may be frequent [188]. The recent elucidation of a molecular alteration responsible for the syndrome of general resistance to glucocorticoids [189] may pinpoint more precise targets for future studies on the DNA and/or mRNA coding for the human glucocorticoid receptor in the tumor. Alternatively, the functional activity of a structurally normal glucocorticoid receptor may be reduced by a variety of intracellular defects. Among many other causes, it is decreased in experimental animal models where v-mos and Ha-ras oncoproteins are overexpressed [190,191]; recent data have elucidated a general mechanism whereby the activated glucocorticoid receptor and the products of the protooncogenes c-*fos* and c-*jun* inhibit each other's action at the gene level [192,193].

A set-point defect might also be caused by the exaggerated activation of cAMP and/or protein kinase C pathways. In vitro studies on rat anterior pituitary cells show that whenever one of these two pathways is stimulated, ACTH suppression by glucocorticoids is diminished [80,90,194]. Increased cAMP formation in AtT-20 cells directly blunts the suppressive effect of glucocorticoids on POMC synthesis through the inhibition of glucocorticoid receptor binding to DNA [195]. A subset of human growth hormone (GH)-producing pituitary tumors is associated with increased production of cAMP [196]; it has been shown to result from the intrinsic activation of their G-protein by a single base mutation which suppresses the GTPase activity of the α-subunit [197]. This precise type of acquired generic alteration has so far not been found in corticotroph tumors [198]. Similarly, although a number of endocrine tumors occur in the Carney complex, including pituitary GH adenomas, no patient with Cushing's disease has ever been described; in that situation, increased activity of the cAMP pathway is induced by mutations of PRKAR1A, the regulatory subunit of PKA. This may be another indication that, in contrast with other types of pituitary adenomas, the cAMP pathway does not actually play a pathogenetic role in the origin of pituitary corticotroph adenomas [199]. An alternate hypothesis, already suggested for other types of endocrine tumors, is that the tumoral cell acquires an abnormal sensitivity to non-CRH hypothalamic neurohormones [136]. In vivo studies have claimed that various hypothalamic factors like thyrotropin-releasing hormone (TRH) and luteinizing hormone (LH)-releasing hormone (LHRH) would increase ACTH release in occasional patients [200–202]. The significance of these results suffers from the inescapable drawback of uncontrolled trials. Very few studies reported the data in vivo and in vitro in the same patient. Few cases, however, have been described which still make it possible that some rare tumor has acquired this unexpected sensitivity [182,203,204]. More and more growth factors also seem to play a role in an autocrine or paracrine fashion within the pituitary [101]; their involvement in the pathogenesis of tumor formation is not yet established.

Recent studies of growth factors, or using the transcriptome approach might hopefully shed some light on the still elusive pathophysiology of these tumors [205,206].

In the context of familial pituitary adenoma syndrome, corticotroph adenomas can be observed in some patients with MEN1 or mutated AIP (see below). These syndromes point to different, specific cell signaling pathways.

THE TUMOR MASS

At the tissue level, the mass of the tumor is another determinant of the final level of ACTH oversecretion. In vitro studies on rat anterior pituitary cells show that increasing glucocorticoids cannot totally suppress the rate of POMC gene transcription [80,93]. Although these studies should not be simply transposed to a pathologic human condition, it should not be totally ruled out that some inescapable POMC gene expression contributes to the unrestrained ACTH secretion, especially when the tumor mass becomes important.

Both clinical and experimental observations show that situations where maximal ACTH secretion is chronically solicited induce an increase in corticotroph cell mass. Rare cases of poorly controlled Addisonian patients have apparently developed pituitary enlargement [207–210]. A large increase in corticotroph cell area is observed in the anterior pituitary of adrenalectomized rats [211,212]. It is possible that glucocorticoids exert a direct inhibitory action on the growth of corticotroph cells [213], their deprivation being a direct stimulus for growth. It is thought, however, that corticotroph cell growth is driven by the action of CRH. Indeed the growth-promoting effect of various hypothalamic neurohormones is well documented, like that of GHRH on GH cells for example, which may proceed through the activation of cellular oncogenes [214]. Long-term administration of CRH in experimental animals also leads to corticotroph cell hyperplasia [215,216] and hypertrophy [211].

To explain the growth of a pituitary corticotroph adenoma on these grounds would imply two necessary conditions: (1) that the adenoma be sensitive to the action of CRH and (2) that CRH be present, as least at some time of the development of the adenoma. Pituitary corticotroph adenomas remain sensitive to the stimulatory actions of CRH [124,125,217,218] and AVP [110,219] in vivo. These actions are used as investigational tools to target the pituitary origin of ACTH oversecretion. In vitro studies have largely confirmed that the tumoral cells are the direct target of these secretagogues [180–184,220]. A synergistic effect of AVP has also been observed [183]. Quantitatively the responses of the tumor cells have rarely been compared to that of normal human corticotroph cells. A single study reported that nine of 16 such tumors had identical sensitivity to CRH as the paired nonadenomatous tissue, and seven had a lower sensitivity [182]. In addition to its action on adenylyl cyclase, CRH has been shown to modulate action potential firing and to increase intracellular calcium on cultured cells obtained from human corticotroph adenomas [221,222].

It may seem paradoxical to invoke a role for CRH in the growth of a corticotroph adenoma when much evidence suggests that it is suppressed in Cushing's disease. This contradiction is only apparent. It is conceivable that, although it is not originally responsible, CRH contributes, at least at the beginning of the disease, to the progression of a pituitary tumor resulting from a clonal event primarily responsible for a set-point defect with secondary tumor growth (Figure 16.10).

DOES A PITUITARY CLONAL EVENT LEAD TO BOTH A SET-POINT DEFECT AND TUMOR GROWTH?

The clonal origin of various human endocrine tumors has been recognized, based on the study of genetic markers borne by the X chromosome in female heterozygous patients. Recent techniques using DNA probes directed at various genetic markers (hypoxanthine phosphoribosyl transferase and/or phosphoglycerate kinase) studied the X-inactivation pattern in peripheral and tumoral tissues through the combined DNA digestion

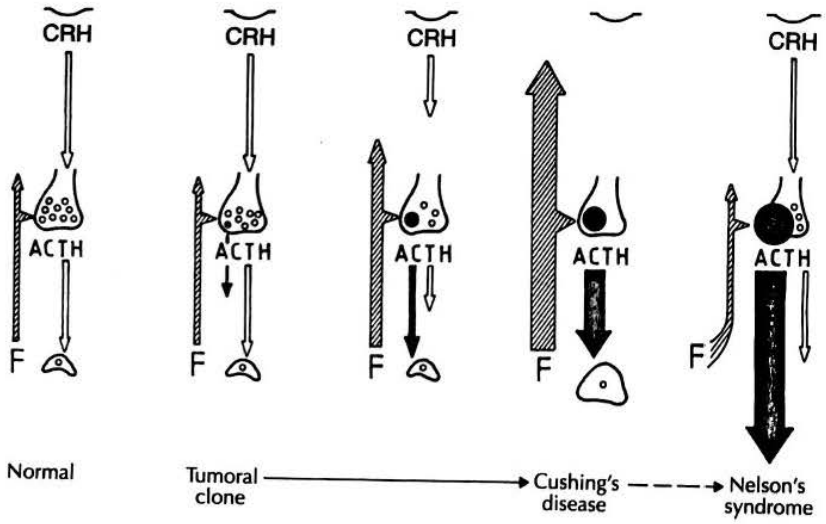

FIGURE 16.10 Pathogenesis of Cushing's disease. Schematic view of a tentative pituitary hypothesis. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; F, cortisol.

with a methylation-specific enzyme and the restriction enzyme giving rise to a restriction fragment length polymorphism. They have shown the monoclonal nature of all nonfunctioning pituitary tumors [223,224]. Recent studies performed on functioning tumors [225] showed a monoclonal pattern in three of three GH-secreting adenomas, four of four PRL-secreting adenomas, and three of four corticotroph adenomas; the fourth corticotroph adenoma was substantially contaminated by interspersed normal adenohypophyseal tissue that may have induced an apparent polyclonal pattern. More recent studies using another anonymous marker of the X chromosome, the M27β probe, have confirmed these results in pituitary corticotroph adenomas [226–228].

Whatever its mechanism, the occurrence of a state of partial resistance to glucocorticoids in a clone of pituitary corticotroph cells could theoretically have the following consequences (Figure 16.10).

1. At the beginning the small tumoral clone would secrete only a minor amount of ACTH with no subsequent increase in cortisol. Persistent CRH action on "cortisol-deprived" clonal cells would constitute an ideal stimulatory condition for their further growth. Thus the adenoma would develop and tumoral ACTH would progressively override nontumoral ACTH with subsequent increased cortisol production and ultimate extinction of hypothalamic CRH.
2. The full-blown expression of Cushing's disease would be attained. This scheme could be extended to the situation where bilateral total adrenalectomy would constitute a further stimulus for tumor progression by restoring normal cortisol (exogenously administered) and eventually CRH.
3. Two conditions that would concur again to stimulate the growth of the tumor, possibly leading to Nelson's syndrome. The set-point defect and the tumor growth potential would be linked. This scheme explains how CRH could have a transient role in the progression of the adenoma. It shows how at some time a pituitary might contain both an adenoma and still normal corticotroph cells, as has been occasionally seen on histologic examination [149–152].

Variants of the Anterior Pituitary Corticotroph Adenoma

PITUITARY CARCINOMAS

Although corticotroph adenomas often show invasive features and sometimes a high mitotic index, the existence of primary pituitary carcinomas is still a subject of debate. The presence of extraneural metastases is required for this diagnosis. Several such patients have been reported [229–232], including cases where immunocytochemistry of distant metastases (liver, bone) proved the presence of POMC peptides [232]. This situation is more frequently, but not exclusively, associated with Nelson's syndrome, occurs equally in males and females, and the mean age is relatively low. Caution should be exerted to eliminate the possibility that an occult nonpituitary tumor secreting ACTH has metastasized to different sites, including the pituitary. Because the growth aggressiveness of the pituitary tumor may be the ultimate prognostic factor [233], methods to predict it via the mitotic index, nuclear aneuploidy, or other means would certainly be helpful.

MIXED ADENOMAS

Concurrent secretion of another pituitary hormone by corticotroph adenomas, although rare, has been reported. It is seldom of clinical significance and most often a chance discovery through global immunocytochemical testing of the surgically removed tissue. Prolactin (PRL) [234], TSH, LH and α-subunits have been found to coexist in an occasional corticotroph adenoma [235].

Curiously, cholecystokinin (CCK) [236], neuromedin U [237], and more recently galanin [238], have been found to be specifically present in corticotroph adenomas as well as in normal human corticotrophs. The significance of this colocalization is unknown. Not unexpectedly other proteins which are ubiquitous biochemical markers of neurosecretory granules such as chromogranin A [239], secretogranin I and II, and 7B2 are present in all pituitary tumors, including corticotroph adenomas [240,241]. Whether they exert any peripheral, autocrine, or paracrine function also needs to be demonstrated. Some of them, like chromogranin A and secretogranin I, have numerous potential proteolytic sites that may be natural substrates for maturation enzymes. In several systems [242,243] including AtT-20 cells [244] a maturation product of chromogranin A, pancreastatin [242], exerts an inhibitory effect on the resident hormone release and may be part of an ultrashort autocrine negative feedback.

Other peptides such as parathyroid hormone (PTH)-related protein [245] and bombesin [246] have been occasionally found associated with pituitary corticotroph adenomas. Again the significance of these associations is not known. Because bombesin has been attributed to a growth effect it may be of pathophysiologic relevance.

SILENT CORTICOTROPH ADENOMAS

The development of immunhistochemistry revealed that some pituitary tumors unexpectedly contained immunoreactive ACTH, although the patients had no clinical or biologic evidence of hypercortisolism [247–250]; hence the name "silent" corticotroph adenomas. Most often these tumors present as macroadenomas, revealed clinically because of their space-

occupying effect. Thorough studies through electron microscopy have separated various subtypes; some are morphologically indistinguishable from the classical basophilic adenoma, others show subtle differences in granule size and loss of some type of microfilaments. In some tumors immunoreactive ACTH cells are rare and associated with other cell populations containing GH, PRL, LH, FSH and TSH [251].

No abnormality has been observed that would alter the quality of the POMC message [166]. The most likely explanation for the clinical silence relies on two molecular bases: the low levels of POMC mRNA on the one hand and the occasional alteration in POMC processing on the other [63,172,173]. However, some tumors show no evidence of any such abnormality. It is then speculated that an intrinsic traffic or export defect is responsible, as suggested by reports of increased lysosomal activity [248].

These tumors emphasize the notion that the growth and secretory activities of a corticotroph tumor need not be concordant. They raise the possibility that growth-promoting factors are operating which are not linked to POMC over-expression. Different types of mutations of Gαs have already been described in a subset of GH-secreting pituitary adenomas [197]. Thus it is likely that variability in the genetic causes of pituitary corticotroph adenomas will be the rule, with a range of consequences. At one end of the spectrum, a given mutation may essentially alter the sensitivity to glucocorticoid; at the other end another mutation may alter mainly the growth regulation leading to a silent corticotroph tumor.

It should be kept in mind that the clinical silence of these tumors should only be asserted after a well-designed hormonal investigation. The secondary occurrence of florid Cushing's disease has indeed been reported in patients who have harbored such tumors for many years [252–254].

Familial Pituitary Adenomas and Cushing's Disease

The multiple endocrine neoplasia type I (MEN I) is a familial disease transmitted as an autosomal dominant trait which combines the occurrence of tumoral lesions in the parathyroid, the pancreas and the pituitary, although all endocrine tissues may not be simultaneously involved [255]. Pituitary lesions have been reported to occur in 15–50% of the cases with a high predominance of PRL and GH hypersecretory syndromes and nonfunctional tumors [256,257]. In a recent study of 16 patients with Zollinger-Ellison syndrome and MEN I, three (19%) were found to have Cushing's disease with biologic and imaging evidence of a pituitary origin [258]. In the three cases the hypercortisolism was mild and might have escaped diagnosis had it not been systematically looked for. This is a unique situation where familial cases of Cushing's disease have recently been reported [259]. Cushing's disease of MEN I must be distinguished from Cushing's syndrome, which often occurs in the sporadic form of Zollinger-Ellison syndrome where an ectopic ACTH syndrome originates from the pancreatic tumoral lesion. The interesting feature of MEN I is the insight on tumor formation that has recently emerged. The pancreatic lesions are also characterized by islet-cell hyperplasia but with concomitant multifocal clonal tumors. Tumor transformation in the pancreas was associated with the deletion of specific DNA regions on chromosome 11, pointing to a possible new antioncogene responsible for endocrine tumor formation induced by the loss of its two alleles [260]. This specific gene alteration, also found in occasional sporadic pituitary tumors, was absent in four examined corticotroph adenomas [261].

The gene coding for aryl hydrocarbon-receptor interacting protein (AIP) was recently found to be involved in familial pituitary adenomas, predominantly GH-secreting [262]; yet, a minor subset of corticotroph adenomas may occur in mutated patients [263,264].

Interestingly, and as already mentioned, no Cushing's disease has been reported in patients with Carney complex [199], or in patients with CDKN1B mutations [264].

Intermediate Lobe Pituitary Adenoma

Besides the classic anterior pituitary corticotroph cells, a second type of POMC-producing cells form clusters of α-MSH immunoreactive cells arranged in follicles in the colloid cyst region in the human gland. They are thought to be the remnants of the fetal intermediary lobe and may generate the cellular cords that penetrate the pituitary posterior lobe [265]. The diversity of POMC-producing cells in the normal human pituitary logically suggested that each of them could give rise to a different subtype of pituitary adenoma with specific localization and secretory pattern, both in terms of POMC peptide molecular forms and dynamic regulation.

Supporting the hypothesis that some human corticotroph adenomas arise from remnants of the pars intermedia are two animal models of Cushing's disease: the dog and the horse both develop the disease spontaneously in association with tumors of the pars intermedia (in at least 30% of the dogs and in all cases of the horses) [136,266,267]. These tumors logically process POMC into essentially intermediate-like peptides; although ACTH is only a minor product, its plasma concentrations reach abnormal levels because of the high biosynthetic activity and the mass of the tumor. The tumoral secretion is unresponsive to the classical regulators of anterior pituitary corticotroph-like dexamethasone, CRH and

vasopressin. As expected, dopaminergic agonists are effective therapeutic agents [268,269].

Evidence for an identical subtype of intermediate-like pituitary adenoma in man has been advanced in a single study [270]. Based on histologic data showing the close association of argyrophil fibers with nests of tumoral cells, and the hormonal work-up showing responses more like those expected of an intermediate lobe tumor, it was suggested that these tumors arose from intermediate lobe remnants, were more often associated with hyperplastic lesions, were less amenable to surgical cure, and were driven by a general hypothalamic defect with decreased dopamine turnover. Investigation of a specific POMC peptide pattern was not performed, and others could not confirm these data [271,272].

Dopaminergic agents have long been claimed to be effective in an occasional patient [203,273], although controlled studies had rather shown that this condition was rare [274,275] and these patients did not necessarily harbor an intermediate lobe pituitary adenoma [276]. Yet more recent studies have added new insight on the presence of dopamine receptors in corticotroph adenomas, using direct assessment by in situ hybridization, quantitative evaluation of mRNA, and direct immunohistochemistry; functional D2 receptors are indeed present in a majority of corticotroph adenomas [277].

Hypothalamus-dependent Cushing's Disease

Although it is becoming increasingly clear that a vast majority of the patients with Cushing's disease harbor a corticotroph adenoma in their pituitary there are still some cases – including patients from Cushing's monograph of 1932 [106] – where pituitary lesions were apparently absent. Therefore two questions remain to be solved: (1) is there a subset of patients with Cushing's disease where pituitary ACTH oversecretion is caused by a primary hypothalamic dysfunction creating simple diffuse corticotroph hyperplasia? and (2) in the vast majority of patients who harbor a pituitary corticotroph adenoma, does the lesion result from prior hyperplasia caused by a primary hypothalamic dysfunction?

IS THERE A SUBSET OF PATIENTS WITH CUSHING'S DISEASE DUE TO PRIMARY HYPOTHALAMIC DYSFUNCTION?

Abnormalities in the quantitative and qualitative aspects of various CNS and anterior pituitary functions have been interpreted as evidence for a primary (and general?) hypothalamic dysfunction that would include CRH overproduction with subsequent ACTH oversecretion. Classically cited are the loss of normal ACTH circadian rhythm, the decreased suppressive effect of glucocorticoids, altered secretory patterns of GH, TSH, PRL and gonadotropins, and modified sleep electroencephalography (EEG) patterns [2,136,278]. A detailed statistical analysis (performed retrospectively on already published studies) showed that the distribution of parameters of plasma cortisol fluctuations was compatible with the existence of two populations of patients with Cushing's disease. It was hypothesized that highly fluctuating cortisol concentrations were of hypothalamic origin, low fluctuations of primary pituitary origin [279]. Also in favor of a primary hypothalamic disorder was the apparent, and sometimes persistent, cure of some patients treated with drugs acting directly at the CNS such as the serotonin antagonist cyproheptadine and the γ-aminobutyric acid (GABA)-ergic sodium valproate [2,136].

All these lines of evidence can be seriously challenged. Many are simply attributable to the hypercortisolic state; altered EEG patterns, abnormal GH, PRL, gonadotropin and TSH secretory patterns all return to normal when the source of hormone excess is removed [2,158,159,161]. The beneficial effect of CNS-directed drugs is debated particularly because it was based on uncontrolled studies; subsequent elegant studies have shown that many with Cushing's disease have spontaneous fluctuations with long-lasting periods of apparent total remission – whether or not the patients are treated with CNS-directed or dopaminergic drugs [280].

Corticotroph cell hyperplasia has been reported in pituitaries of patients with Cushing's disease [153–155,281,282]. Because CRH exerts a growth-stimulatory action on corticotroph cells in animals [215,216] and because corticotroph cell hyperplasia has been observed in pituitaries obtained from patients with Cushing's syndrome resulting from chronic CRH oversecretion by hypothalamic [283] or ectopic [284,285] tumors, this histologic finding is generally taken as a strong argument for CRH involvement [286]. Establishing this histologic diagnosis is of utmost difficulty [14]. It should not be accepted when obtained on limited surgical materials. Some adenomas may escape surgical exploration because of an ectopic location, and the difficult histologic diagnosis of corticotroph hyperplasia certainly requires that the whole gland be thoroughly examined since the corticotroph cells are not scattered randomly in the gland, but rather show clusters of densely aggregated cells [147,287]. If this histologic diagnosis can be demonstrated under the necessary scrutiny in a patient with the clinical and biologic features of Cushing's disease, then it probably offers the best evidence of CRH dependence. Yet it would still need to be proven that CRH is of hypothalamic origin, and not from an occult ectopic source, and if so, that it is not merely a functional and transient hypothalamic CRH dysfunction associated with disorders like depression, chronic stress and general resistance to glucocorticoids.

Even the finding of corticotroph cell hyperplasia is not definitive proof that it is CRH-mediated. Evidence

has been provided for the existence of primary multinodular corticotroph hyperplasia [288]. Thus the ultimate proof still requires the unambiguous demonstration that hypothalamic CRH is indeed overproduced. Plasma CRH is normal or low in patients with Cushing's disease, although the significance of these peripheral plasma values may not be absolutely relevant [289,290]. CRH rather appears to be low in the cerebrospinal fluid (CSF) of patients with Cushing's disease [291], in contrast with depressed patients [292,293]. Although CRH analogues that exert an antagonist activity have been used in the rat [294], such a molecule remains to be constructed for human studies. It certainly would constitute a unique heuristic tool and the sole way to definitively establish a pathophysiologic role for CRH in Cushing's disease.

DOES CORTICOTROPH CELL HYPERPLASIA PRECEDE ADENOMA FORMATION?

That hyperplasia often precedes the formation of a clonal tumor has long been recognized in animal models and in humans, and brilliantly demonstrated by the recent use of powerful transgenic animal models [295]. Site-directed expression of the SV40T antigen in the mouse pancreatic β-cell first induces hyperplasia of the endocrine cells [296]. As a result of a second and spontaneous event, an occasional clonal β-cell tumor develops. It is thought that this experimental model somehow reproduces the long-proposed two-step theory of Knudson for spontaneous oncogenesis [297]. In humans the familial form of medullary thyroid cancer (Sipple syndrome) provides the best example of a naturally occurring endocrine cancer where tumoral transformation is preceded by a state of general hyperplasia of C cells [298]. The molecular mechanisms responsible for the different steps have been unravelled in privileged situations in humans, such as the retinoblastoma. In most other cases they are still unknown. The local expression of angiogenic compounds may be the tumor-promoting factor [299]. On these grounds it is interesting to remember that a recently characterized angiogenic factor, vascular endothelial growth factor or vasculotropin, was isolated from pituitary folliculostellate cells [300,301] and the mouse corticotroph cell line AtT-20 [302].

Increasingly, the pituitary is becoming the target of site-directed oncogenesis in various transgenic animals. Transgenic mice expressing the human GHRH gene develop selective hyperplasia of the GH cells [303,304]. It will be of major interest to observe if authentic tumors eventually develop with time. Various genes have been targeted to pituitary corticotroph cells in transgenic mice using transgenes placed under the promoter of the rat POMC gene [305,306]. Expression of SV40T antigen provoked marked development of both anterior pituitary corticotrophs and intermediate-lobe melanotrophs [307]; whether true clonal tumors secondarily develop is not yet known. Other approaches have been used recently that create experimental models of Cushing's disease in transgenic animals [308]. ACTH-producing pituitary tumors were generated with the polyoma early region promoter linked to a cDNA encoding the polyoma large T antigen [309]. Chronic ACTH and corticosteroid overproduction was induced by targeting the expression of an antisense message to the glucocorticoid receptor type II [310], and in transgenic mice over-expressing CRH [311]. In this latter experimental model pituitary corticotroph hyperplasia was observed. It will be crucial to determine if adenomatous lesions ultimately develop.

In humans, the evidence that hyperplasia precedes the formation of a pituitary adenoma is still scant; although pregnancy is a condition with PRL cell hyperplasia, no association is clearly found to suggest that it is a risk factor for subsequent development of a PRL-secreting tumor. The ectopic GHRH syndrome is almost always associated with GH cell hyperplasia when pituitary examination is performed [312]. Similarly, pituitary enlargement occurring in states of long-lasting peripheral hormone deprivation (hypothyroidism, hypogonadism) is, as a rule, associated with hyperplasia and reverses with adequate treatment. Whether true tumors have really developed under such conditions remains to be established unequivocally.

The evidence that primary CRH hyperactivity may be responsible for corticotroph cell hyperplasia secondarily initiating the formation of an adenoma is based on theoretical, histologic and clinical grounds: (1) CRH exerts a growth-stimulatory action on corticotroph cells; (2) associations of a corricotroph adenoma and corticotroph hyperplasia have been found [149–152]; and (3) an increased number of stressful events have been found in patients with Cushing's disease [313,314]. It suggested that prolonged CRH overactivity eventually led to the formation of the adenoma. Clinical experience does not indicate that depressed patients are particularly at risk for Cushing's disease. More interesting are the patients whose disease recurs after "successful" removal of a pituitary adenoma [129,315]. It is speculated that the original hypothalamic CRH overactivity had been transiently silenced by the hypercortisolism, and recovers its activity after removal of the pituitary adenoma. In fact, there is simply no way to be sure that the recurrence is not due to the regrowth of a small amount of tumoral cells that had escaped the surgeon's skill and which can be removed by a second pituitary surgery [316,317].

Thus, although such a mechanism remains a possibility (Figure 16.11), there is certainly at the present time no definitive proof that corticotroph cell

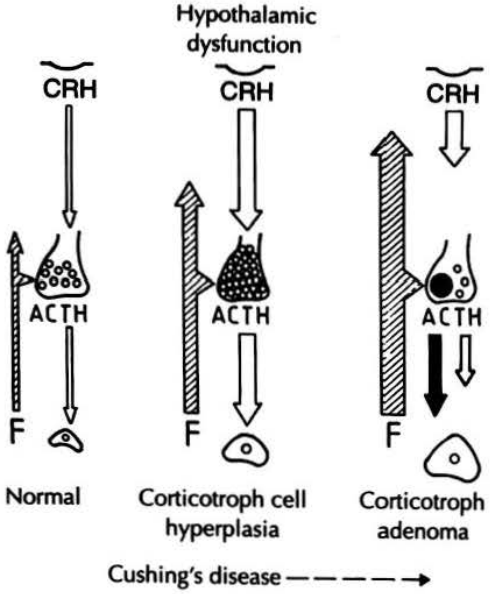

FIGURE 16.11 Pathogenesis of Cushing's disease. Schematic view of a tentative hypothalamic hypothesis. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; F, cortisol.

hyperplasia is a prerequisite condition leading to the formation of a pituitary adenoma. Progress in this field might come from new experimental approaches generating chronic pituitary stimulation by CRH in animals [311,318] and whenever an anti-CRH analogue will be available for clinical studies.

EFFECTS OF CHRONIC ACTH AND POMC PEPTIDE OVERSECRETION

Effects on the Adrenal

Effects of ACTH on Corticosteroid Secretion

Steroidogenesis in the zona fasciculata and the zona reticularis of the adrenal cortex is regulated predominantly, if not exclusively, by ACTH. Binding of the pituitary peptide to its specific membrane receptor induces an immediate secretion of glucocorticoids, androgens and mineralocorticoids [319,320]. The primary mediator of ACTH action is cAMP and cAMP-dependent protein kinase; protein kinase C does not directly mediate the actions of ACTH although interactions between the two kinase systems have been described [321].

Because little steroid is stored in the gland, increased secretion is the reflection of increased synthesis [320]. ACTH primarily acts to increase the rate-limiting step of steroid synthesis (the conversion of cholesterol to pregnenolone) by enhancing the accessibility of cholesterol to the substrate-binding site of cytochrome P450. This action requires a rapid protein synthesis and a labile protein called steroidogenesis activator polypeptide (SAP) has recently been characterized [322]. In the long term, ACTH action also involves a stimulatory effect on the expression of various key enzymes of steroidogenesis, most likely at the transcriptional level [323,324].

In contrast with some other endocrine functions in humans, chronic adrenocortical stimulation by ACTH does not induce a desensitization state. Indeed the opposite occurs; the adrenocortical response is amplified [325]. This long-recognized phenomenon had been attributed to a "trophic" effect of ACTH though recent knowledge has shed a molecular explanation. Adrenocortical cells exposed to ACTH in vitro acquire an increased number of ACTH receptors and an increased rate of protein G_s expression [326–329]. Thus the binding of ACTH and the transducing apparatus are both amplified, explaining the higher sensitivity and the greater response potential of chronically stimulated cells.

Fascinating results have recently unmasked an autocrine network that operates on adrenocortical cells. In response to ACTH various growth factors are secreted. Insulin-like growth factor-I (IGF-I) is secreted [330,331] and acts on its own receptors to stimulate the differentiated functions of adrenocortical cells [332,333]. In contrast, transforming growth factor-β (TGF-β) exerts inhibitory effects, its receptors being also regulated by ACTH. Moreover, ACTH favors the release of angiogenic factors such as fibroblast growth factor (bFGF) and IGF-II, thus stimulating the growth of the adrenals and of their vascular system as well [321].

Although much work remains to be done to unravel the physiologic relevance of each individual system and their integrated implications in the final adrenocortical response, these autocrine factors stress the importance of the adrenal glands as potential amplifiers of corticotroph activity. In comparison with normal cells, hyperplastic adrenocortical cells of Cushing's disease have particular qualities: (1) they are more sensitive to low doses of ACTH; and (2) their response to ACTH stimulation is higher and longer [325,334]. If Cushing's disease is defined as a set-point defect at the pituitary level, it is conceivable that increased responsiveness of chronically stimulated adrenal glands may lower the amount of ACTH needed to maintain the same degree of cortisol oversecretion. Diminished ACTH secretion would thus occur in parallel with adrenal hyper-responsiveness, sometimes to the point where plasma immunoreactive ACTH reaches the lower limit of sensitivity of a given RIA. This reciprocal interaction between the adrenals and the pituitary adenoma may explain why no good correlation is found between plasma ACTH levels and the level of cortisol overproduction in patients with Cushing's disease [335].

The mechanisms of adrenal androgen secretion grossly parallel those of cortisol [320]. Thus

dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS) and Δ-4-androstenedione are elevated in Cushing's disease [336]. Their peripheral transformation to testosterone and dihydrotestosterone may lead to a moderate state of androgen excess in females [337]. Dissociation between cortisol and adrenal androgens is observed, however, in the particular situation of patients resuming normal corticotroph function after successful pituitary surgery. DHEAS remains suppressed for months or years after plasma cortisol has normalized [336].

The action of ACTH on adrenal mineralocorticoids is more complex. In the zona glomerulosa ACTH acutely stimulates aldosterone release [338]. Yet this action is only transient, since increased concentrations of cortisol in the adrenal cortex inactivate cytochrome P450 11-β-corticosterone methyl oxidase. In contrast, in the zona fasciculata and reticularis the stimulatory action of ACTH is permanent. Thus 11-deoxycorticosterone (DOC), corticosterone (B), and often 18-OH-DOC, are elevated whereas aldosterone and 18-OH-B are normal or slightly suppressed in parallel with low plasma renin activity. The concentration of plasma zona fasciculata mineralocorticoids (DOC) is directly correlated to that of ACTH and participates as one determinant in the mechanism of high blood pressure [338,339].

Various physiologic agents have recently gained a new interest as possible regulators of ACTH action at the adrenal level or even as regulators themselves. Angiotensin II, serotonin [340], interleukins and as yet unidentified monocyte products [341] have shown some apparent stimulatory actions. Atrial natriuretic factor inhibits the action of ACTH both on the zona glomerulosa and the zona fasciculata [342]. Peptides with anti-ACTH action (corticostatins) have been isolated from rabbit lung and peritoneal neutrophils which are unrelated to POMC [343]. Although the pathophysiologic relevance of these observations remains elusive they may offer some speculative thoughts on new therapeutic approaches.

Effects of Non-ACTH POMC Peptides on Corticosteroid Secretions

N-terminal fragment, γ3-MSH, β- and γ-LPH, β-MSH, α-MSH and β-endorphin have all been shown to exert some effect on adrenal secretions [344–350]. In comparison with ACTH much higher concentrations were required, raising some doubt about their real significance and possible minor contamination (less than 0.1%) of purified preparations [344]. An aldosterone-stimulating activity of the N-terminal fragment [35,349], β-LPH [346], β-endorphin [348], β-MSH [347], α-MSH [345] and γ3-MSH [350] has been reported. Specific receptors to Lys-γ3-MSH have been described in the rat adrenal [351] where it would act essentially as a synergic molecule with ACTH. The clinical relevance of these results remains somewhat uncertain, primarily because of the weak intrinsic action of the peptides, and also because peptides like γ3-MSH and β-MSH are not normally found within normal or tumoral human pituitaries [168,352].

Besides, in patients with Cushing's disease, increased plasma immunoreactive Lys-γ3-MSH has been occasionally reported in some patients with idiopathic hyperaldosteronism [353], a finding which could not be confirmed by others in the same type of patients nor in other patients with dexamethasone-suppressible hyperaldosteronism. As to the somewhat elusive pituitary aldosterone-stimulating factor (ASF), it appears to be unrelated to POMC [354].

Although often suggested, the definite demonstration that some abnormal fragments of POMC processing, preferentially generated in nonpituitary tumors responsible for the ectopic ACTH syndrome, somehow enhance the secretion of mineralocorticoids by the adrenals still remains to be made. Similarly, that cortical adrenal androgen-stimulating hormone (CASH) – or joining peptide 1–12 – is a specific regulator of adrenocortical androgens has not been confirmed [355]. ACTH 7–38 or corticotrophin-inhibiting peptide (CIP) exerts an anti-ACTH action [356]. Although occasionally described in some pituitary extracts it is not a significant product of POMC in the human normal or tumoral pituitary.

Effects of ACTH on Adrenocortical Growth

Hypophysectomy results in adrenal cortex atrophy that is restored by the sole administration ofACTH [320]. Thus, in vivo, ACTH is the predominant if not the exclusive trophic factor for the adrenals. Prolonged in vivo stimulation with chronic ACTH administration or oversecretion eventually leads to an increase in total adrenal protein and RNA synthesis. Cell proliferation is indicated by an increase in total DNA [320]; the resulting adrenocortical hyperplasia participates in the amplified response of the chronically stimulated gland, and the weight of each gland can be greatly increased.

The exact mechanism whereby ACTH promotes adrenocortical growth still remains somewhat mysterious, since in vitro studies show a paradoxical negative effect of ACTH on adrenocortical cell proliferation [321]. As already mentioned, the growth-stimulatory effect of ACTH in vivo most likely proceeds through the activation of a local and complex network of autocrine growth factors and their own receptors which appear to trigger the expression of early cellular protooncogenes such as c-*fos* [357]. Although a number of other substances, including POMC-peptides such as γ3-MSH [358], have been shown to exert some adrenocortical growth effect

they do not appear to be sufficiently potent to exert a direct effect in vivo.

Extra-adrenal Effects of ACTH and POMC Peptides

ACTH, β- and γ-LPH all contain a common heptapeptide (Met-Glu-His-Phe-Arg-Trp-Gly) which bears the melanocyte-stimulating activity present within POMC [28]. As measured in the frog skin bioassay the three molecules have essentially the same intrinsic bioactivity [359]. The more potent peptides (x100) α-MSH and β-MSH_{5-22} are not formed in normal and tumoral anterior pituitaries in humans. They may be generated in some nonpituitary tumors responsible for ectopic ACTH syndrome [352]. Thus in Cushing's disease and in Nelson's syndrome high plasma levels of ACTH, β-LPH and γ-LPH all participate, for essentially the same part, in the hyperpigmentation.

The same core heptapeptide is responsible for the lipolytic activity of the LPHs and ACTH. This effect is species-specific and observed predominantly in the rabbit. Human (and rat) adipocytes are essentially not responsive.

Because a small, but definite, amount of β-endorphin is formed in the anterior pituitary the question has arisen of the potential effect of this highly powerful analgesic. Very high levels of authentic nonacetylated, presumably fully bioactive, β-endorphin have been measured and chemically identified in the plasma of some patients with Nelson's syndrome. These patients showed no evidence of analgesia and had no response after naloxone administration [360]. This observation emphasizes the total lack of analgesic action of circulating β-endorphin which cannot cross the blood–brain barrier to act on its CNS receptors. CSF β-endorphin levels were low or normal in patients with Cushing's disease and even in Nelson's patients with highly elevated plasma levels [361].

PATHOLOGY OF THE ADRENAL IN CUSHING'S DISEASE

Simple Diffuse Hyperplasia

The most common adrenocortical lesion of Cushing's disease is bilateral simple diffuse hyperplasia [4]. The two glands are symmetrically (and generally moderately) enlarged, weighing between 5 and 12 g each at operation. The glands are yellow or brown and the cortex appears regularly widened on section. On light microscope examination a wide inner zone of compact zona reticularis cells separated from an outer zone of clear cells is observed. The zona glomerulosa is not changed. The cells themselves usually appear normal. Already in this form of hyperplasia small nodular lesions are found which emphasize the essential continuity of this condition with the nodular hyperplastic form with large, macroscopic nodules [362].

Multinodular Hyperplasia

Although this definition is somewhat arbitrary, it is generally used whenever one or several macroscopic (visible to the naked eye) yellow nodules are present [4,6].

Such glands, in general, have a greater weight than in simple diffuse hyperplasia. The size of the nodules displays an extremely wide range of variation, from a few millimeters to several centimeters. Although as a rule they occur in both glands, marked asymmetry is occasionally seen, which may falsely indicate an autonomous adenoma-like lesion. In contrast to the autonomous adenomas, a constant feature that must be thoroughly examined, is that the attached cortex which lies in between the nodules is always hyperplastic [4]. With the light microscope, nodular cells show features not dissimilar to those of the hyperplastic regions with alternate collections of compact and clear cells.

Controversy still exists on the mechanism leading to nodular formation, its possible implication as a transient state leading to the formation of autonomous adenomas, and even adrenal carcinomas [337–342]. A large body of evidence suggests that in most cases multinodular hyperplasia is an anatomical variant that has kept its normal ACTH dependency: (1) unilateral adrenalectomy of a highly predominant and asymmetrical gland fails to cure the hypercortisolism [369]; (2) in many cases a fine hormonal evaluation is consistent with a pituitary source of ACTH as the responsible drive of cortisol oversecretion [367]; and (3) finally the finding and removal of a pituitary microadenoma has convincingly been shown to cure such patients [370]. It is assumed that multinodular hyperplasia results from long-standing ACTH stimulation of the adrenal cortex [371].

In other cases the situation is less clear, particularly because the ACTH dependency of the hypercortisolism is in question [372–374]; plasma ACTH is undetectable, the 17-hydroxycorticosteroids not stimulated by the metyrapone test, and cortisol secretion poorly or not at all suppressed by the classic high-dose dexamethasone test. None of these data are alone sufficient to prove that ACTH secretion is suppressed. A nondetectable plasma ACTH level simply may be under the lower limit of the assay and still be present in sufficient amounts to stimulate a large mass of adrenal cells [375,376] that have been shown to be even more sensitive to ACTH than simple hyperplastic cells [377]. Some patients who did not respond to the classic

high-dose (8 mg/day) dexamethasone suppression test subsequently responded to a higher dose of 16 or 32 mg [378]. It thus appears that the "autonomy" of cortisol secretion may be only apparent and not actual in many cases; and hormonal tests must be interpreted with the view that a large mass of highly ACTH-sensitive adrenocortical cells may somehow modify the classic limits of their responses [375,376,378].

Yet there remain some cases of authentic multinodular hyperplasia where the most thorough investigations have failed to detect the slightest indication of basal or stimulated (CRH) ACTH activity, including inferior petrosal sinus [379] or after bilateral adrenalectomy [380]. In these cases an ACTH-independent bilateral macronodular adrenal hyperplasia must be diagnosed [380]. The cause of this syndrome remains elusive. Privileged observations have suggested that "transition from pituitary-dependent to adrenal-dependent Cushing's syndrome" [368,381,382] may occur, providing a tentative explanation if not a definite proof. An alternate hypothesis is that some as yet unidentified non-ACTH factors exert a stimulatory action on normal – or more likely adenomatous – adrenocortical cells [379,380].

Cushing's Disease and Adrenocortical Carcinoma

The development of an adrenocortical carcinoma has been reported in exceptional patients with long-lasting Cushing's disease and multinodular hyperplasia [366,383]. In one case the patient had evidence of a 5-mm pituitary cystic basophil adenoma found at autopsy [384]. Similar, and exceptional, observations of occasional malignant adrenocortical lesions have been made in patients with poorly controlled congenital adrenal hyperplasia [383]. They raise the question of the possible role of ACTH on the generation or, more likely, the growth promotion of a concurrent adrenocortical adenoma. Convincing evidence that chronic ACTH stimulation may eventually generate an autonomous adrenocortical lesion is still lacking.

Adrenal Rests

Accessory adrenocortical tissue is often found in ectopic sites [383]. Classically it contains only the cortical component of the gland. The most usual sites include the celiac plexus, the kidney, the gonads, the broad ligaments, the epididymis and the spermatic cord. They may also occur beneath the liver capsule.

Capsular extrusions are collections of adrenocortical cells just outside the capsule of the adrenal glands. They are thus distinct from adrenal nodules. Histologically they appear to contain only zona fasciculata cells. They are ACTH-sensitive and may develop concomitantly with diffuse adrenocortical hyperplasia.

These accessory adrenocortical tissues probably explain why persistent cortisol secretion is not exceptional after an apparent total bilateral adrenalectomy [385]. They may be reactivated by the chronic stimulatory effect of highly elevated ACTH plasma levels and even become the source of excess steroid secretion in Nelson's syndrome.

OTHER CAUSES OF CUSHING'S SYNDROME

Understanding the pathophysiologic mechanisms of these conditions is essential to the principles of the differential diagnosis of Cushing's syndrome.

ACTH-dependent Spontaneous Cushing's Syndromes

CRH-secreting Tumors

CRH-secreting tumors have recently been recognized as a cause of chronic pituitary ACTH oversecretion, and hence Cushing's syndrome [285].

Rare hypothalamic tumors have been described such as gangliocytomas [283]. More patients have presented with ectopic CRH syndrome. The most frequent nonhypothalamic tumors responsible for CRH secretion have been the prostate, small-cell lung cancers, colon carcinomas, nephroblastoma, thyroid medullar carcinomas and bronchial carcinoids [285,386]. Pituitary tissue obtained in such patients exhibited the expected corticotroph cell hyperplasia [283–285]. In general the patients presented with mild clinical features of hypercortisolism.

Although, theoretically, chronic CRH hypersecretion by a nonhypothalamic tumor might induce pituitary ACTH oversecretion, some pathophysiologic aspects of the syndrome remain ambiguous. First, CRH is only a weak ACTH stimulator [387], CRH infusion in normal volunteers for three consecutive days induced only a slight ACTH and cortisol rise, although plasma CRH values were extraordinarily high, up to 10 000 pg/ml [388]. Moreover, the placenta, which is a "physiologic" source of ectopic CRH, induces very high plasma CRH values (up to 1000 pg/ml) during the last trimester of pregnancy [290], yet plasma free cortisol again is only slightly elevated [389,390]. The poor stimulatory action of circulating CRH on ACTH secretion in these two conditions is tentatively explained by two reasons: (1) the down-regulation of CRH receptors at the pituitary level by glucocorticoids [391–393]; and (2) the buffering effect of large amounts of circulating CRH-binding protein is similar in males and females, and independent of estrogen action [394,395]. Second, most patients with

Cushing's syndrome due to ectopic CRH production had much lower plasma CRH values [285,290,396,397] than those observed in pregnant women. Third, when the CRH-secreting tumors could be examined in most cases they were found to contain ACTH and/or other POMC products as well [386,397,398].

Thus, except for a single case where a metastasis located at the median eminence [285] might have been the source of excess CRH acting directly at the pituitary level (surprisingly, in this patient, cortisol was not suppressed by dexamethasone), skipping the buffering effect of the CRH-binding protein [395], there is still some question as to whether moderately elevated plasma CRH levels originating from an ectopic source can ultimately induce a state of chronic ACTH oversecretion. The definitive proof should be accepted when removal of a tumor harboring only CRH (and not ACTH) eliminates Cushing's syndrome.

Probably because of the limited number of reported cases and the pending questions regarding its pathophysiologic mechanism, ectopic CRH syndrome has not been ascribed a clear basal or dynamic specific hormonal pattern.

Besides CRH, ectopic secretion of bombesin has been claimed to induce pituitary ACTH oversecretion leading to another cause of ectopic Cushing's syndrome with a somewhat analogous mechanism [399]. This proposal remains to be confirmed.

Ectopic ACTH Syndrome

Probably first reported by Brown in 1928 [400], the existence of ectopic ACTH syndrome was definitively established by Liddle's group in the 1960s [401]. Since that time a number of reviews have documented its prevalence as a cause of Cushing's syndrome, its various clinical presentations and its specific hormonal pattern [402–404].

Recent advances in the molecular aspects of ACTH biosynthesis and POMC gene expression have shed new light on its pathophysiologic mechanism [405]. POMC gene expression is a ubiquitous phenomenon which normally occurs in many nonpituitary tissues [52–59]; a highly dominant mode of POMC gene expression proceeds through a transcription initiation starting at the 5′ end of the third exon generating a short, truncated, POMC RNA that contains the coding region for ACTH but lacks that for a signal peptide of the precursor and which therefore is nonfunctional [55,57,58]. For a nonpituitary tissue, or tumor, to produce ectopic ACTH syndrome several essential qualitative and quantitative conditions must be met.

1. A shift in POMC gene transcription initiation must occur which directs the generation of a pituitary-like POMC mRNA, the translation product of which may enter the secretory pathway.
2. A maturation process must occur which releases at least some $ACTH_{1-39}$.
3. Ultimately, if some genuine $ACTH_{1-39}$ happens to be properly formed it must be secreted in excess, a quantitative aspect which depends on the rate of gene transcription on the one hand, and the amount of functional tumor mass on the other.

The molecular mechanisms of ACTH oversecretion in pituitary and nonpituitary tumors are schematically shown in Figure 16.12. In pituitary tumors the overall process of POMC gene expression appears qualitatively unaltered, yet exaggerated; in nonpituitary tumors

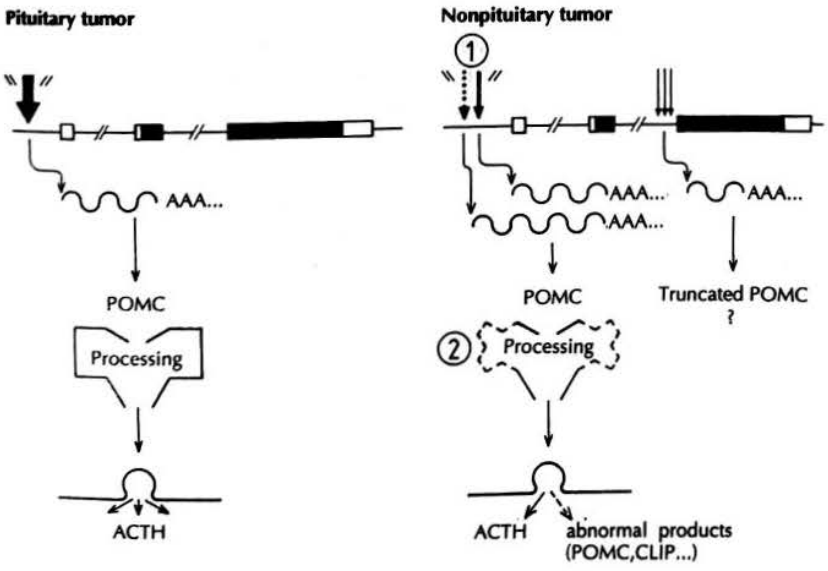

FIGURE 16.12 Schematic presentation of the molecular mechanisms of proopiomelanocortin (POMC) gene expression and adrenocorticotropic hormone (ACTH) oversecretion in a pituitary tumor (left panel) and a nonpituitary tumor (right panel).

various types of POMC transcripts are formed and different maturation processes operate that are appropriate for the resident hormone precursor of the given tissue (e.g., procalcitonin in a medullary thyroid carcinoma, progastrin-releasing peptide in a bronchial tumor) and more or less efficient for the ectopic POMC. Hence an abnormal maturation pattern of POMC is a classic, although inconstant, feature of ectopic ACTH syndrome. POMC may be poorly processed [62,406,407] or abnormal fragments such as CLIP and hβ-MSHSH$_{5-22}$ may be generated [61,352,408,409]. These processing abnormalities diminish the tissue's ability to secrete authentic ACTH – the sole bioactive peptide in terms of steroidogenesis – and somehow protect the patients from the consequences of the tumor production. They also provide the investigator with subtle molecular clues that an ACTH-dependent Cushing's syndrome may originate from a nonpituitary source. The V3 vasopressin receptor is abundantly expressed on carcinoid tumors also producing ACTH [85], in contrast to small-cell lung carcinomas. In these latter tumors with ectopic ACTH secretion, aberrant transcription of POMC may be directly related to the neoplastic phenotype [413].

Another hallmark of ectopic ACTH syndrome is its production being totally unresponsive to glucocorticoid feedback, providing the basis for hormonal investigation. Apparently this lack of glucocorticoid sensitivity is not due to a lack of glucocorticoid receptor within the tumor [410]. Apart from exceptional cases [396,411,412], these tumors are also unresponsive to CRH. Because the long-standing hypercortisolic state has appropriately suppressed the pituitary ACTH, the patients do not respond to the CRH test in vivo, in contrast to patients with Cushing's disease [218].

Although the classic sources of ectopic ACTH syndrome are tumors derived from endocrine tissues [403] recent observations imply mononuclear cells as possible alternate sources. These data should be analyzed in view of the recent report that POMC gene expression and ACTH production occur normally in some circulating mononuclear cells [59,414].

Cortisol Hyper-reactive Syndrome

The case was recently described of a patient who exhibited some clinical features of Cushing's syndrome but had low levels of plasma and urinary cortisol [415]. Cellular studies revealed that his tissues were abnormally sensitive to the action of glucocorticoids; higher affinity and higher response of aromatase to glucocorticoids were found in isolated adipocytes. Although ACTH was low and poorly reactive to the CRH test it is not clear whether the small amount of secreted cortisol is still under the control of inappropriately secreted pituitary ACTH.

ACTH-independent Spontaneous Cushing's syndrome

Primary Adrenocortical Tumors

Primary adrenocortical tumors cause approximately 20% of the cases of spontaneous Cushing's syndrome in adults [2]. Benign adenomas and adrenocortical carcinomas distribute evenly. As for Cushing's disease, a female preponderance is noted (Table 16.2). Chronic glucocorticoid excess induces an appropriate suppression of pituitary ACTH. The contralateral and the nontumorous ipsilateral adrenals are both atrophic [4,383]. Cortisol secretion is autonomous and unresponsive to either glucocorticoid deprivation or administration. Most benign adrenocortical adenomas respond to exogenously administered ACTH, and most adrenocortical carcinomas do not [16]. Whereas benign tumors are usually small and secrete exclusively cortisol, malignant tumors are much larger, and secrete a whole array of steroid precursors and androgens. Removal of a benign adrenal adenoma induces an immediate and definitive cure with often transient, sometimes long-lasting, hypocorticotropism. Malignant adrenocortical tumors are highly aggressive; in most series the survival rate is only 20% 5 years after diagnosis [16,17].

Other Adrenocortical Disorders

PRIMARY PIGMENTED NODULAR ADRENAL DISEASE (PPNAD)

This rare condition occurs primarily in childhood [416]. Cortisol oversecretion is autonomous with hormone dynamics essentially similar to those encountered in cases of adrenocortical adenomas. Pituitary ACTH is suppressed, unresponsive to the CRH test or to cortisol deprivation (metyrapone test), and cortisol secretion is unresponsive to the dexamethasone suppression tests. However, this primary adrenal disorder is driven by bilateral adrenocortical lesions; the two glands harbor numerous nodular lesions that appear typically brown or black. The adrenal's size is classically not increased. Histologically, the nodules consist of typical compact adrenocortical cells with eosinophilic cytoplasm containing brown pigment. The adrenocortical tissue which lies in between the nodules has been variously described as normal or more often atrophic; the latter aspect separates this condition from the more usual and ACTH-dependent multinodular hyperplasia. Other features add to the concept that adrenocortical nodular dysplasia is a separate entity. It may be part of a more complex clinical spectrum called the Carney complex which associates myxomas of the heart, skin, or breast, pigmented skin lesions, endocrine tumors and peripheral nerve tumors (schwannomas), and which is often a hereditary condition transmitted

as a Mendelian autosomal dominant trait [417,418]. It was recently shown that germline mutations of the regulatory subunit R1-α of the protein kinase A (PKA) were present in a majority of such patients [419,420].

ACTH-INDEPENDENT BILATERAL MACRONODULAR ADRENAL HYPERPLASIA

Already mentioned, this condition suggests that non-ACTH factors may induce cortisol hypersecretion by nodular and hyperplastic adrenocortical glands [379,380]. Exceptional familial cases have been reported [421]. A recent report convincingly demonstrates that such adrenocortical lesions had acquired an inappropriate sensitivity to gastric inhibitory polypeptide that stimulated cortisol release in vivo and in vitro, also explaining why the patient had high plasma cortisol increases after meals [422,423]. This led to the concept of various "illegitimate" receptor expressions on some adrenal tumors: β-adrenergic-, vasopressin-, LH-receptors inducing variable clinical phenotypes and pharmacologic responses [423a].

In McCune-Albright syndrome, nodular hyperfunction may occur in different endocrine tissues including the adrenal glands where it can lead to hypercortisolism. It has been demonstrated that this syndrome is caused by a somatic mutation of the α-subunit of the G-protein, which activates adenylyl cyclase [424]; thus cAMP production is constitutively activated and cortisol overproduction is genuinely autonomous in the affected areas of the gland.

Gonadal Tumors

Exceptional cases of cortisol-secreting testicular and ovarian tumors have been described [425,426]. Whether these originated from ectopic adrenocortical cells is of speculative interest.

Iatrogenic Cushing's Syndromes

Exogenous Glucocorticoids

By far the most frequent cause of Cushing's syndrome is iatrogenic [427]. Patients given high doses of glucocorticoids invariably develop the clinical features of Cushing's syndrome, the severity of which depends on many variables, including the intrinsic glucocorticoid activity of the given drug, its in vivo bioavailability, the dose and duration of treatment, the mode of administration and the personal sensitivity of each individual [428]. Drug administration may be factitious [429,430] and raise serious difficulties in the differential diagnosis, particularly with the recently described cortisol hyper-reactive syndrome [415]. The syndrome may also result from large-dose administration of progestins, which possess a glucocorticoid action [431] or from ritonavir (a protease inhibitor that inhibits P4503A4, and is used to "boost" levels of other protease inhibitors) coadministration with glucocorticoids in HIV-infected patients [432]. In all these situations pituitary ACTH is suppressed and the adrenals are atrophic.

Exogenous Cortrosyn

Cortrosyn may be administered chronically in patients as an antiinflammatory or antiedematous agent. It will invariably induce the clinical features of Cushing's syndrome with highly elevated cortisol, adrenal androgens and DOC. In a way similar to that which occurs in ectopic ACTH syndrome, pituitary ACTH (and other POMC-peptides) will be suppressed.

CLINICAL FEATURES

On a historical note, it has been proposed that the first published case of Cushing's disease was "a near miss" mistakenly described by Osler in 1899 as having "an acute myxoedematous condition ..." [433,434]. The unequivocal description of this condition is attributed to the pioneering work of his most famous student Harvey Cushing, who published the case of Minnie G. first in 1912 [435]; and later again in his classical monograph of 1932 [106] with 11 more cases. Minnie G. was

> a young (16y) woman ... of most extraordinary appearance. Her round face was dusky and cyanosed, and there was an abnormal growth of hair, particularly noticeable on the sides of the forehead, upper lip, and chin. The mucous membranes were of bright colour despite her history of frequent bleeding. Her abdominous body had the appearance of a full-term pregnancy ... numerous purplish striae were present over the stretched skin of the lower abdomen ... the peculiar tense and painful adiposity affecting face, neck and trunk was in marked contrast to her comparatively spare extremities ... (the) ... most striking feature was the rapidly acquired adiposity of peculiar distribution in an amenorrheic young woman ...

DESCRIPTION OF THE CONDITION

Centripetal fat deposition is the most common manifestation of glucocorticoid excess and often the initial symptom of the patient [2,3,436]. Although weight gain is classic it may be minimal and the peculiar distribution of adipose tissue readily distinguishes it from simple obesity. Fat accumulates in the face and the supraclavicular and dorsocervical fat pads, leading to the typical moon faces and buffalo-hump, most often accompanied by facial plethora. It may exhibit inflammatory features with hot and reddish skin and may be slightly painful [106]. This acquired habitus change is best evidenced by comparison with anterior

photographs. Fat also accumulates over the thorax and the abdomen, which becomes protuberant. Development of lipomatosis in various situations has been occasionally described and may induce a reversible widening of the mediastinum on chest X-ray [437]. Abnormal fat distribution is of variable degrees; it is probably the most sensitive symptom of Cushing's disease, being exceptionally absent [438,439]. It disappears rapidly and totally after cortisol hypersecretion is reduced. The fine pathophysiologic mechanism that determines fat redistribution probably lies in the differential sensitivity of central and peripheral adipocytes to the opposite lipolytic and lipogenic actions of cortisol excess on the one hand, and secondary hyperinsulinism on the other [2].

Less frequent, but certainly crucial, are the clinical features that pertain to the protein-wasting effect of cortisol. Absent in simple obesity they have a high diagnostic value and must be thoroughly searched for at examination.

1. Skin thinning due to the atrophy of the epidermis and the underlying connective tissue may be mild and is best appreciated by rubbing the skin gently over the tibial crest. In some patients the skin is so fragile that it can be scratched simply by removing a strip of adhesive tape. Skin thinning and tension over accumulated fat both account for the plethoric appearance of the face and the purple aspect of striae due to the streaks of capillaries, which almost become visible. Striae are indeed present in many patients and are most commonly located on the abdomen and flanks, but also on the breasts, hips and axillae. In contrast with the usually whitish and small striae often seen after pregnancy or rapid weight gain, the striae of Cushing's disease are typically purple to red, and wide (>1 cm). Almost 62% of patients complain of easy bruisability, whereas it is relatively uncommon in simple obesity [439]. The minimal trauma generate multiple ecchymotic lesions or purpura especially on the forearm; blood collection often results in large ecchymotic lesions. Minor wounds heal slowly and are the source of postoperative complications at the incision site. The most superficial wounds, especially frequent on the lower extremities, may lead to indolent infection and ulceration that take months to disappear. Lower-limb edema is frequent and does not always result from congestive heart failure but rather from increased capillary permeability. Protein wasting is responsible for a generalized tissue fragility. Surgeons usually find the tissues tear easily. Spontaneous ruptures occur, mainly of tendons.
2. Muscle wasting is frequent and characteristically proximal leading to fatiguability, muscle atrophy occurring mostly in the lower limbs [440]. It is found on formal testing in about 60% of patients [439]. Disappearance of muscle mass may become apparent and measurable; it contrasts with the truncal obesity. The weakness may be so severe as to prevent the patient from getting up from a chair without help.
3. Bone wasting results in general osteoporosis. Cushing's disease is associated with bone loss and an increased risk of fractures. The prevalence of osteoporosis (T score −2.5 SD or lower) assessed by bone mineral density using dual-energy X-ray absorptiometry is about 40% [441]. Patients have a low Z score in the lumbar spine (with a mean of −1.59 SD when taking into account the most important studies) and in the femoral site (−1.1 SD) [441–449]. Particularly vulnerable is the vertebral body; loss of bone density is almost invariably present when searched by sophisticated means such as dual-photon-absorptiometry. Compression fractures of the spine are evident on plain X-rays in about 20% of the patients and almost half of the patients complain of backache [439]. Neurologic complications almost never happen. In contrast, kyphosis and loss of height, sometimes dramatic (up to 20 cm), are frequent. Pathologic fractures can occur elsewhere, particularly in the ribs and pelvis [441]. Demineralization is readily visible on skull X-rays and shading of the dorsum sellae is quite common, indicating cortisol action, rather than an expanding pituitary adenoma. Renal stones, as a consequence of hypercalciuria, are present in 15% of cases [439]. In children, Cushing's disease almost invariably provokes growth retardation, if not growth arrest. Without treatment, the final adult height is reduced and peak bone mass lowered.
4. The patient with chronic hypercortisolism has impaired defense mechanisms against infections. There is no recent study on Cushing's disaese dealing with this subject, but in the 1950s [3], severe infectious complications were reported in 42% of untreated patients. Banal bronchopulmonary infections may take a most aggressive, life-threatening course. Superficial mucocutaneous infections are extremely frequent, such as tinea versicolor and ungueal mycosis, which will only subside with control of the hypercortisolism.
5. A majority of patients have high blood pressure [372,450–452]. It may occasionally be severe, inducing cardiac hypertrophy and eventually congestive heart failure. The pathophysiologic mechanism of hypertension in Cushing's disease is complex and multifactioral due to both the glucocorticoid effect of cortisol [453,454] and to the mineralocorticoid effect of cortisol and DOC [455].

Well-defined carotid wall plaques are detected in one-third of patients with active or cured Cushing's disease [456]. Increased susceptibility to both arterial and venous thrombosis is also present due to lipid [457] and coagulation [458,459,460] disturbances. A systematic review on 15 studies in 476 patients with Cushing's syndrome, including 398 patients with Cushing's disease [460], reported a risk of 1.9 and 2.5% thromboembolic complications not provoked by surgery, mostly occurring during persistent hypercortisolism or relapse. Hypercoagulability was suggested by high levels of factor VIII, factor IX and von Willebrand factor, and by evidence of enhanced thrombin generation but the overall effect on fibrinolysis remains unclear [460]. Cardiovascular complications are the major threats of the disease and contribute greatly to its morbidity and mortality rate [3,18,19,461,462]. Successful removal of the pituitary microadenoma reduces high blood pressure [463].

6. Hirsutism due to a slight excess of adrenocortical androgens is extremely frequent in women. Moderate hair growth is visible on the face (upper lips, chin, sideburns), and less often on the chest, breasts, abdomen and upper thighs. A dorsal lanugo is often observed; some degree of acne and seborrhea are frequent. Frank virilism (temporal hair loss, coarsening of the voice, clitoral hypertrophy) occasionally occurs and would rather point to another cause of Cushing's syndrome, especially an adrenocortical carcinoma. Excess adrenal androgens and cortisol both suppress gonadotroph function, resulting in an array of gonadal dysfunctions. Most female patients have oligomenorrhea or amenorrhea, and infertility is frequent. Rare patients may also have concomitant hyperprolactinemia [464,465]. In male patients the curtailed gonadotroph function [466] induces a dramatic fall in testosterone which is not compensated by the increased adrenocortical androgens. It results in loss of libido and diminished sexual performance. Loss of sexual hair and reduced testis size are observed. Gynecomastia does not usually develop.
7. Psychic disturbances are extremely common. They are highly variable both in their expression and severity and do not correlate with the intensity of the hypercortisolism. They are most often mild, limited to anxiety, increased emotional lability and irritability of unwarranted euphoria. Sleep disorders are also frequent. Severe psychotic symptoms may occur such as depression, manic disorders, delusions and/or hallucinations, and may ultimately lead to suicide. No clean link is found between the premorbid psychologic status and the type of morbid psychologic manifestations [467]. In many cases controlling the hypercortisolism results in dramatic improvement with complete disappearance of the psychic manifestations. Impairment in short-term memory and cognition is common [20,468–470]. The quality of life is reduced in patients with Cushing's disease using the generic short form 36 (SF-36) autoquestionnaire, an integrated measure of physical and psychological well-being or other questionnaire [19,471–475]. The quality of life score improves partially after treatment [471,473–476]. A score of Cushing symptoms was inversely related to SF-36 [473]. Other identified factors for worse quality of life among various studies are active hypercortisolism, female gender, young age at diagnosis, recent hospitalization, adrenal insufficiency, hypopituitarism, anxiety and depression.

The skin, as already mentioned, is often plethoric, especially on the face. Hyperpigmentation is almost never observed in the usual, uncomplicated forms of Cushing's disease, where ACTH and LPH plasma levels are only moderately elevated. In contrast it is a frequent symptom of patients whose treatment is directed primarily at the adrenals, especially after total bilateral adrenalectomy.

Miscellaneous clinical features have been claimed to be associated with Cushing's disease and reported as classic manifestations, although they now appear rare and not obviously related to glucocorticoid excess. These include thirst and polyuria in the absence of glycosuria or severe hypokalemia, and a tendency to exophthalmos, which might result from orbital fat accumulation. Cataract is rarely present unless as a secondary consequence of diabetes mellitus [439]. Although exceptional, symptoms related to the mass effect of a pituitary tumor (headache, visual defect, pituitary insufficiency) should be looked for systematically.

DIAGNOSIS OF CUSHING'S DISEASE

Routine Laboratory Tests

Routine laboratory tests may provide some clue to the diagnosis, their major interest being to measure the severity of the disease which is not only related to the rate of cortisol secretion but also, for each individual, to their personal sensitivity to glucocorticoids. They will be most useful for the follow-up of treated patients.

Altered counts of circulating leukocytes are frequent, showing increased neutrophils and decreased lymphocytes and eosinophils [436]. Significantly increased hemoglobin, although classically reported, is actually rare. Serum electrolytes are usually normal. In severe

cases hypokalemia, alkalosis and hypernatremia develop in response to high levels of cortisol and DOC.

Impairment of glucose tolerance is frequent, between 39 and 94% depending on the studies which include all causes of Cushing's syndrome [450]. Reduced glucose tolerance (defined as blood glucose levels 7.8–11.1 mmol/L 2 h after an oral glucose tolerance test) is reported in 21–64% of patients with Cushing's syndrome; diabetes mellitus (defined by fasting blood glucose levels at or above 7.0 mmol/L or 11.1 mmol/L or greater 2 h after an oral glucose tolerance test) is reported in 20–47% of patients with Cushing's syndrome [439,477,478]. Rarely, patients develop ketosis and may require transient insulin treatment. The mechanisms of the diabetogenic effect of cortisol are well known and increased insulin plasma levels reflect the state of insulin resistance.

Plasma calcium and phosphate are usually normal and a mild hypercalciuria is reported in up to 40% of cases [436]. Lipid abnormalities are encountered in 37.5–52% of patients with Cushing's syndrome [3,457,478]. These are most often mild, showing a slight increase in triglycerides or combined hyperlipoproteinemia, especially in patients with impaired glucose tolerance.

Chest X-ray and electrocardiogram are normal except in cases of rib fractures and cardiac enlargement due to high blood pressure. Kidney function and liver tests are normal. Serum immunoglobulin G (IgG) has been reported to be slightly depressed. Bone mass is reduced in most patients, as are biochemical markers of bone formation like osteocalcin [443–447,479].

Clues to Clinical Diagnosis of Chronic Hypercortisolism

The clinical features of hypercortisolism cover a wide spectrum of symptoms and signs. Many, such as obesity, high blood pressure and psychologic disturbances, are extremely common and yet Cushing's disease is rarely their cause. Thus several authors have attempted to characterize each sign and/or symptom according to its sensitivity and specificity by comparing its prevalence in patients and in suspected (obese) subjects [436,438,439], the sensitivity of a sign or symptom being the percentage of patients with Cushing's syndrome who present it; the specificity of a sign or symptom being the percentage of subjects without Cushing's syndrome who do not present it. As shown in Table 16.3 [438] abnormal fat distribution (central obesity) is the most sensitive sign, and evidence of protein wasting (osteoporosis, myopathy) is highly specific. In the absence of fat redistribution, the likelihood of Cushing's disease is slim; in the presence of protein wasting, weight gain is highly suggestive of Cushing's syndrome.

TABLE 16.3 Prevalences of Clinical Features of Cushing's Syndrome among 211 Patients in whom the Syndrome was Suspected.

Clinical Feature	Patients with Cushing's Syndrome	Patients without Cushing's Syndrome
Osteoporosis*	0.64	0.03
Central obesity*	0.90	0.29
Generalized obesity*	0.03	0.62
Weakness*	0.65	0.07
Plethora*	0.82	0.31
WBC $\geq$11 000/mm^3*	0.58	0.30
Acen*	0.52	0.24
Striae (red or purple)*	0.46	0.22
Diastolic blood pressure $\geq$105mmHg*	0.39	0.17
Edema (pitting)*	0.38	0.17
Hirsutism*	0.50	0.29
Ecchymoses*	0.53	0.06
Serum K^+ $\leq$3.6 mEq/L*	0.25	0.04
Oligomenorrhea	0.72	0.51
Headaches	0.41	0.37
VPRC $\geq$49	0.37	0.32
Females	0.65	0.77
Abnormal GTT	0.88	0.77
Age $\leq$35 years	0.55	0.52

** Prevalences differed significantly ($p < 0.05$) in the two groups.*
WBC, white blood cells; VPRC, volume of packed red cells; GTT, glucose tolerance test.
From Nugent et al. [438].

This scheme provides a most useful guide that will prove highly fruitful for the clinical approach to many suspected cases.

Particular Clinical Presentations

Difficulties for Diagnosis

Many patients with Cushing's disease present with a highly suggestive combination of symptoms and signs, as just described, while in other cases, the clinical picture is less clear and often misleading for several reasons.

1. In some patients, the clinical features are less complete and sometimes one symptom may predominate. An occasional patient has been misdirected for months or even years to rheumatologic, cardiologic, or psychiatric clinics before it is realized Cushing's syndrome is

responsible for the symptomatology. Some patients exhibit the syndrome only partially, and one symptom can dominate the whole picture. Mild forms may be mistaken for all sorts of ill-defined conditions, such as polycystic ovary syndrome, idiopathic hirsutism, idiopathic cyclic edema and essential hypertension. The reported prevalence of Cushing's syndrome is between 1 and 9.5% in screening studies of patients with type 2 diabetes [21,24,25], between 0.5 and 1% in patients with hypertension [480,481] and as high as 10.8% in patients with osteoporosis [26].

2. At both extremes, the intensity of the disease generates diagnostic difficulties. Mild forms may be mistaken for many ill-defined conditions, including polycystic ovary syndrome, essential hypertension, idiopathic cyclic edema [87] and idiopathic hirsutism. Alternatively, a rare case of authentic Cushing's disease may present with symptoms so severe (including profound myopathy and hypokalemia) that they will irresistibly suggest the ectopic ACTH syndrome.
3. Most patients with Cushing's disease exhibit some fluctuation of cortisol secretion, others display a truly cyclic pattern (Figure 16.13) [280,483–488]. Episodes of active hypercortisolism are separated by periods of normal pituitary–adrenal activity of varying lengths. Some exhibit a fairly regular pattern of episodic hypercortisolism and complain of "swelling" from time to time [489]. A slight delay in obtaining the necessary blood, salivary, or urine samples to establish the hypercortisolism may allow the diagnosis to be missed. The simplest way to make this diagnosis is to educate the patient to collect a 24-hour or overnight [280] urine sample or bedtime [490,491] saliva sample at the time when they feel that symptoms have recurred.
4. In the mild forms of Cushing's disease, the diagnosis is often less apparent in men than in women. It is claimed that some persistent testicular androgens offer a better protection against the protein-wasting effect of cortisol.
5. In rare instances the first presenting symptoms will be those of a pituitary tumor. Careful evaluation of a macroadenoma might clearly indicate a state of ACTH hypersecretion in a patient who had no evident feature of chronic hypercortisolism. These findings may even be secondarily encountered during the careful monitoring of what was primarily diagnosed as a nonfunctional pituitary adenoma, stressing the need for prolonged follow-up of such patients [252–254].
6. Quite exceptionally, Cushing's disease may be recognized in the systematic work-up of a patient with MEN I or during family screening of familial isolated pituitary adenoma due to AIP mutation (see above).

Cushing's Disease in Children

In children, Cushing's disease almost invariably provokes growth retardation, if not growth arrest [8,9,492]. A decrease in growth rate may be the sole symptom in mild forms of the disease where the final diagnosis is often delayed. However, weight gain with centripetal obesity, like in adults, is present in most cases. Hormonal and imaging data have no particular aspect at this age. The occurrence of highly aggressive pituitary tumors has been reported [493,494]. The most usual treatment is selective adenomectomy by the

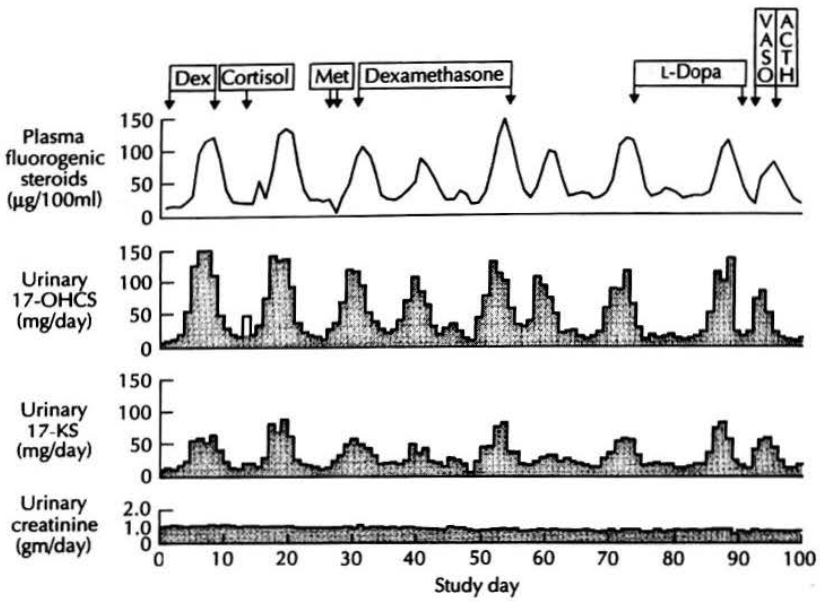

FIGURE 16.13 A case of cyclic Cushing's disease. Summary of the patient's daily morning plasma cortisol levels and daily 24-h urinary excretion of 17-hydroxycorticosteroids (17-OHCS) and 17-ketogenic steroid (17-KS) over a 100-day study period. *From Brown et al. [483].*

transsphenoidal route with a success rate of about 45–85% [495,496].

Cushing's Disease in Pregnant Women

Pregnancy occurs rarely in a hypercortisolic woman because of the hypofertility associated with this condition. To date less than 100 well-documented cases have been reported with Cushing's syndrome of all causes [497–500]. It should be outlined that an abnormal preponderance of adrenocortical tumors is found in most series during pregnancy.

In mild cases of Cushing's disease the clinical diagnosis may be obscured by features frequently present in pregnancy such as weight gain, high blood pressure, abdominal striae and impaired glucose tolerance. However, the presence of exaggerated morphological changes, virilism and especially catabolic features and hypokalemia, should raise suspicion.

The physiologic modifications of pituitary–adrenal homeostasis in normal pregnancy also hamper the biologic diagnosis. The normal and slight hypercortisolic state of late pregnancy may be difficult to distinguish from that of genuine Cushing's disease. Probably the best indicators would be the indices of free plasma cortisol (24-hour urinary cortisol excretion and salivary cortisol) showing a definite increase – in comparison with standard values at the same stage of pregnancy – and a lack of circadian rhythm [389,501,502]. Increased free testosterone may also be of help. Advancing normal gestation is associated with increasing loss of suppressibility after dexamethasone. No studies have developed a diagnostic threshold for interpretation of dexamethasone tests. Pregnant patients with ACTH-independent Cushing's syndrome do not consistently have low (<10 pg/ml; 2.2 pmol/L) or suppressed (<5 pg/ml; 1.1 pmol/L) plasma ACTH values. The ACTH values must be interpreted with caution. When in doubt between ACTH-dependent Cushing's syndrome and adrenal Cushing's syndrome, the 8 mg overnight dexamethasone suppression test may be useful [499]. The efficacy of the CRH stimulation test for differential diagnosis between Cushing's disease and adrenal Cushing's syndrome, and between Cushing's disease and ectopic ACTH secretion is unknown due to the limited number of reported cases. However, the responsiveness to CRH in pregnant women with Cushing's disease appears to be conserved, particularly in the first months of pregnancy. Its safety, its criteria and its diagnostic value have not been established [499]. The benefit of magnetic resonance imaging (MRI) during the first trimester must be carefully weighed because of its potential teratogenicity. Moreover, MRI should be performed without contrast agent (gadolinium) given the uncertainty about its safety, which limits considerably its sensitivity for detecting microadenomas. Macroadenomas appear more frequent than in nonpregnant patients. A typical image of pituitary adenoma (greater than 6 mm) with concordant biological tests makes the diagnosis of Cushing's disease highly likely.

Hypercortisolism during the course of pregnancy is associated with a high rate of maternal and fetal complications. Maternal complications occur in more than two-thirds of cases. The two main ones are hypertension and impaired glucose tolerance, reported respectively in 68% and 25% of cases. A pre-eclampsia or eclampsia is seen in 10% of cases. The cardiovascular, psychiatric and bone complications are rare, about 5% each. Of 136 observations collected in the review of the literature by Lindsay et al. [499], two maternal deaths were reported. Fetal complications are frequent, especially prematurity and intrauterine growth retardation (approximately 45% and 20%, respectively). Miscarriages and stillbirths were reported in 5% and 6% of cases, respectively, and at least three deaths of newborns. Adrenal insufficiency and fetal virilization are rare. Malformations do not seem more frequent. There is no Cushing's syndrome in newborns, confirming the protective effect of placental 11-beta hydroxysteroid dehydrogenase type 2. Symptomatic treatment of diabetes and high blood pressure is always necessary. Among the 40 cases of pregnant women with Cushing's disease compiled by Lindsay [499], only 20% underwent transsphenoidal pituitary surgery and 17% had an adrenalectomy during pregnancy, mainly in the second trimester (the period during which the fetal risk is lower and laparoscopy for adrenal surgery is still feasible). Metyrapone and ketoconazole, two inhibitors of cortisol synthesis, are theoretically contraindicated, but isolated observations or small series have been reported. Under metyrapone, an improvement of hypercortisolism was observed in ten patients, but the stimulation of precursors with mineralocorticoid activity may be responsible for hypertension, and it may provoke fetal hypoadrenalism [503].

Most anticortisolic drugs are contraindicated: metyrapone has been rarely used [503]. Transsphenoidal surgery should be a relatively safe procedure during pregnancy but until now it has only been reported once [504]. Few cases of spontaneous resolution after delivery have been reported [505].

Course of Cushing's Disease

Until recently Cushing's disease was a most severe condition with high morbidity and mortality rates. In older series, Cushing's disease ultimately led to death in a majority of untreated patients. Cardiovascular complications [3] were the predominant causes followed by infections and suicide [18,19,461,462]. In an epidemiological study during the period from 1975 through 1992

in Spain in 49 patients affected by Cushing's disease, among which disease remission was obtained in around 80% of cases during these years, a four-fold higher mortality was demonstrated, mainly due to cardiovascular diseases, and associated with age, persistence of hypertension and impaired glucose tolerance [18]. More recent studies are much more optimistic in cured patients after transsphenoidal surgery with a long-term mortality not significantly different from that in the general population [19,461,462]. In an investigation during the period from 1985 through 1995 in Denmark in 45 patients with Cushing's disease who had been cured through transsphenoidal neurosurgery, only one had died. The standard mortality ratio was not significantly different from that in the control population [19]. However, in the same study, in 20 patients with persistent hypercortisolism after initial neurosurgery, six had died which represents a five-times higher mortality rate than normal population [19]. The ultimate prognosis of Cushing's disease depends upon the severity of the hypercortisolic state and the aggressiveness of the pituitary tumor.

Several factors determine the severity of the hypercortisolic state in a given patient: the level of cortisol overproduction, the duration of hypercortisolism, and above all, some intrinsic and so far not fully identified factors that establish a different set-point of peripheral glucocorticoid sensitivity for each individual, and in the same individual in different target tissues. This explains the variable clinical features that are observed in patients with similar indices of hypercortisolism, and that in the same individual a defined target tissue seems to suffer more than the others. Because most, if not all, patients can now be cured of their disease (or at least hypercortisolism may be controlled) the practical question has become whether the clinical manifestations of Cushing's disease are reversible after successful treatment. Some patients truly are rejuvenated. As a rule younger patients obtain more benefit from a cure than older patients. In the latter group skin changes, muscle wasting and osteoporosis improve less obviously. Only recently have studies with dual-photon absorptiometry shown that suppression of hypercortisolism seemed to induce a rise in bone mass [506]. Therefore, because some effects of chronic hypercortisolism, especially in older adults, induce changes that are not easily reversible, not only should these patients be treated aggressively, they should also be treated rapidly.

The growth potential of the pituitary tumor may be another determinant of the final prognosis. Rare cases of spontaneous cure of Cushing's disease have been reported. They are thought to result from infarction and/or calcification of a pituitary adenoma [507,508]. In a minority of patients tumor growth seems to be boosted by bilateral total adrenalectomy, eventually leading to Nelson's syndrome. This rare occurrence is unpredictable. It is another argument that pinpoints the pituitary as the more logical and first target of therapeutic strategies.

DIAGNOSTIC PROCEDURES

Two steps should be used in the diagnostic approach. First, establish that chronic hypercortisolism or Cushing's syndrome is present; second, identify its cause with its specific prognostic and therapeutic implications.

It is essential that the investigative work-up be done in a coordinated, and somewhat compulsive, fashion with the clear assumption that a diagnostic certainty is the best assurance of an appropriate treatment.

The greatest difficulties are encountered when therapeutic procedures have been prematurely initiated in patients who subsequently turn out to have been misdiagnosed. Assessment of the pituitary–adrenal axis at that time may present insurmountable obstacles. Only then is it regretted that sufficient time had not been allocated to perform the initial work-up. This approach requires a skilled nursing staff, well trained to perform basal and dynamic hormonal evaluations, as well as indisputable steroid and peptide assays. Sophisticated imaging techniques must also be capable of identifying inconspicuous anatomic lesions that can be as small as a few millimeters in diameter.

HORMONAL EVALUATION

Establishing the Hypercortisolic State

The numerous and nonspecific clinical features of chronic hypercortisolism explain why Cushing's syndrome is often considered; the low incidence of this syndrome explains why it is exceptionally confirmed. It was thus essential to develop the means to assess the cortisolic state and to identify, for a given individual, whether it is inappropriately high. An ideal parameter would be that which shows no overlap between normal subjects, including obese, and patients with a hypercortisolic state, whatever the cause.

Baseline Measurements

LATE NIGHT SERUM CORTISOL

Serum cortisol is easily measured by competitive protein-binding assay or currently, by more specific immunoassays [509]. As a group, patients with Cushing's syndrome have higher morning serum cortisol values [510], yet around 50% fall within the normal

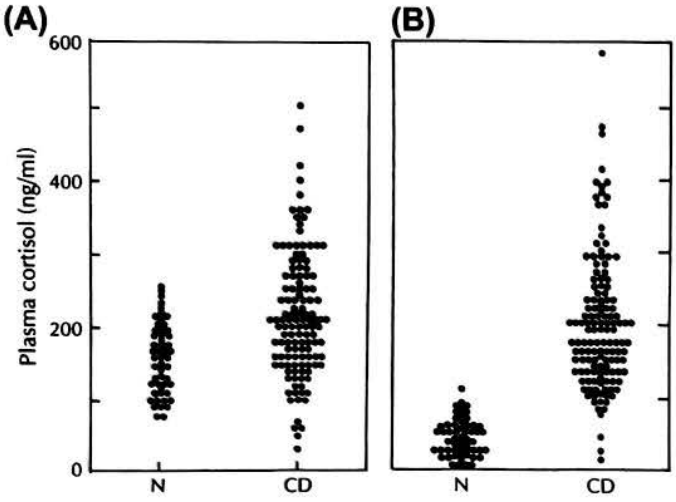

FIGURE 16.14 Plasma cortisol of the same 125 patients with Cushing's disease (CD) were obtained at 8:00 a.m. (A) and 8:00 p.m. (B) They are compared with those of normal subjects (N) at the same times.

range [450,511,512]. Because patients with Cushing's syndrome typically lack a normal circadian rhythm this overlap progressively disappears during the day (Figure 16.14). Studies obtained from multiple series showed that normal values were found in 17% of 182 patients between 4:00 and 9:00 p.m. but in only 3.4% of 147 patients at 11:00 p.m. [509]. A single sleeping midnight serum cortisol of <50 nmol/L (1.8 μg/dl) effectively excludes Cushing's syndrome; an awake midnight serum cortisol of >207 nmol/L (7.5 μg/dl) is highly suggestive of Cushing's syndrome [25,513–517] Late evening serum cortisol has a good sensitivity but suffers from two drawbacks: (1) normal subjects show frequent fluctuations of serum cortisol and so do patients with Cushing's syndrome [118–120,517,518]; thus a trough value in a patient may overlap with an occasional burst of cortisol in a normal subject, including in the evening; and (2) serum cortisol measures the total (free + bound) circulating hormone; it will therefore be altered whenever corticosteroid-binding globulin (CBG) concentration varies [517,518].

Repeat serum cortisol measurements are used to assess the lack of circadian rhythm in patients with Cushing's syndrome. Various sampling frequencies – at least six per day – and mathematical paradigms have been proposed to define a normal, and an abnormal, circadian pattern [512,519]. They do not help so much to establish the diagnosis as to appreciate the ultimate quality of a therapeutic regimen. In theory, an ideal treatment should not only control the hypercortisolism but also restore a qualitatively normal pituitary–adrenal homeostasis, including a normal circadian rhythm. Plasma cortisol may be used as the end point of the various suppression tests (see below); it is inescapable in patients with chronic renal failure [520].

Some authors have used continuous blood withdrawal with a peristaltic pump to measure the integrated concentration of plasma cortisol over different lengths of time. It was found that the 24-hour integrative plasma cortisol reliably separated normal subjects from patients with Cushing's syndrome [115,116]; then a shortened collecting period over 6 hours proved equally efficient, provided that it was performed between 8:00 p.m. and 2:00 a.m. [521]. This approach is probably more interesting as a research tool than as a routine laboratory procedure; further curtailing the time of withdrawal would ultimately end up at an equally efficient and certainly less cumbersome single blood collection at 11:00 p.m.

Blood free cortisol is the best indicator of the cortisolic state. Not only is it biologically relevant, but it is also a highly sensitive parameter. Since plasma CBG is not totally saturated at normal plasma cortisol values a further elevation increases the ratio of free/bound cortisol. Whenever cortisol production increases, variations of free plasma cortisol are amplified in comparison with those of total plasma cortisol [522,523]. Measurement of blood free cortisol requires sophisticated techniques which cannot be easily performed.

LATE NIGHT SALIVARY CORTISOL

Salivary cortisol concentration is a reliable indicator of blood free cortisol [524]. It offers a convenient, nonstressful way of sample collection, even in outpatients (Figure 16.15) and a special device has been developed to measure its integrated concentration over time [525]. Many studies would suggest that it can readily substitute for serum cortisol with an at least equal performance [490,502,514,517,518,526–528].

24-HOUR URINARY CORTISOL EXCRETION (URINARY CORTISOL)

Basal urinary collections have long provided the sole index of adrenocortical activity of a given individual. The 17-hydroxycorticosteroids measured by the Porter and Silber reaction and/or the 17-ketogenic steroids secondarily measured by the Zimmerman reaction have been extensively used. They suffer from several limitations. First, as many as 25% of obese subjects overlap with patients with Cushing's syndrome [509]. This overlap is reduced when the excretion of 17-hydroxycorticosteroids is expressed in mg/day and mg/g of urinary creatinine [511]. Second, situations of increased (obesity, hyperthyroidism) or decreased (hypothyroidism) cortisol metabolism induce parallel variations in urinary 17-hydroxycorticosteroids which do not correspond to a hyper- or hypocortisolic state but simply to adaptive changes in cortisol production rate

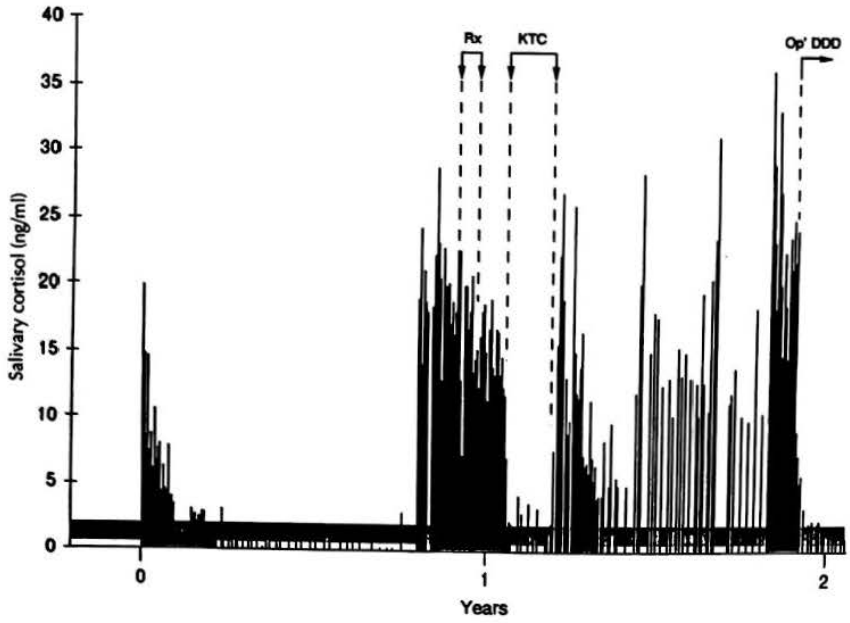

FIGURE 16.15 Two-year follow-up of 10:00 p.m. salivary cortisol in a patient with Cushing's disease. Spontaneous fluctuations of the disease and the response to various therapeutic regimens are observed. R_x, pituitary radiotherapy; KTC, ketoconazole. The horizontal black bar indicates the normal range of 10:00 p.m. salivary cortisol.

[529–531]. Drugs that accelerate or derive cortisol metabolism in the liver (phenyl-hydantoin, barbiturates, 1,1-dichloro-2–(o-chlorophenyl)-2-(*p*-chlorophenyl)-ethane (*op*'DDD)) alter the measured urinary 17-hydroxycorticosteroids without (or before for *op*'DDD) altering the production rate and the plasma levels of cortisol [518,532,533]. For these reasons the baseline urinary 17-hydroxycorticosteroids should no longer be used to diagnose Cushing's syndrome.

In contrast with cortisol metabolites, 24-hour urinary cortisol excretion is an almost ideal marker of the cortisolic state. Urinary cortisol is measured by competitive protein-binding assay after extraction, or by immunoassay. Because it is correlated with the levels of blood free cortisol, urinary cortisol excretion has several invaluable qualities: (1) it is biologically relevant, being a reflection of how much biologically active, i.e., free, cortisol has been circulating over the last 24-hour period [534]; (2) it is a highly sensitive marker, since whenever the cortisol production rate increases two-fold, urinary cortisol excretion increases four-fold, whereas the 17-hydroxycorticosteroids and plasma cortisol increase only two-fold (Figure 16.16); and (3) it is not altered in obese patients, in estrogen-treated females, or by drugs or conditions that modify cortisol metabolism [511,535]. A number of studies have verified that the theoretical advantages of urinary cortisol excretion actually offer a practical gain for the diagnosis of Cushing's syndrome [509]. An almost perfect distinction is obtained between patients with Cushing's syndrome and normal subjects, provided that the urine collection is well done, and that the laboratory has validated its normal values in a large population of normal subjects

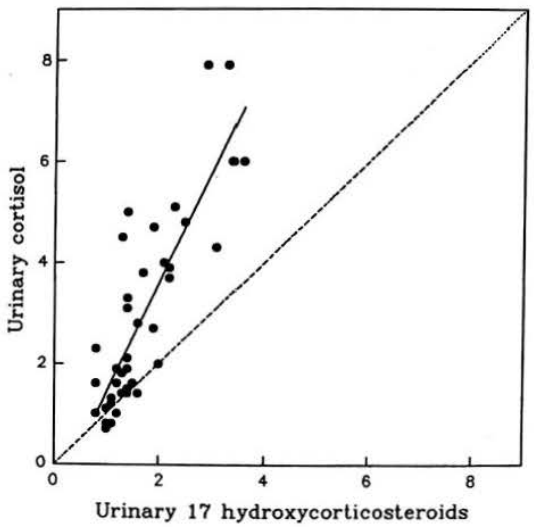

FIGURE 16.16 Correlation between the relative variations of urinary cortisol and 17-hydroxycorticosteroids in response to the stimulatory action of RU 486 in patients with Cushing's disease.

(Figure 16.17). This single basal measurement has a diagnostic accuracy comparable to the reference low-dose dexamethasone suppression tests [512,536,537]. Variations on the theme have been developed that measure urinary cortisol excretion over short (and different) periods of time to obtain a circadian pattern. Unsurprisingly, urinary cortisol excretion shows the same variations as plasma cortisol [538]. Vesperal (20–24-hour) [539] or overnight [280] urinary cortisol excretion have proved excellent diagnostic tools; they offer an obvious gain in convenience for the work-up or the follow-up of outpatients.

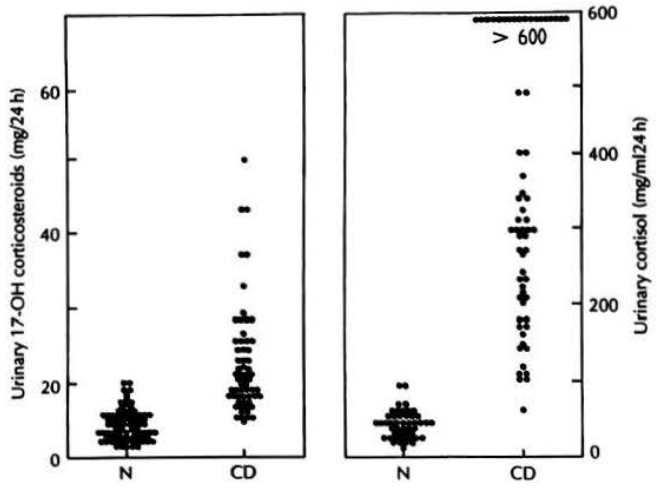

FIGURE 16.17 Baseline urinary 17-hydroxycorticosteroids and cortisol in the same 61 patients with Cushing's disease (CD) are compared with the values obtained in normal subjects (N).

CORTISOL PRODUCTION RATE

Daily cortisol production rate has an excellent sensitivity, since all patients with Cushing's syndrome have, as expected, increased cortisol production rates [509]. It has several disadvantages: (1) it is a difficult and cumbersome procedure requiring the administration of labeled isotopes; and (2) it has a poor specificity since cortisol production rate appropriately rises whenever cortisol metabolism is increased [511]. This adaptive reaction simply maintains the eucortisolic state. The recent development of a more practical analytic procedure using a stable isotope may renew this approach [540].

Suppression Tests

These tests were established in the late 1950s and early 1960s at a time when the sole measurement of baseline urinary 17-hydroxycorticosteroids offered a poor predictive value to separate normal subjects from patients with Cushing's syndrome.

THE CLASSIC LOW-DOSE DEXAMETHASONE SUPPRESSION TEST

The scientific knowledge on pituitary adrenal regulation had led Liddle to surmise that "... the fact that in Cushing's syndrome cortisol secretion is manifestly excessive implies that the normal restraint on pituitary or adrenal function is not operating properly ... " To test this hypothesis it was "... desirable that the steroid selected for the purpose of suppressing ACTH be one which did not itself contribute appreciably to the level of 17-hydroxycorticoids in the urine ... " [123]. Thus synthetic steroid analogues were selected because of their high glucocorticoid potency. It was anticipated that they would suppress ACTH when given in minute amounts as compared to the daily amount of normally secreted cortisol. It was established that 0.5 mg dexamethasone, given every 6 hours for eight doses (2 mg/day) induced almost complete suppression of urinary 17-hydroxycorticosteroid excretion on the second day of administration in normal subjects (<2.5 mg/g urinary creatinine). In contrast, almost all patients with Cushing's syndrome, whatever the etiology, maintained relatively high levels (>2.5 mg/g urinary creatinine), indicating that their feedback mechanism indeed was " ... not operating properly ... " Thus this low dose of dexamethasone allowed a convenient and efficient means to separate patients with, and subjects without, Cushing's syndrome [123,509,511,531].

In adults or in pediatric patients weighing more than 40 kg, dexamethasone is given in doses of 0.5 mg for 48 h, at 6 h intervals, beginning at 09:00 h on day 1 (i.e., at 09:00, 15:00, 21:00 and 03:00 h) and obtaining serum cortisol at 09:00 h, 6 h after the last dose of dexamethasone or beginning at 12:00 h (i.e., at 12:00, 18:00, 00:00 and 06:00 h) and obtaining serum cortisol at 08:00 h, 2 h after the last dexamethasone dose [541]. For pediatric patients weighing less than 40 kg, the dose is adjusted to 30 μg/kg/d (in divided doses) [9].

Initially appreciated through measurement of the urinary 17-hydroxycorticosteroids, the suppressive effect of the low-dose dexamethasone test has been subsequently evaluated by measuring urinary cortisol excretion (normal response: less than 27 nmol/24 h; 10 μg by 24 hours) or morning serum (normal response: less than 50 nmoles/L; or 1.8 μg/dl) or salivary cortisol collected precisely 2 hours after the last oral dose of dexamethasone, with an equal diagnostic accuracy [518,537,542,543].

Analysis of multiseries gathering several hundred cases showed that less than 5% of patients with Cushing's syndrome had normal suppression, but as many as 30% of subjects (and only an exceptional subject) without the syndrome failed to suppress normally. Furthermore, drugs that accelerate dexamethasone metabolism by induction of CYP3A4 may falsely elevate serum and urinary cortisol (false-positive responses); conversely, drugs that impair dexamethasone metabolism by inhibition of CYP3A4 may falsely decrease serum and urinary cortisol (false-negative responses).

Some authors have proposed improvement the test by tailoring the dose of dexamethasone according to body weight and administering 20 μg/kg each day rather than a fixed dose of 2 mg/day [511]. In children, it is certainly essential to adjust the dexamethasone dose [544], usually to body surface area at 2 mg/day per 1.73 m^2 for the classic low-dose dexamethasone suppression test. Since it was designed, the classic low-dose dexamethasone suppression test has been considered the most reliable means to confirm or rule out the diagnosis of Cushing's syndrome[509].

THE OVERNIGHT 1 MG DEXAMETHASONE SUPPRESSION TEST

Based on the same theoretical grounds, numerous alternate suppression tests have been proposed which attempted to offer some practical advantage over the classic approach. Probably the most popular is the overnight 1 mg dexamethasone suppression test [545]. Dexamethasone (1 mg) is administered orally between 11:00 and 12:00 p.m. and serum cortisol is measured the next morning between 8:00 and 9:00 a.m. This test is simple in outpatients. In normal subjects plasma cortisol values will be suppressed below a definite limit (established by each laboratory, and depending on the assay method). The recommended cutoff is 50 nmoles/L (or 1.8 μg/dl or 18 ng/ml), which achieves high sensitivity rates and specificity rates of 80% [517,518]. Drugs that modify dexamethasone metabolism are the same as those seen for the classic low-dose dexamethasone suppression test. A number of series have established the high sensitivity of the test, since only an exceptional patient with Cushing's syndrome will suppress normally [509].

Unfortunately the test's specificity is not good, since it has been found positive (lack of normal suppression) in as many as 13% of obese subjects and in 23% of hospitalized or chronically ill patients [509]. It may also be falsely positive in women taking estrogen. Thus, although it does not show the same diagnostic accuracy as the classic low-dose dexamethasone test it is still highly useful, and convenient, to eliminate diagnosis of Cushing's syndrome in outpatients.

As an alternative to blood collection, salivary cortisol has been used to assess the suppressive effect of the overnight 1 mg dexamethasone test with essentially similar results [490]. Further studies are needed to establish whether it will improve the specificity of the test.

Different authors have used different criteria to establish the cutoff point for normal suppression; it may be chosen as the upper limit of the normal range, the mean plus 1, 2, or 3 standard deviations, or even arbitrarily. These manipulations have implications, since raising the cutoff point increases the test specificity (less false-positive), but at the same time decreases the sensitivity (more false-negative). A sound philosophy [546] is probably to use stringent cutoff points that tend to lower the specificity since it is more acceptable to restudy a subject with suspected Cushing's syndrome than to miss the diagnosis. These limitations apply to the interpretation of other tests. A reasonable approach to diagnosis sometimes necessitates repeating tests, which must always be correlated with the clinical observation.

OTHER SUPPRESSION TESTS

Some have proposed suppression of cortisol by intravenous dexamethasone infusion in order to avoid (hypothetical) variations due to interindividual differences in the rate of dexamethasone absorption. Various dosages and times of administration have been used that all successfully achieved the desired separation between patients with Cushing's syndrome and normal (or obese) subjects [547–549].

In the field of dexamethasone suppression the ingenuity of many investigators has led to the development of many different tests. The result is extraordinarily reassuring since, even if some tests are better, all work essentially in the same manner and therefore offer an effective confirmation to the hypothesis that initiated this approach. The trick really is to use a potent glucocorticoid agonist and to titrate its dosage of administration so that it will be sufficient to totally suppress cortisol production of all normal subjects (including obese), yet insufficient to totally suppress cortisol production in all patients with Cushing's disease and, of course, in those with other causes of Cushing's syndrome.

Establishing the ACTH-driven Hypercortisolic State

When the diagnosis of Cushing's syndrome has been unequivocally obtained, its etiological investigation relies, above all, upon the appreciation of the corticotroph function.

Plasma ACTH

The first successful approach was that of Liddle's group in 1961 [108] who used the Lipscomb and Nelson [107] ACTH bioassay "... in search of a definitive answer to the question of whether the pituitary secretes abnormal quantities of corticotropin (ACTH) in Cushing's disease ... " The answer was positive, and was rapidly confirmed by others.

A few years later Berson and Yalow chose the ACTH assay as one of their first RIAs developed in humans and immediately applied this new method to the investigation of the pituitary–adrenal axis [64]. Until recently ACTH RIA has been the method of choice to assess corticotroph function because of its sensitivity and specificity [550].

ACTH is rapidly destroyed in blood by enzymes. Special care is necessary to obtain adequate plasma samples for RIA. The RIA itself presents difficulties that pertain to the low plasma concentrations, the strong affinity of ACTH for absorption to glassware, a tendency for the labeled tracer to undergo incubation damage and interference of plasma with a given antiserum. There is therefore an absolute need that both blood collection and ACTH RIA be performed expertly. The diagnostic implications of plasma ACTH determination are too important to allow uncertainty of sampling and testing.

Because various antisera will be directed against various epitopes of the molecule, some discrepancies have been observed between different RIAs, and between a given RIA and the bioassay [550,551]. Over the years many reliable RIAs have been developed using either extracted or unextracted plasma [550].

Other means to measure plasma ACTH have been developed. Radioreceptor assays [552] and the cytochemical or redox bioassay [553] have the advantage of measuring bioactive ACTH. The redox bioassay offers extraordinary sensitivity that is at least 100 times better than that of most RIAs. However, these two theoretical advantages are not really needed for diagnostic investigation of Cushing's syndrome and thus cannot compete with the ease of RIAs.

IRMAs, in contrast, offer theoretical and practical advantages as well over classical RIAs [171,554,555]; the sensitivity is somewhat better than that of most RIAs, although in the same order of magnitude (0.2 pmole/L or 1 pg/ml at best). The specificity is improved by definition since only intact $ACTH_{1-39}$ is measured. Although ACTH fragments are not measured by IRMAs they may interfere with the assay system at high concentrations by saturating the first antibody. Failure to recognize this may lead to erroneous interpretation [556]. Most important is the convenience of the IRMAs, which can be performed on unextracted plasma, with results obtained rapidly – within 24 hours – on a wide range of plasma values. Blood manipulations require fewer precautions than with RIAs since the "sandwich" effect protects the ACTH molecule during the incubation. Recent studies have largely confirmed the validity and efficacy of ACTH IRMAs which yield results which correlate almost perfectly with those obtained by the best RIAs in the same plasma samples [555]. Thus, because of their unique practical convenience it is anticipated that ACTH IRMAs are the method of choice for evaluating plasma ACTH.

In normal subjects, morning plasma ACTH constantly ranges between <2.2 and 17.6 pmol/L (<10 and 80 pg/ml) in most laboratories. Numerous series have shown that patients with Cushing's disease have morning plasma ACTH levels that tend to be slightly elevated; ACTH is almost always measurable, between half and two-thirds of the patients have values within the normal range, and the values of the others usually do not exceed 40 pmol/L (or 180 pg/ml) (Figure 16.18) [112,113,509]. Thus, morning plasma ACTH does not fully separate patients with Cushing's disease from normal, just like morning plasma cortisol. This overlap disappears when ACTH is measured later in the day [557]. At this stage of the diagnostic procedure this overlap really is not troublesome since the goal is to separate the different causes of Cushing's syndrome. In that prospect the information is invaluable; that ACTH plasma level is merely measurable is totally inappropriate and unquestionably indicates that the hypercortisolic state is ACTH-driven. It eliminates the patients whose Cushing's syndrome is secondary to an autonomously secreting adrenocortical tumor. In this latter situation pituitary ACTH secretion is appropriately suppressed and ACTH plasma levels are invariably undetectable [509], that is, under the lower sensitivity limit of the RIAs. It does not eliminate the patient with ectopic ACTH syndrome. If at least one of the two ACTH measurements is greater than 15–20 pg/ml (3.3–4.4 pmol/L) during the hypercortisolism phase, it is very likely that the Cushing's syndrome is ACTH-dependent. If there is any doubt, it is advisable to carry out a CRH test or even a high-dose dexamethasone suppression test and a CT scan of the adrenal glands [25,518].

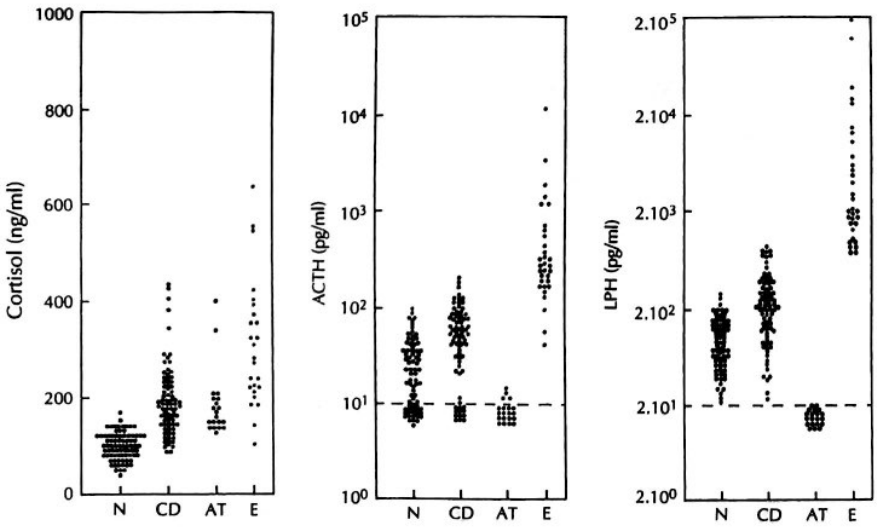

FIGURE 16.18 Morning plasma values for cortisol, adrenocorticotropic hormone (ACTH) and lipotropin (LPH) in the same blood sample obtained from normal subjects (N), patients with Cushing's disease (CD), patients with a cortisol-secreting adrenocortical tumor (AT) and patients with ectopic ACTH syndrome (E).

Plasma Non-ACTH POMC Peptides

Almost all natural POMC peptides have been measured in human blood: the N-terminal fragment [168,558,559], joining peptide [38,560], β-LPH and γ-LPH [113,561–563] and β-endorphin [564,565]. Since they are secreted concomitantly with ACTH by the pituitary corticotrophs the plasma levels of any of them constantly show almost perfect correlations with all the others (Figure 16.19). The molar plasma ratio of two POMC fragments is usually close to one, with slight differences related to different metabolic clearance rates. Thus it is not surprising that plasma determination of any non-ACTH POMC fragment provides the investigator with essentially the same diagnostic accuracy as that of ACTH itself (see Figure 16.18).

On a practical basis LPH RIAs have been the most popular non-ACTH POMC fragments studied. They were the first available but also offer several practical advantages. In contrast with ACTH, both β-LPH and γ-LPH are extremely stable in blood; immunoreactive plasma LPH values will remain unchanged in blood kept at room temperature for 24 hours [117], so that handling of blood collection is much less troublesome. Because there are large species differences in the common amino terminal region of the β- and γ-LPHs, these molecules are highly antigenic and antibodies with high affinity may be easily raised. Hence direct LPH RIA in a small volume (50 μl) of unextracted plasma is readily feasible [117]. Like ACTH, plasma immunoreactive LPH tends to be slightly elevated in Cushing's disease, and its value completely discriminates patients with Cushing's disease and patients with autonomous cortisol-secreting adrenocortical tumors who always have undetectable plasma values [113]. Other reports have shown essentially identical results using the N-terminal fragment, the joining peptide, or the β-endorphin RIAs.

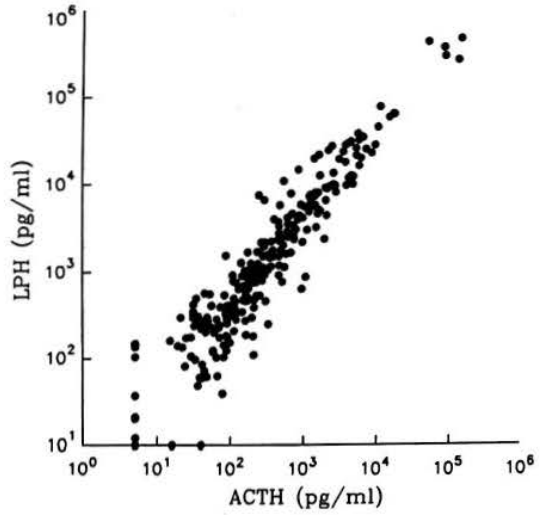

FIGURE 16.19 Correlation between plasma immunoreactive lipotropin (LPH) and adrenocorticotropic hormone (ACTH) baseline values in patients with Cushing's disease and Nelson's syndrome.

Plasma Adrenocortical Androgens

The pattern of adrenocortical steroid secretion provides some clues in the etiologic work-up [2]. Because ACTH also regulates adrenocortical androgen secretion the latter tend to be elevated in Cushing's disease (and ectopic ACTH syndrome), whereas they are decreased in benign adrenocortical adenoma since this tumor typically produces only glucocorticoids that suppress pituitary ACTH secretion [336,337,566]. These notions are most helpful for the diagnosis of female patients: low plasma testosterone, DHEAS and Δ-4-androstenedione will be suggestive of a benign adrenocortical adenoma. Slightly elevated androgens will point to Cushing's disease but will not rule out ectopic ACTH syndrome. Highly increased androgens will suggest an adrenocortical carcinoma [16].

Establishing the Pituitary Origin of the ACTH-driven Hypercortisolic State

At this point it becomes necessary to discriminate between patients whose ACTH oversecretion is of pituitary (Cushing's disease) or nonpituitary (ectopic ACTH syndrome) origin.

Over the years various approaches have been developed to find the source of ACTH oversecretion in Cushing's syndrome. Essential clues are provided by sophisticated measurement of corticotroph function that, besides the quantitative and qualitative assessment of its baseline levels, will also require dynamic manipulations and sometimes invasive tracking investigations. Because many of these procedures were primarily set to eliminate an adrenocortical tumor as well, this aspect will also be discussed briefly.

Baseline Corticotroph Function

PLASMA ACTH LEVELS

The ACTH plasma level by itself provides a first clue to the source of ACTH oversecretion. Patients with ectopic ACTH syndrome tend to have higher levels than patients with Cushing's disease. Yet the overlap between the two groups is wide, notably in corticotroph macrodenomas, ACTH values may be high [63,113,509] and further investigations are needed for a clear separation (Figure 16.18).

PLASMA NON-ACTH POMC PEPTIDES

Altered POMC maturation is common in nonpituitary tumors, and decidedly unusual in pituitary corticotroph adenomas [60,62]. This subtle mechanism may be

useful in detecting abnormal POMC fragments in blood that would pinpoint, although not identify, a nonpituitary origin of the ACTH oversecretion.

Partial degradation of ACTH into CLIP is fairly common in nonpituitary tumors (see Figure 16.8) [61]. CLIP escapes detection by most (sufficiently specific) ACTH RIAs as well as by ACTH IRMAs. Since the LPHs are unaffected by the altered POMC processing the plasma LPH/ACTH ratio is increased [113].

Gel filtration chromatography and/or high-pressure liquid chromatography (HPLC) of plasma samples or extracts will also detect abnormal molecular forms of ACTH, or unusual molecules like β-MSH_{5-22} [352]. Elegant multitargeted IRMA systems have been developed that can recognize by simple direct plasma assays the occurrence of an abnormal POMC processing at a given location on the precursor molecule [567,568].

These approaches have their limitations, however. Besides being only available to highly specialized laboratories, their sensitivity is low and their specificity also is not perfect. Rare cases of pituitary macroadenoma have been described which convincingly did not process POMC normally [63,172,173].

TUMOR MARKERS

Some tumours that cause ectopic ACTH secretion have powerful tumor markers, which must be measured if there is any doubt, such as pheochromocytomas (urinary and plasma-free normetanephrine and normetanephrine), medullary thyroid carcinoma (calcitonin) and gastrinomas (gastrin). Chromogranin A had a positive predictive value of 83% and a negative predictive value of 70% for the diagnosis of EAS in an NIH study on six patients with occult ectopic ACTH secretion diagnosed by central venous catheterization, 11 patients with histologically proven ectopic ACTH secretion and 25 patients with Cushing's disease [569]. Other tumor markers may be measured: calcitonin and its derivatives (for other neuroendocrine tumors than medullary carcinoma), urinary 5-hydroxyindoleacetic acid, glycoprotein alpha-subunit, free hCG beta-subunit, carcinoembryonic antigen, neuron-specific enolase, vasoactive intestinal peptide, glucagon, etc., have less diagnosic accuracy.

Dynamics of the Corticotroph Function

THE CLASSIC HIGH-DOSE DEXAMETHASONE SUPPRESSION TEST

The purpose here is to test the pituitary-dependency of the hypercortisolic state. In the classic [123] test dexamethasone is given orally at the dose of 2 mg every 6 hours (8 mg/day) for 2 days. Urinary corticosteroids are measured on the second day of dexamethasone administration and compared with their pretreatment (control) value. In the original paper of Liddle, all patients with Cushing's disease decreased their urinary 17-hydroxycorticosteroid to less then 50% of control values, whereas all those with adrenocortical tumors failed to reach this level of suppression [123]. Since this report it has been stated by many authors that all patients with Cushing's disease should suppress to less than 50% of their control values on the high-dose dexamethasone suppression test. Actually "... the degree of suppression is not crucial, as long as it is beyond the day-to-day fluctuations observed during control periods. In response to large doses of dexamethasone ... most (patients with Cushing's disease) exhibit decreases to less than 50% of their control values. A few, however, have been known merely to exhibit decreases to 70 to 80% of their control values" [531].

Numerous series have confirmed that whereas some 60–85% of patients with Cushing's disease suppress to less than 50% of their control values on the high-dose dexamethasone test, no rigid cutoff level should be given that rules out the diagnosis of Cushing's disease [25,509]. The specificity can be improved using a cutoff of cortisol suppression greater than 90%, although a specificity of 100% can never be attained [25,518,570]. In some patients with authentic Cushing's disease this level of suppression (50–60% of baseline) could be obtained only by administering much higher doses of dexamethasone, sometimes up to 32 mg/day [378,509]. Some data suggest that these patients have a more severe disease as judged by baseline plasma and urinary cortisol [571].

If this test has a great specificity to eliminate autonomous secreting adrenocortical tumors, it is somewhat less powerful to eliminate ectopic ACTH syndrome where an apparent suppression is not rarely observed [412,509,572].

Comparing previous and current dexamethasone urinary corticosteroid values does not only evaluate the effect of dexamethasone, but also the effect of time. Many tumors have spontaneously cycling or fluctuating activities that may induce large variations over a 2 day period. Thus an ACTH-secreting nonpituitary tumor that would spontaneously decrease its activity at the time when the test is performed could be erroneously interpreted as being suppressed by dexamethasone [573]. Alternatively a paradoxical increase in cortisol secretion has been occasionally observed in patients with authentic Cushing's disease, a phenomenon best explained by spontaneous fluctuations of the disease [483,484,574]. The only way to avoid this flaw for a single individual would be to obtain repeated urinary values to establish the degree of spontaneous variations, and/or to repeat the test itself to evaluate whether it is reproducible.

The high-dose dexamethasone test often confirms information already available from the classic low-

dose dexamethasone test. Many patients with Cushing's disease who, by definition, fail to completely suppress on the low-dose test, however exhibit a definite decrease in their urinary corticosteroids. Thus, at the same time these patients exhibit an abnormal degree of resistance to the suppressive effect of glucocorticoids, which is the hallmark of Cushing's disease.

Some variations have been brought to the classic test which measure urinary cortisol instead of 17-hydroxycorticosteroid [570] or morning plasma cortisol before and after the 2 days of dexamethasone administration, with essentially the same diagnostic accuracy.

THE OVERNIGHT 8 MG DEXAMETHASONE SUPPRESSION TEST

In this test 8 mg of dexamethasone is given orally as a single dose at 23:00 or 24:00 h and the 08:00 h or 09:00 h serum cortisol the next morning is compared with that of the previous (control) day. The proposed cutoff point for a positive response (suppressibility) is a serum cortisol decrease to 50% or less of its control value [575]. With this arbitrary criterion, two studies gathering 73 patients with Cushing's disease, eight with adrenocortical tumors and 12 with ectopic ACTH syndrome, compared this overnight test directly with the classic high-dose dexamethasone suppression test performed in the same patients. This test appeared at least as efficient if not better, with 89% sensitivity and 100% specificity for the diagnosis of Cushing's disease [576]. That this overnight suppression test reaches higher diagnostic accuracy than the classic test may be explained by the simple fact that it is a stronger one since the 8 mg dose is given as a single administration. In the same manner as for the classic test, there is no theoretical reason to fix a rigid cutoff at a 50% decrease.

Others have studied the acute variations of plasma cortisol during dexamethasone infusion [577] to discriminate pituitary-dependent Cushing's disease.

THE METYRAPONE TEST

The purpose of this test is not dissimilar to that of the high-dose dexamethasone suppression test since it also evaluates the pituitary-dependency of the adrenocortical hyperfunction. The approach is just the inverse, i.e., to observe the corticotroph response to cortisol deprivation.

In the classic test [578] 750 mg of metyrapone is given every 4 hours for six doses. Urinary (24-hour) 17-hydroxycorticosteroids are measured the day before, the day of and the day after metyrapone administration. Normal subjects usually show at least a two-fold rise in urinary 17-hydroxycorticosteroids on the treatment day or on the day after, compared with the day before, reaching values above 10 mg/day [578,579]. Results obtained from more than 100 patients with Cushing's disease from different studies showed that virtually all patients responded to metyrapone with an increase in urinary 17-hydroxycorticosteroids. The sensitivity of the test reaches almost 98% [509]. In many patients an explosive response is obtained (up to 100 mg/24 h). Thus, failure to respond to metyrapone essentially excludes the diagnosis of Cushing's disease.

If this test has a great specificity to eliminate adrenocortical tumors it is less powerful to eliminate ectopic ACTH syndrome [572]. When the diagnostic accuracies of the metyrapone and the classic high-dose dexamethasone suppression tests are compared, similar figures are obtained, indicating that they merely address the same question as to whether the pituitary is involved.

Alternate methods have been proposed including the use of single-dose metyrapone tests [448,580]. Measuring plasma 11-deoxycortisol to assess the response to metyrapone in Cushing's syndrome requires special precautions. In Cushing's disease spontaneous and short fluctuations in ACTH activity may blunt the plasma 11-deoxycortisol increase. Whatever the cause of Cushing's syndrome, blockade of the 11-β-hydroxylase will automatically, and inevitably, increase plasma 11-deoxycortisol whether or not an ACTH rise is triggered. In the case of an adrenocortical tumor, the plasma 11-deoxycortisol increase will remain much lower than that observed in Cushing's disease. However, in patients with ectopic ACTH syndrome, especially if ACTH plasma levels are high, plasma 11-deoxycortisol will rise to levels identical with those reached in Cushing's disease, even though the ACTH levels do not change [581]. These flaws are avoided in the classic metyrapone test evaluated on the urinary 17-hydroxycorticosteroids, because they are the sum of cortisol and 11-deoxycortisol metabolites they will only rise if ACTH secretion increases. Thus it is highly recommended that the metyrapone test be performed in its classic setting with the total urinary 17-hydroxycorticosteroids as the best parameter.

The LPH plasma levels rise in response to metyrapone in patients with Cushing's disease and could help to discriminate them from those with ectopic ACTH syndrome where no response is observed [581]. The true diagnostic value of this approach is not well established.

DIRECT ASSESSMENT OF PITUITARY ACTH RESERVE

Several secretagogues that act specifically on the corticotroph cell in the normal subject have been used in Cushing's syndrome with the assumption that they would only trigger further ACTH secretion if the latter were of pituitary origin.

THE LVP TEST LVP is a synthetic peptide analogue of AVP that exhibits the same agonistic activity on the

pituitary V_1 receptors. It is administered as a bolus intramuscular injection of 10 IU. Plasma cortisol and ACTH are measured before, and up to 60 minutes after, injection [111]. LVP has long been the sole ACTH secretagogue with a direct pituitary action. Its use has been limited because of its low stimulatory activity in comparison with its effects on smooth muscle V_1 receptors which generate gastrointestinal symptoms, general pallor and high blood pressure. These unwanted effects contraindicate its use in older subjects or patients at risk for coronary heart disease or glaucoma. In the diagnostic work-up its major interest is to achieve a complete separation between patients with adrenocortical tumors, where ACTH levels remain undetectable, and the rare patients with Cushing's disease who have low-to-undetectable baseline ACTH and in whom LVP invariably restores measurable plasma ACTH [113]. The use of LVP to discriminate between Cushing's disease and ectopic ACTH syndrome has not been established. The LVP test is abandoned in clinical practice because of the high incidence of side effects, and the substitute use of the desmopressin test.

THE DESMOPRESSIN TEST More recently the desmopressin (1-deamino-8D-arginine vasopressin) (which is a V2 and V3 agonist) has been used as a more potent ACTH secretagogue in Cushing's disease, with less side effects. Response is assessed by measuring ACTH, serum cortisol and possibly salivary cortisol, at various intervals – 30 minutes before, basally, 15, 30, 45 and 60 minutes after intravenous administration of 10 μg of desmopressin with serial blood samples obtained from an indwelling catheter inserted in a forearm vein. Patients have to restrict fluid intake for the remainder of the day to avoid water overload. Adverse effects are limited to a short-lived flushing sensation, a transient tachycardia, mild and transient decrease in blood pressure, headache, abdominal pain, or weight increase. There are several criteria for interpretation in the literature (parameter: ACTH or cortisol; threshold: a relative increase of more than 35–50% in ACTH or of more than 20–36% in cortisol) [25]. The desmopressin test induces a positive ACTH response in ca. 85% of patients with Cushing's disease [582–584]. Yet, since the V3 receptor is expressed in as many as 30% of ectopic tumors secreting ACTH [87,584], the usefulness of the desmopressin test is limited in the differential diagnosis of ACTH-dependent Cushing's syndrome. It might be more interesting in the postoperative assessment to predict recurrence after pituitary surgery as normal subjects rarely respond to the test [25,585,586].

THE CRH TEST A major breakthrough was achieved with the isolation, characterization and synthesis of ovine CRH in 1981 [71]. This discovery opened up new avenues to investigators of corticotroph function in humans [290,586,587]. Thorough studies in normal subjects rapidly showed that the ovine peptide was active in humans, eliciting an ACTH response that was definitively higher than that elicited by LVP, but still lower than that obtained after insulin-induced hypoglycemia [387,588]. Dose–response studies indicated that administration of 10 μg/kg body weight maximally stimulated cortisol release, while larger amounts could induce still higher ACTH responses [387, 589]. Synthetic ovine CRH (in many countries including the United States of America) or synthetic human CRH (in Europe) is administered intravenously at a dose of 100 μg in adults or 1 μg/kg body weight in children. Response is assessed by measuring ACTH, serum cortisol and possibly salivary cortisol 30 minutes before, baseline, 15, 30, 45 and 60 minutes after administration of CRH with serial blood samples obtained from an indwelling catheter inserted in a forearm vein. The test is well tolerated; the few side effects are mild facial flushing and neck tightness. In contrast with normal subjects, the time of day when the test is performed has no particular implication in Cushing's syndrome, but for convenience it may be performed in the morning. Data comparing the ovine and human CRH peptides in the same patients confirm that the former has a higher potency and provides a better diagnostic accuracy, largely outweighing the theoretical advantage of using a homologous peptide, at least for a single-dose testing [590].

The theoretical promise of the CRH test relies on it being a potent and specific stimulator of pituitary ACTH, thus allowing a better separation between patients with Cushing's disease, adrenocortical tumors, and, especially, ectopic ACTH syndrome (Figure 16.20) [218].

As with many tests confusion arises with the various ways different authors not only administer CRH but also appreciate an "exaggerated" or a "flat" response [586]. Criteria for a positive or negative response are seldom defined. In a survey of ten published series [218,591–599], Kaye and Crapo [576] developed their own criteria from these combined data: a positive response would be a relative increase of more than 50% in ACTH or of more than 20% in cortisol; a negative response would be a relative increase of less than 50% in ACTH or of less than 20% in cortisol. With these criteria, the sensitivity and specificity of the CRH test for the diagnosis of Cushing's disease would be 80 and 95% using the ACTH response, and 91 and 95% using the cortisol response. There is a general agreement that the test has a high diagnostic accuracy which compares favorably with that of the classic high-dose dexamethasone suppression test [600]. In evaluating 100 patients with Cushing's disease and 16 patients with ectopic ACTH secretion, a single a.m. CRH stimulation was

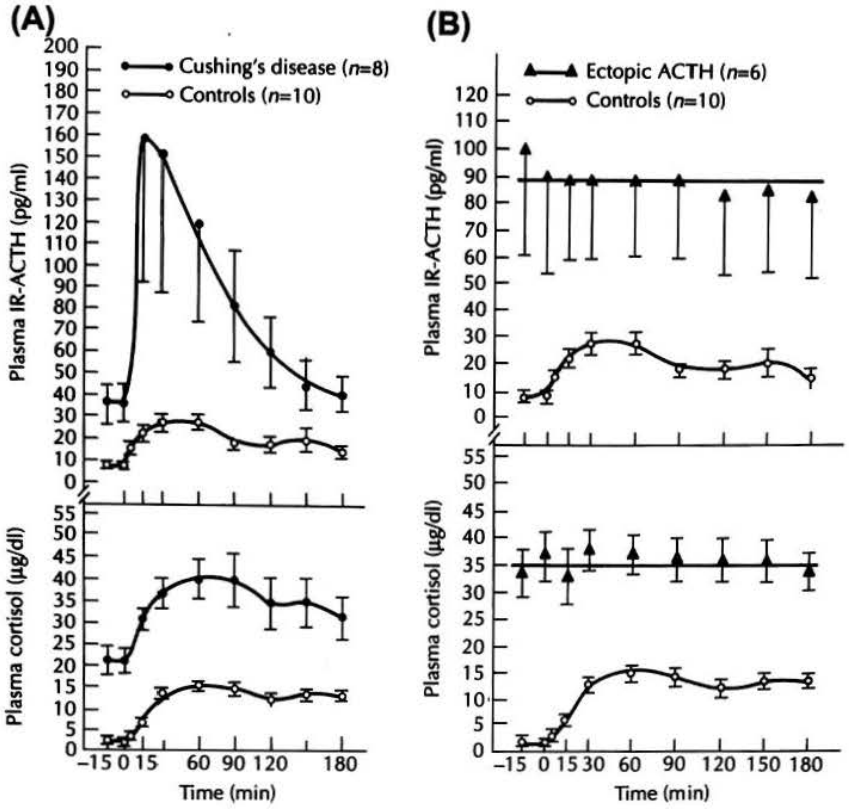

FIGURE 16.20 Responses of plasma immunoreactive (IR) adrenocorticotropic hormone (ACTH) and cortisol to corticotropin-releasing hormone (means ± SEM) in eight untreated patients with Cushing's disease. (A) six patients with Cushing's syndrome due to ectopic ACTH secretion; (B) and ten controls. *From Chrousos et al. [196].*

performed. Seven percent of patients with ACTH-dependent Cushing's did not respond, while no patient with ectopic ACTH secretion responded to CRH [601]. A positive response is highly suggestive of Cushing's disease, and the stronger the response the higher the probability. In another study with 101 patients with Cushing's disease and 14 patients with ectopic ACTH secretion, the sensitivity and specificity of the human CRH test for the diagnosis of Cushing's disease was 70 and 100% using a relative increase of more than 105% in ACTH, and 85 and 100% using a relative increase of more than 14% in cortisol response [602].

There is a general agreement that the test has a high diagnostic accuracy which compares favorably with that of the classic high-dose dexamethasone suppression test [600]. In the series where both tests were applied to the same patients, similar diagnostic accuracies were found [592,595,603–606]. In some cases however, the two tests did not agree, leading some authors to advocate a combined-test strategy to achieve the correct diagnosis. Authentic cases of Cushing's disease that did not respond to CRH have been reported by several authors and account for the 86% sensitivity of the test [576]. The stimulatory action of CRH can be strengthened by the synergistic effect of AVP or its V_1 analogues [607]. Data obtained with a combined CRH/AVP test showed that all patients with Cushing's disease responded positively [608]. A desmopressin-CRH test may be more discriminatory, with a specificity of 80–100% [609,610].

Occasional patients with ectopic ACTH syndrome exhibited an apparent positive response [412,592]. In some cases it may be questioned whether the ACTH increase originated from the tumor or the normal pituitary. Diagnostic difficulty is particularly important in the exceptional cases of bronchial carcinoid tumors that also respond to the high-dose dexamethasone suppression test and metyrapone [412]. Whether these responses are apparent or real is not proved, although in vitro studies tend to indicate that an occasional non-pituitary tumor may be authentically CRH-responsive [396]. These rare cases may lead to unwarranted pituitary surgery for a mistakenly proposed diagnosis of Cushing's disease. They are the ones which would justify systematic bilateral inferior petrosal sinus sampling.

That the pituitary tumor responsible for Cushing's disease further increases its ACTH secretion in response to CRH is further evidence of its intrinsic relative resistance to glucocorticoids. Indeed, the state of chronic hypercortisolism should normally suppress CRH action on the corticotrophs. Thus it is not surprising to observe that the CRH and the high-dose dexamethasone suppression tests each provide

essentially the same diagnostic accuracy [600], since both assess the relative insensitivity to glucocorticoids of the pituitary tumor.

Tracking the ACTH Source: Bilateral Inferior Petrosal Sinus Sampling

The availability of reliable plasma ACTH RIAs – and more recently IRMAs – has prompted the development of invasive sampling procedures aimed at collecting blood draining immediately from the pituitary gland. The goal of this approach is two-fold: first to establish whether ACTH oversecretion is of pituitary or nonpituitary origin; and second, and in the case of Cushing's disease, to lateralize the pituitary location of a microadenoma.

A reliable technique requires that blood sampling be done close enough to the pituitary, that is, within the inferior petrosal sinus. Because a pituitary adenoma will lateralize its secretion in the ipsilateral inferior petrosal sinus it is essential that both sinuses be catheterized. Both sinuses and peripheral blood must be sampled simultaneously [586].

A central-to-peripheral ACTH gradient is calculated by the ratio of ACTH plasma levels in the inferior petrosal sinus with the highest level over ACTH in the peripheral blood. In patients with Cushing's disease this gradient is almost always over 2, with a mean of 15. In patients with ectopic ACTH syndrome this gradient is almost always lower than 1.7. Some have reported that the test can be improved by simultaneous CRH stimulation which increases the gradient [611–613]. A basal central:peripheral ratio of >2:1 or a CRH stimulated ratio of >3:1 is indicative of Cushing's disease. Analysis of different series and individual case reports all confirm the great diagnostic power of the procedure to discriminate between Cushing's disease and ectopic ACTH syndrome with a sensitivity and a specificity of 94% [611–617,619–626].

The main causes of false-negatives are inferior petrosal sinus sampling that is not sufficiently selective, plexiform vascularization in at least one sinus, abnormal venous drainage of an adenoma which is intrasphenoidal but not strictly intrasellar and intermittent ACTH secretion in a case of Cushing's disease. Most of these causes may be avoided by refering to an experienced neuroradiologist [627], checking catheter position with a venous angiogram and measuring prolactin, along with ACTH [628,629]. The main causes of false-positives are investigation done during a period of normal cortisol levels in the presence of a tumor causing ectopic but intermittent ACTH secretion [629], and ectopic tumor secretion of CRH [629,630]. Some false positives may be avoided by checking the consistency of hypercortisolism during the days before the procedure and preceding the catheterism by careful investigations for an endocrine tumor.

Although somewhat invasive, particularly considering the general vascular fragility of patients with Cushing's syndrome, there are few serious side effects reported. Venous thromboembolism, sixth nerve palsy, veinous subarachnoid hemmorhage, brain stem infarction [616,631,633–635]. The slight discomfort of the technique [632] is largely overcome by its diagnostic accuracy. This investigation should only be considered and performed in centers where there is a great deal of experience in the matter.

Some propose cavernous sinuses [636] or jugular venous sampling [637–639] and some substitute CRH by desmopressin, without major adverse effects during or after the procedure [640,641].

In the case of Cushing's disease this procedure also helps to localize the pituitary microadenoma by evaluating the sinus-to-sinus ACTH gradient [620,621,642]. A gradient over 1.5 lateralizes the adenoma to the pituitary half draining in the ipsilateral sinus with the highest ACTH level. Some have reported that the test can be improved by simultaneous CRH stimulation which increases the gradient [611–613]. Variable efficacy of this approach has been reported, often with a high success rate [576]. In fact this technique is not diagnosis-directed; its goal is to help the neurosurgeon remove a pituitary microadenoma that would not be readily picked up by imaging techniques and possibly not seen at surgery. Successful blind hemihypophysectomies directed by sampling lateralization have been claimed [611,621]. Obvious difficulties are anticipated in previously operated patients, and with macroadenomas and microadenomas situated centrally. Incorrect lateralizations have been reported [615,616,642] in patients with Cushing's disease. Several authors have recently reported that other pituitary hormones (PRL, GH, α-subunit) colateralize with ACTH on the ipsilateral sinus draining the microadenoma [620,643–645]. Since these non-ACTH peptides were not detected by immunohistologic studies in the removed adenomas they raise the question of a nonspecific effect of the adenoma on pituitary blood flow, or of an as yet undemonstrated local, and general, paracrine effect of the tumor. An incorrect position of the catheter might explain a colateralization of ACTH and prolactin.

IMAGING TECHNIQUES

The Pituitary

Skull X-ray and Tomograms

Because most pituitary corticotroph adenomas are small, gross deformation of the pituitary sella is rarely

encountered [646] in untreated Cushing's disease. They may be demonstrated in patients who develop Nelson's syndrome, and in the rare patients with an initial macroadenoma. Skull X-rays will often show evidence of osteopenia of the dorsum sellae, and provide the neurosurgeon with useful indications on the bone landmarks and state of pneumatization of the sphenoidal sinus.

CT Scanning

With the development of pituitary surgery as the treatment of choice for Cushing's disease, preoperative localization of a pituitary adenoma is more important. CT has been for a long time the only imaging technique for the pituitary gland. With coronal images and an adequate method of injection, CT can achieve a sensitivity no higher than 50% [130,134,576,647–649]. The microadenoma will appear as a hypodense round lesion; a mass effect on the pituitary stalk and diaphragma will depend on the size of the lesion. The specificity of CT is not perfect since abnormal images are not infrequent and may provide false-positive results in patients with other causes of Cushing's syndrome [650,651]. CT scanning also easily recognizes the exceptional initial macroadenoma (Figure 16.21).

FIGURE 16.21 A huge corticotroph macroadenoma detected by computed tomography scan in a patient with mild clinical features of Cushing's disease and a cyclic evolution.

MRI

This technique has significantly improved our ability to detect pituitary microadenomas in Cushing's disease. Several studies have shown that many patients with a negative CT have a positive MRI [652–654]. Pituitary MRI should be performed in all patients with ACTH-dependent Cushing's syndrome.

Pituitary MRI consists of sagittal and coronal T_1-weighted images, coronal T_2-weighted spin echo MRI images in thin sections before gadolinium, followed by a dynamic coronal T_1 sequence beginning simultaneously with the contrast injection, and, as required, a T_1-weighted 3D gradient echo sequence (ED3D). Typical of a microadenoma is a hypointense signal better delimited after enhancement (Figure 16.22). It is often easier to detect (and sometimes visible only) after gadolinium injection on dynamic sequences or in ED3D sequence. Although this is very rare, corticotropic adenoma should be sought outside the intrasellar region (in the cavernous sinuses, sphenoidal sinuses, nasopharyngeal area) if the pituitary has no focal signal abnormality that is suggestive of adenoma. MRI may reveal pituitary adenoma in no more than 36–78% of cases in adult series [576,625,626,652,653,655–657]. Furthermore, false-positives occur where there is pituitary incidentaloma and artefact [625,658]. In addition, MRI determines the size of the adenoma, how it relates to the cavernous sinuses and whether or not it is invasive. The imaging will guide the surgeon, avoiding the need for overly aggressive surgery and the accompanying complications. The imaging will provide a picture of air distribution in the sphenoid sinus, will identify sphenoid

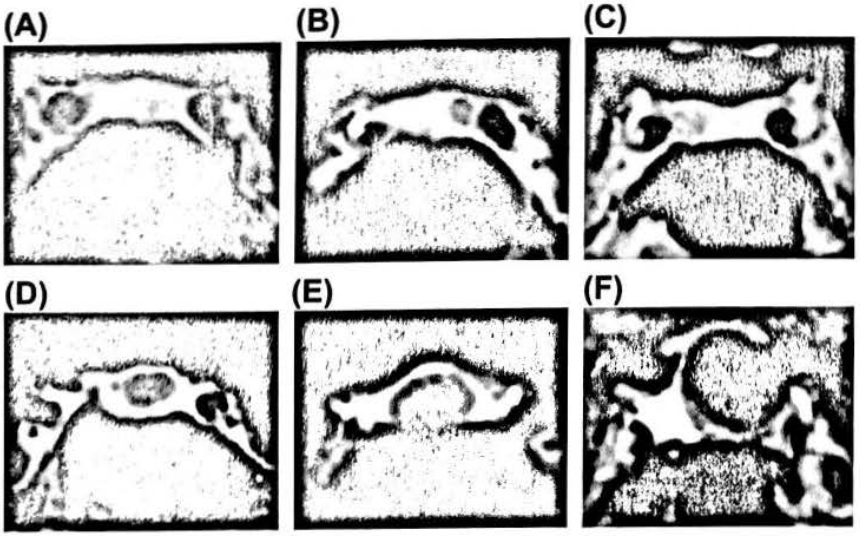

FIGURE 16.22 Pituitary magnetic resonance image in Cushing's disease. T_1-weighted images obtained in the coronal plane with gadolinium enhancement. Adenomas of increasing size are depicted from (A) to (F).

sinusitis, empty sella syndrome, carotid ectasia, etc., which will all influence surgical strategy.

The Adrenals

The main, and crucial, goal of adrenal imaging in Cushing's syndrome is to rule out an adrenal tumor. Adrenal imaging may be indicated if doubt persist between ACTH-dependent and ACTH-independent Cushing's syndrome in cases of sometimes low ACTH levels, with a CRH test and a high-dose dexamethasone suppression test. It is advisable to carry out an adrenal imaging before bilateral adrenalectomy [25,450,518].

Standard X-ray, tomography, ultrasonography and retroperitoneal pneumography have all been outrated by the highly effective and noninvasive techniques using MRI and especially CT scanning.

As a result of chronic stimulation by excess ACTH the two adrenal glands develop hyperplasia exhibiting CT features that will be essentially the same in Cushing's disease and ectopic ACTH syndrome. As a rule both glands are moderately enlarged; there is no reliable measure of the adrenals but a loss of normal concavity of their borders is considered pathologic. Occasional nodules may be present, probably more frequently in Cushing's disease than in ectopic ACTH syndrome. Macronodular hyperplasia develops in up to 15% of patients with Cushing's disease [369]. In cases where it is highly asymmetrical with a predominant macronodule on one side, an erroneous diagnosis of adrenocortical adenoma may be made that possibly leads to an unwarranted and unsuccessful unilateral adrenalectomy. Great care should be exerted to analyze the contralateral gland which, in contrast with a benign adrenocortical adenoma, will not show the characteristic features of adrenal atrophy [369]. This is also the rare situation where adrenal scintigraphy with iodocholesterol [659] will be of help in definitively proving the bilateral, although asymmetrical, functional lesions in the case of macronodular hyperplasia, in contrast with the strictly unilateral isotope uptake by a benign adrenocortical adenoma [660,661].

No adrenocortical tumor large enough to cause Cushing's syndrome, i.e., >1.5 cm, should escape detection by CT. A benign adrenocortical adenoma is readily visible in the fat-filled perirenal area of these patients and it is essential to appreciate the atrophic aspect of the contralateral gland by comparing its thickness with that of the diaphragma crus [379]. Adrenocortical carcinomas, as a rule, are characteristically large and partly necrotic tumors. They may contain calcifications or hemorrhagic areas. At this stage MRI can be used as the most sensitive method in the preoperative assessment of vascular patency of the inferior vena cava and of locoregional invasion (liver, kidney and pancreas) using sagittal and coronal planes.

PITFALLS IN DIAGNOSIS

Drug Interactions

A more extensive description of drug interactions is provided in Chapter 9.

Inducers of High CBG Plasma Levels

High estrogen states, as encountered in pregnancy and in oral contraceptive treatment, induce increased plasma CBG levels. This modification is accompanied by a parallel increase in plasma cortisol [662]. Persistence of a normal pituitary–adrenal axis is easily demonstrated by other indices; free plasma cortisol and salivary cortisol are normal and have normal circadian variations, while 24-hour urinary cortisol excretion is normal. Although false-positive responses to the overnight 1 mg dexamethasone suppression test are occasionally observed, the classic low-dose dexamethasone test is normal [509,511]. In late pregnancy the situation is more complex due to additional factors that profoundly modify the pituitary–adrenal homeostasis (see below).

*op'*DDD and/or some of its metabolites have been shown to have estrogen-like actions [663]. In some individuals highly elevated plasma CBG levels may obscure a proper evaluation of the drug's action on cortisol production. Urinary and/or salivary measurements bypass this potential pitfall.

Liver Enzyme Inducers

Several drugs have the common property of inducing liver enzyme activations that accelerate the metabolism of endogenous and/or exogenous steroids and of some pharmacologic agents [429].

*op'*DDD [533], rifampicin [664], phenytoin [665] and barbiturates [666] divert cortisol metabolism toward 6β-hydroxycortisol. This highly polar compound escapes the extraction usually performed on urine samples, artifactually lowering the result of the Porter and Silber assay. This explains why the urinary 17-hydroxycorticosteroids drop within a few days after the onset of *op'*DDD treatment, whereas plasma cortisol remains unchanged until the delayed adrenolytic action of the drug begins its effect, generally only after a few weeks.

Anticonvulsants like phenytoin and barbiturates also accelerate dexamethasone metabolism [532]. Patients on these drugs have false-positive low-dose dexamethasone suppression tests [509]. In some patients with suspected Cushing's syndrome it may be difficult to interrupt their anticonvulsant treatment. It has been proposed to monitor the test with concomitant measurement of plasma dexamethasone [667]; alternatively, because cortisol metabolism is less accelerated a suppression test has been calibrated where plasma corticosterone suppressibility is assessed after oral

administration of 50 mg cortisol at midnight [668]. In such patients, however, basal urinary cortisol excretion is normal.

Antiglucocorticoids (RU 486)

Although this newly developed drug is used primarily as an antiprogesterone it also exerts an antiglucocorticoid action that is readily observed within a few hours after a single oral administration [669,670]. As expected, plasma and urinary cortisol are elevated and suppressibility by dexamethasone is altered. Because of the long duration of action of the drug, this state of general glucocorticoid resistance is still noticeable up to 3 days after single-dose administration [671]. Pilot studies have been performed where patients received long-term therapy with RU 486 (200–400 mg/day) up to several months, for breast cancer. A two- to three-fold plasma cortisol increase was observed after 2 weeks of treatment which plateaued thereafter and was not modified by the 1 mg overnight dexamethasone suppression test [672].

This increased pituitary–adrenocortical activity is an adaptive, and appropriate, response to the state of drug-induced glucocorticoid resistance. As expected, no clinical feature of hypercortisolism is observed.

Glucocorticoids

A rare patient may present with clinical features of glucocorticoid excess while on glucocorticoid treatment for an inflammatory disease, and also have an endogenous cause of Cushing's syndrome. The diagnosis may be easily made in the case of an autonomously secreting adrenocortical tumor. It is theoretically much more difficult in the rare case where Cushing's disease is suspected, since the abnormal pituitary ACTH secretion may have been somewhat sensitive to the suppressive effect of exogenous steroids.

Glycyrrhetinic Acid

Glycyrrhetinic acid, a hydrolytic product of glycyrrhizic acid, has long been recognized as a causative agent of a pseudohyperaldosteronism syndrome. Its mechanism of action has been unraveled [673]. The compound inhibits the enzyme 11-β-hydroxysteroid dehydrogenase which mainly converts cortisol to the inactive cortisone. Because this enzyme activity is present in the kidney, its inhibition induces a local excess of cortisol which will act, in a spill-over mechanism, on the kidney mineralocorticoid (or glucocorticoid type I) receptor and exert a mineralocorticoid-like effect [674]. As a consequence of the blockade of cortisol metabolism in the kidney urinary cortisol is increased; plasma cortisol is unchanged. Thus urinary cortisol is a false indicator of the cortisolic state in subjects under liquorice abuse [675].

Intercurrent Pathologic States

Simple obesity has long been a major diagnostic problem when urinary 17-hydroxycorticosteroids were the usual markers of adrenocortical activity [526]. Obesity per se induces an increased metabolic clearance rate of cortisol [529,530]. As an adaptive and appropriate response the cortisol production rate is increased with increased urinary cortisol metabolites. It has now been clearly demonstrated that the more appropriate parameters of baseline cortisol homeostasis (plasma and salivary cortisol, circadian rhythm and urinary cortisol excretion), and the classic low-dose dexamethasone suppression test are all normal in simple obesity [509].

Hyperthyroidism also accelerates cortisol metabolism with the same consequences as simple obesity, with urinary 17-hydroxycorticosteroids being elevated. Other parameters of cortisol homeostasis remain normal. Hypothyroidism induces the opposite abnormalities, i.e., low urinary 17-hydroxycorticosteroids [572].

Chronic renal failure has been mistakenly associated with abnormal glucocorticoid regulation, including diminished suppressibility by dexamethasone [677]. Because urinary measurements are evidently useless special caution must be applied to the sole possible plasma measurements. Polar metabolites of cortisol accumulate in blood to such high levels that they may significantly interfere in some cortisol assays that are not sufficiently specific. With the necessary precautions, including plasma extraction or highly specific immunoassay, plasma cortisol is normal and normally suppressible by the classic low-dose dexamethasone test [520,676,679]. Correct assessment of the pituitary–adrenal axis may be further hampered by the finding of increased plasma LPH levels [175,520], especially in hemodialysis patients, due solely to a decreased plasma clearance of LPH [680]. Plasma ACTH remains normal [520]. Patients with HIV infection, especially if treated with protease inhibitors, may exhibit features of pseudo-Cushing's syndrome, including fat pads and central obesity. Appropriate ACTH/cortisol suppression after dexamethasone excludes the diagnosis of Cushing's disease [678].

An exceptional patient has been reported who had both Addison's and Cushing's diseases [681]. The diagnosis was achieved by demonstrating the lack of normal circadian ACTH rhythm (which is normally preserved in Addison's disease) under precise conditions of cortisol administration.

Hypercortisolic States without Cushing's Syndrome

Various pathologic or physiologic conditions may be associated with biochemical, and sometimes clinical,

TABLE 16.4 Hypercortisolic States without Cushing's Syndrome

Functional hypothalamic CRH oversecretion
Depression
Anorexia nervosa
Alcoholism
Chronic stress
Strenuous exercice
Nonhypothalamic CRH oversecretion
Pregnancy
General insensitivity of glucocorticoids
Familial resistance to glucocorticoids
Pregnancy
RU 486

CRH, corticotropin-releasing hormone.

evidences of endogenous glucocorticoid excess (Table 16.4). In these situations, increased cortisol production is thought to be driven by pituitary ACTH oversecretion secondary to CNS disorder or to an appropriate adaptive reaction. This functional hypercortisolic state (sometimes called "pseudo-Cushing") is usually mild and transient and regresses with its cause. Hence it is not classically regarded as a cause of genuine Cushing's syndrome, but has long been recognized and best studied in depressed patients.

Depression

Patients with severe endogenous depression often exhibit biochemical stigmata of hypercortisolism [291]. Plasma cortisol and urinary steroids excretion are increased, and are not suppressed normally on the classic low-dose dexamethasone test. Activation of the pituitary–adrenal axis is fairly specific of depression among other conditions of primary affective disorders and may be observed in as many as 40–60% of patients in some series [290]. These observations eventually led to the routine use of differently designed and debated dexamethasone suppression tests as a biologic means for both the diagnosis and the follow-up of such patients.

Normal or slightly increased plasma ACTH levels indicate that the disorder is pituitary driven. A fine evaluation of the hypothalamic–pituitary–adrenal axis of depressed patients has recently shed new light on the pathophysiologic mechanism leading to the hypercortisolic state of this disorder [682]. Depressed patients have an attenuated plasma ACTH response to CRH in comparison with normal controls, yet their basal plasma cortisol levels are elevated, and respond normally to CRH. These results indicate that the pituitary corticotroph is intrinsically normal, the attenuated ACTH response to CRH showing that they are sensitive to the negative feedback of increased cortisol levels (just the reverse happens in Cushing's disease). The normal cortisol response to CRH, in spite of blunted ACTH rise, is compatible with hyperplasia and hyper-responsiveness of the adrenal cortex, as has been independently reported by others in depressed patients [683]. The dynamics of ACTH are similar to those observed in normal subjects administered long-term CRH infusion [388] and therefore point to the hypothalamus or suprahypothalamic regions as the primary cause of ACTH oversecretion. Although the source and significance of CSF CRH is debated [290], the finding that depressed patients have increased CSF CRH levels [292], which eventually appear to correlate positively with the degree of pituitary–adrenocortical overactivity [293], has been taken as a further indication that this condition is due to a hypothalamic dysfunction with CRH overproduction.

Whatever the exact pathophysiologic mechanism, the hypercortisolic state that accompanies depression often creates a serious diagnostic problem. A depressed patient may present with obesity, mild hirsutism, slight hypertension and moderate glucose intolerance. Although none of these is by itself absolutely conclusive, several features may more or less distinguish between transient functional hypercortisolism and true Cushing's syndrome with secondary depression. Classically in depression:

1. The hypercortisolic state is clinically and biologically mild. Urinary cortisol excretion almost never exceeds three times the upper limit of normal [684];
2. The circadian pattern of plasma cortisol levels is less disrupted and sometimes a phase-shift phenomenon is merely observed [685,686];
3. Cortisol response to insulin-induced hypoglycemia is present in depressed patients in contrast to patients with Cushing's syndrome of any cause, including Cushing's disease [121,122,509];
4. ACTH response to CRH is attenuated in contrast to the exaggerated response of Cushing's disease, a wide overlap, nevertheless is observed [682]; (see also further Tests to distinguish between "pseudo-Cushing" and Cushing's syndrome)
5. Finally, imaging investigations should find no evidence of adrenocortical or pituitary tumor.

Cases have been reported where depression preceded the occurrence of true Cushing's disease, raising the question of possible pathophysiologic role for CRH, and further complicating the diagnostic issue.

Anorexia Nervosa

Anorexia nervosa is associated with an array of neuroendocrine disorders among which sustained hypercortisolism is frequent (see Chapter 18) [291]. Increased urinary

cortisol and lack of normal suppression by the classic low-dose dexamethasone test may be found. Clinical features of hypercortisolism are absent probably because of the mild hypercortisolic state and the lack of sufficient substrates, more likely than because of a hypothetic down-regulation of glucocorticoid receptors. The fine evaluation of ACTH and cortisol response to CRH in underweight patients with anorexia nervosa reveals patterns very similar to those observed in depressed patients [687]. Together with the finding of an increased CSF CRH level in anorexia nervosa [688,689] these data point to a hypothalamic or suprahypothalamic origin of pituitary–adrenocortical overactivity in anorexia nervosa. An exceptional case has been reported where authentic Cushing's disease with a pituitary adenoma found at surgery occurred 2 years after the onset of anorexia nervosa [690]. In contrast with depressed patients there is generally no clinical hesitation for the diagnosis. Abnormal corticotroph dynamics are corrected with weight restoration, and they might simply represent a nonspecific manifestation of inanition [691].

Alcoholism

Patients with chronic alcoholism may present with clinical and biochemical features of glucocorticoid excess creating a pseudo-Cushing's syndrome [692–694]. General fatigue, diminished muscle strength, plethoric facies, truncal obesity and abdominal striae may be encountered, which all mimic the typical clinical features of Cushing's syndrome [372]. A diagnosis which is further supported by the finding of increased plasma cortisol and urinary steroid excretion, a disrupted circadian rhythm and lack of normal response to the classic low-dose dexamethasone suppression test.

Alterations of the hypothalamic–pituitary–adrenal axis consistent with a hypothalamic origin are found in patients under chronic alcohol abuse, yet they are mild and present only in a minority of patients. Thus, it remains to be determined whether these functional abnormalities are associated with the propensity for alcohol abuse, are caused by ethanol intake possibly through a decrease of 11-β-hydroxysteroid dehydrogenase activity [695,696], or simply related to a common CNS disorder also responsible for depression.

Whatever the mechanism involved, alcoholic pseudo-Cushing's syndrome is a real diagnostic challenge. The simplest and most effective way to avoid a false diagnosis is to think of alcoholism and to observe the nice parallel decrease and normalization of cortisol indices and liver function tests during alcohol withdrawal in hospitalized patients [692–694,697].

Stress

Transient states of glucocorticoid excess without clinical stigmata commonly accompany an array of stressful conditions. They are thought to represent normal adaptive activation of the hypothalamic–pituitary–adrenal axis. Many such situations are encountered, including surgery, test-taking, various acute and chronic illnesses, terminal illnesses, extended burns and diabetes mellitus [509,698,699]. The simple stress of hospitalization has been claimed to increase glucocorticoid secretion. These observations emphasize the absolute need to await the resolution of any stressful intercurrent condition before initiating a proper diagnostic evaluation.

Strenuous Exercise

Slight alterations of the pituitary–adrenal axis may be encountered in response to physical exercise [700]. In a recent study, moderate elevation in baseline plasma cortisol and a blunted ACTH and cortisol response to CRH were observed in normal men running more than 45 miles per week [586].

Pregnancy

Normal pregnancy is associated with a profound hormonal turmoil that significantly alters glucocorticoid homeostasis (see also Chapter 17).

In the first months of pregnancy increased estrogens induce a two- to three-fold rise in plasma CBG that reaches a maximum at about 3 months and plateaus thereafter [662]. This generates a parallel rise in plasma cortisol but, in a similar manner to that observed in women on estrogen contraception, it does not induce a true hypercortisolic state since plasma free cortisol, and salivary and urinary cortisol remain within the normal range.

With time more significant alterations develop that culminate in the last trimester when unequivocal features of a hypercortisolic state are found, at least from a biochemical viewpoint. Mean unbound and salivary cortisol and urinary cortisol excretion show a two- to three-fold increase [389,390,501]. Thirty percent of women have 24-hour urinary cortisol excretion above the upper limit of normal, nonpregnant women, and most have an abnormal response to the classic low-dose dexamethasone suppression test [701].

The mechanism and consequences of this slight state of authentic hypercortisolism are not totally understood. However, major advances have been made in recent years which illuminate this intriguing problem.

The normal placenta has been identified as a large and physiologic site for CRH gene expression [702], depositing enormous quantities of the peptide into the maternal blood flow. Plasma CRH levels in late pregnancy may attain peaks of several thousand picograms per milliliter in comparison with the picograms per milliliter range in normal, nonpregnant women [394]. Although a large proportion of circulating CRH is bound to a carrier protein [703,704], strikingly elevated

levels of free and bioactive CRH circulate at this time. Under such conditions and because plasma ACTH levels in pregnant women show a moderate but significant rise of about two- to three-fold [701], it first seemed logical to charge placental CRH as a natural culprit. The situation, however, is not as clear; no correlation has been found between plasma CRH and pituitary–adrenal parameters [389]. Conservation of a perfectly normal, although slightly shifted upward, circadian rhythm of salivary cortisol is in sharp contrast with the steady-state of high plasma CRH levels [389]. It points to an unrestrained hypothalamic drive that continues to operate and which overcomes both peripheral CRH and the expected negative feedback of increased plasma free cortisol. Whether it is related to a direct action of hypothalamic CRH at the pituitary, to AVP, or to an as yet unidentified factor, is unknown.

Progesterone exerts an antiglucocorticoid action on rat pituitary corticotroph cells [705]; it has been hypothesized that prolonged and highly elevated progesterone levels induce a state of relative and general glucocorticoid resistance [389]. This would explain the slight shift in plasma free cortisol with conserved normal circadian rhythm, the abnormal dexamethasone suppressibility, and also the absence of peripheral clinical features of hypercortisolism. This hypothesis is reinforced by the recent finding that the change in salivary cortisol after delivery correlated with the increase in serum progesterone concentration in late prenancy [389].

Some have suggested that increased plasma ACTH in pregnancy might result from placental secretion [701] and/or a reviviscent intermediate lobe of the pituitary [706,707].

Familial Resistance to Glucocorticoids

This newly recognized syndrome was first identified in a patient with hypertension and hypokalemia [708]. Fine hormonal evaluation showed no evidence of aldosterone oversecretion or adrenocortical enzyme blockade. Instead evidence of glucocorticoid excess was found; plasma cortisol and urinary cortisol were elevated. Suppression of plasma cortisol by increasing doses of dexamethasone was abnormal with a shift to the right of the dose–response curve demonstrating the relative resistance of the pituitary to the negative glucocorticoid feedback in a manner similar to that observed in Cushing's disease [709]. Two features were different; there was a normal circadian rhythm of plasma cortisol, and a total absence of clinical features of hypercortisolism. These observations suggested that the state of glucocorticoid resistance was not restricted to the pituitary, but was general. This hypothesis was reinforced by the finding of decreased glucocorticoid binding affinity of the patient's fibroblasts [709], and recently established by the cloning of the glucocorticoid receptor in an affected patient. A single base substitution (A→T) at position 2054 changed Asp_{641} to Val within a highly conserved and hitherto supposedly functional region of the ligand-binding domain of the receptor, explaining the loss of affinity [189]. This rare familial syndrome has now been identified in several families, amounting to about 20 such patients. The clinical and biochemical features severely affect the patients with homozygous defects. Increased activity of the pituitary–adrenal axis is an adaptive, and thus appropriate, reaction. The hypertension and hypokalemia are explained by the increased mineralocorticoid activity due to excess DOC and cortisol acting on the normally sensitive mineralocorticoid receptor.

Tests to Distinguish between "Pseudo-Cushing" and Cushing's Syndrome

These tests have been developed primarily to distinguish between the functional hypercortisolism of depression and genuine Cushing's syndrome.

THE COMBINED DEXAMETHASONE–CRH TEST

The hypothesis is that patients with pseudo-Cushing's syndrome are under chronic CRH stimulation due to their stressful situation and show a blunted response to exogenous CRH after dexamethasone administration. The initial protocol consisted of giving dexamethaone in doses of 0.5 mg for 48 h, at 6 h intervals, beginning at 12:00 h (i.e., at 12:00, 18:00, 00:00 and 06:00 h) and obtaining serum cortisol at 08:00 h, and then administering ovine-sequence CRH (1 μg/kg) i.v. at 08:00 h, 2 h after the last dexamethasone dose. The plasma cortisol value 15 min after CRH is expected to be greater than 38 nmol/liter (or 1.4 μg/dl or 14 ng/ml) in patients with Cushing's syndrome, but to remain suppressed in normal subjects and in patients with pseudo-Cushing's syndrome. Recent studies [710–713] reported lower specificity of the Dex-CRH test than the initial publication by the National Institutes of Health (NIH) group [714] such that this test gave no better results than the repeated assessment of the other screening tests. The cutoff proposed with ovine CRH cannot be extended to human CRH, widely used in Europe, which stimulates ACTH and cortisol secretion less than the ovine CRH. Furthermore, the interval between dexamethasone and CRH is longer than in the NIH protocol if the dexamethasone dose starts at 10:00 h instead of 12:00 h. Lastly, human CRH is expensive, just as is the 48 h hospitalization often needed to strictly respect the dexamethasone test protocol. A better diagnostic accuracy was obtained with a cortisol threshold of 70 nmol/L (25 ng/ml or 2.5 μg/dl) or with an ACTH threshold of 5.9 pmol/L (27 pg/ml) 15 min after ovine CRH (1 μg/kg, maximum 100 μg) at 08:00 h, 2 h after the last dose of dexamethasone [711].

THE CRH TEST

A recent Italian study [715], attempted to rehabilitate the CRH test in the differential diagnosis between ACTH-dependent Cushing's syndrome and pseudo-Cushing, first described by the NIH group [682]. Using a combination of two hCRH test parameters they showed that ACTH-dependent Cushing's syndrome can be diagnosed with specificity and sensitivity both over 90% [715].

THE DESMOPRESSIN TEST

In two publications of Italian groups (Milan and Naples), the desmopressin test has a good diagnosis accuracy (94%) with a threshold of ACTH of 6 pmol/L (27 pg/ml) [710,716], that was comparable to that of the dexamethasone-CRH test, even in patients with only mild hypercortisolism. Besides, desmopressin is cheaper than CRH and the desmopressin test is more convenient than dexamethasone-CRH which required 2 days' hospitalization. Furthermore, the desmopressin test may be useful in the differential diagnosis of ACTH-dependent Cushing's syndrome, and above all in the post-transsphenoidal survey of Cushing's disease. Like the dexamethasone-CRH test and perhaps the CRH test, the desmopressin test may prove useful for patients with mild hypercortisolism and normal ACTH levels, in whom the differential diagnosis has narrowed to Cushing's disease or pseudo-Cushing. Unlike the CRH test, dexamethasone-CRH and desmopressin tests cannot be applied in patients with ACTH-independent Cushing's syndrome.

Normal Suppression with the Classic Low-dose Dexamethasone Test in Authentic Cushing's Disease

It has been estimated that a minority of patients (5%) with authentic Cushing's disease suppress normally with the classic low-dose dexamethasone test, and thus are false-negatives [509]. Returning to the original data provides a simple explanation [123]. The first trials to titrate the daily amount of synthetic glucocorticoid necessary to suppress urinary 17-hydroxycorticosteroids used two different molecules with different potencies, namely Δ^1-9α-fluorocortisol and Δ^1-16α-methyl-9α-fluorocortisol (or dexamethasone). Titration curves readily show that complete suppression requires 2 mg/day of the first compound, but only $\leq$1 mg/day of dexamethasone. Curiously, in the original paper both drugs are used under the same generic name "ΔFF" leading the author to anticipate that "... although these two compounds were used interchangeably in the present study, it is possible that smaller doses of dexamethasone would be appropriate, in view of the apparent greater potency of this agent" [123].

In occasional patients with Cushing's disease, normal suppressibility by the classic low-dose dexamethasone test has been attributed to a decreased metabolic clearance rate for dexamethasone [717,718]. Simultaneous measurements of plasma endogenous cortisol and dexamethasone provide a means to evaluate suppressibility in comparison with the dose–response curve obtained in normal patients [668,718].

Other causes of apparently normal suppression in Cushing's disease are encountered in the rare patients with true cyclical episodes of hypercortisolism whenever the test is performed during a quiescent phase.

Etiologic Pitfalls

Cushing's Disease Mimicking an Autonomous Adrenocortical Tumor

The classic situation is that of a patient with Cushing's syndrome with apparent autonomous cortisol oversecretion [372]: urinary 17-hydroxycorticosteroids fail to suppress with the classic high-dose dexamethasone test, basal ACTH is low or undetectable and adrenal imaging reveals a unilateral adrenal mass. When a unilateral adrenalectomy is performed, although transiently ameliorated, the hypercortisolism inevitably recurs, allowing a correct and a posteriori diagnosis of Cushing's disease in its macronodular hyperplastic form. This situation is not uncommon and the diagnosis would be correctly established if strict criteria were used.

1. Establishing autonomous cortisol production requires indisputable proof. It has previously been noted how the high-dose dexamethasone test should be interpreted with more subtlety and how it must be sometimes strengthened [378]. The ACTH RIA may be insensitive and undetectable basal plasma ACTH levels may become detectable with a better assay or after LVP or CRH stimulation [113]. A metyrapone test may also be of help showing a rise in urinary 17-hydroxycorticosteroids. Thus, in many cases what appears as an autonomous adrenocortical activity does not resist a closer, and stronger, examination.
2. The second aspect that needs thorough evaluation is adrenal imaging. Although it may be highly asymmetrical to the point of mimicking a unilateral adrenocortical tumor, the macronodular hyperplasia of Cushing's disease is not accompanied by contralateral atrophy, as is the case in a true autonomous adrenocortical adenoma [369]. Thus careful examination of the contralateral gland on CT scan almost invariably confirms the diagnosis. It may be in this rare situation where iodocholesterol scanning may be helpful, showing asymmetrical but bilateral isotope uptake [660,661].