Laboratory Aids in the Diagnosis of Pituitary Tumors

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ABSTRACT

It is the aim of this review to acquaint the reader with the techniques often used in evaluating pituitary function and to show how they help in the diagnosis of pituitary tumors and the discernment of their systemic effects.

Introduction

The pituitary gland is affected by a variety of tumors which usually manifest themselves by compression of the optic chiasm or alteration of endocrine function. The clinician may not be able to make a specific histological diagnosis, but he can assess the extent of pituitary malfunction quite accurately and initiate appropriate therapy.

Pituitary hormone secretion is a dynamic event, varying throughout the day and night in response to many endogenous and exogenous stimuli. These changes are exquisitely controlled by central nervous system mechanisms whose final common pathway is in the hypothalamus. In fact, most procedures used in testing anterior pituitary function affect the central nervous system primarily and the pituitary secondarily through its vascular connections with the hypophysiotropic areas of the hypothalamus. Depending on clinical circumstances, the physician has three major laboratory criteria for determining adequacy of hormonal function:

1. Basal secretion.—Hormone secretion may vary diurnally such as adrenocortical hormone (ACTH) or monthly such as luteinizing hormone (LH) or follicle stimulating hormone (FSH) and may vary with food ingestion, with sleep or with a wide variety of physiologic and non-physiologic stimuli. A single basal serum level of a pituitary hormone is therefore often inadequate for diagnostic purposes.

2. Effect of provocative stimuli on secretion.—Hormone secretion in excess of the basal rate is required in many situations. This ability to respond adequately to provocative stimuli is characteristic of a healthy pituitary secretory system.

3. Effect of inhibitory stimuli on secretion.—Pituitary hormone secretion is normally inhibited, and serum hormone concentration reduced, in response to physiologic stimuli which affect each of the hypothalamic-pituitary trophic hormone systems. The stimulus may be a metabolic

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event, a rising serum level of target organ hormone or changes in the level of a metabolite. The exogenous alteration of a specific stimulus in a direction normally associated with hypersecretion of the target hormone (e.g., administration of a glucocorticoid or of thyroid hormone) results in decreased secretion of the appropriate trophic hormone (ACTH or thyrotropin [TSH]) in healthy subjects.

Patients with pituitary tumors may present with various groups of symptoms that include bitemporal visual field defects, amenorrhea in women and impotence in men, hypersecretory syndromes (including acromegaly, galactorrhea, and Cushing's syndrome), trophic hormone deficiencies (hypopituitarism), or any combination of these. To use laboratory aids properly for diagnosing these tumors and assessing their hormonal effects, physicians must be acquainted with the normal dynamic changes in pituitary hormone secretion.

**Evaluation of Growth Hormone Secretion**

**Basal Growth Hormone Secretion**

The dual hypothalamic control of human growth hormone (HGH) secretion by the anterior pituitary and the great number of endogenous and environmental factors that normally influence HGH secretion make it difficult to define what is "normal" for basal secretion and serum concentration. A highly simplified outline of the neural control mechanisms, including the speculated sites at which brain catecholamines may affect growth hormone secretion in man, is shown in figure 1.

Basal serum levels of HGH are slightly higher in women than in men, the upper limit of normal being approximately 10 and 6 millimicrograms (nanograms, ng) per ml, respectively. There are two difficulties with assigning normality to a given basal serum concentration. First, many normal people have undetectably low basal HGH levels, as do most patients with growth hormone deficiency. Second, exercise, stress, prolonged fasting and recent sleep-induction all stimulate HGH secretion and elevate serum HGH levels. To avoid misinterpretation, it is necessary to measure HGH responses to provocative and inhibitory stimuli.

**Growth Hormone Stimulation**

*Insulin-induced hypoglycemia.* Insulin-induced hypoglycemia is the most consistent stimulus to HGH release. Two fasting control blood samples are drawn at 15 minute intervals with the patient resting comfortably, and then 0.1 unit per kg of crystalline insulin is administered intravenously. Blood samples are then collected every 15 minutes for 90 minutes. Blood glucose and HGH levels are determined on each specimen. A 50 percent fall in blood glucose is required for meaningful results. A positive (normal) HGH response consists of a rise of 5 ng per ml or more in serum HGH above baseline.

For the patient's safety a saline infusion is kept running throughout the study, and the test is terminated by an intravenous bolus of 50 percent glucose. Since patients with hypopituitarism are quite sensitive to insulin, some workers have recommended a smaller insulin dose (0.05 U per kg) for suspected cases of pituitary failure; only if the fall in blood sugar is inadequate (i.e., the concentration remains greater than 50 percent of basal level) is the higher dose then attempted on another occasion.

*Arginine infusion test.* The protocol for arginine infusion test is similar to the insulin test except that 0.5 gms per kg of arginine is infused over 30 to 45 minutes. The rise in serum HGH, as in the induced-hypoglycemia study, should be at least 5 ng per ml over the basal serum level.

*Other provocative tests.* L-Dopa, glucagon, vasopressin, stress and pyrogen have all been proposed and tested in the evalua-
tion of HGH secretion. None has any particular advantage over the two tests mentioned, and most have proven less reliable. A positive HGH response to a single provocative stimulus suggests an intact secretory mechanism. Because failure to respond to an individual stimulus can occur in normal subjects, negative responses to several stimuli (insulin hypoglycemia, L-dopa, arginine infusion) best confirm the diagnosis of HGH deficiency.

**GROWTH HORMONE INHIBITION**

Suppression of HGH secretion in normal subjects is easily accomplished by administration of 100 gm of glucose orally. Serum HGH levels decline within a half-hour, remain low for up to three hours and then rise above basal levels from four to five hours after the glucose load. This HGH rebound after the initial suppression is apparently related to the normal fall in blood sugar concentration at the third and fourth hours after orally administered glucose. Baseline HGH levels are obtained in overnight-fasted, rested patients under nonstressed conditions. This avoids the potent stimulus to HGH secretion of stress. Blood samples are taken half-hourly for three hours, then hourly to the sixth hour if both phases of the diphasic response are to be measured. Normal subjects should have serum HGH reduced to less than 5 ng per ml by the induced hyperglycemia before the normal rebound occurs. Because acromegalic patients fail to suppress their serum HGH concentrations to this level, oral glucose loading is an essential test when this diagnosis is clinically uncertain.

**Acromegaly**

The clinical abnormalities that characterize acromegaly are produced by chronic hypersecretion of HGH and by pituitary enlargement. These abnormalities can be classified as local, metabolic, visceral and acral. Local effects produce headache and bitemporal field defects. Metabolic consequences include deterioration of glucose tolerance and hypertension. Visceral abnormalities include enlargement of the major organs of the body, deepening of the voice, thickening of the tongue and ominous cardiomegaly that is associated with increased frequency of congestive heart failure and cardiac deaths. Finally, acral changes include the enlargement of hands and feet, the coarsening of facial features and the prognathism, malocclusion and increased spacing of the teeth that may allow physicians to make the right diagnosis instantly.

Though the diagnosis is sometimes apparent at a glance, there are good reasons for proper laboratory testing to make a definitive diagnosis. First, there are many questionable cases of patients with rugged features combined with one or more suggestive findings. Second, the disease is treatable by removing the HGH-secreting pituitary adenoma, particularly with the newer methods of trans-sphenoidal hypo-
physectomy—and many of the chronic disfiguring and disabling features are reversible. Finally, the once-prevalent notion of "burned-out" acromegaly (inactive disease after long-standing activity) has been proven false by persistently elevated serum HGH levels in such patients: in these instances the patient, not the disease, has burned-out.

The characteristic abnormality of patients with acromegaly or pituitary gigantism (the juvenile variant of chronic hypopituitarism found in patients whose epiphyses are still open) is a persistently elevated basal serum HGH concentration which is not decreased to less than 5 ng per ml by an oral glucose load. The test is performed using a standard 100 gm oral glucose challenge, and blood samples are collected for HGH concentration before glucose administration, and at half-hour intervals for two to three hours.

Most interesting has been the unexpected variation (physiologic or paradoxical) in blood HGH concentrations brought on by provocative and inhibitory stimuli, demonstrating that HGH secretion in acromegaly is often nonautonomous and under deranged hypothalamic control. It is known that brain catecholamines play an important role in hypothalamic mechanisms controlling HGH secretion by the anterior pituitary and that L-dopa (a precursor of dopamine) normally stimulates such secretion and raises the serum HGH concentration. It has been found that acromegalic subjects characteristically have no increase, or more commonly a paradoxical decrease, in serum HGH after oral L-dopa (figure 2). These results (1) re-emphasize the frequently non-autonomous nature of HGH hypersecretion in acromegaly, (2) reinforce the concept of deranged hypothalamic control in acromegaly and (3) suggest that in this disease acutely raised levels of brain catecholamines may inhibit secretion of GRF (the reverse of the normal state) or stimulate secretion of somatostatin, or both, causing blood levels of HGH to be suppressed.

GROWTH HORMONE DEFICIENCY

Laboratory evaluation of possible hypopituitarism must include evaluation of HGH secretion. The patterns of trophic hormone deficiency suggest that HGH secretion is the function most frequently impaired as anterior pituitary deficiency
unfolds. Clinically, disordered sexual function (particularly amenorrhea) is most commonly encountered. Physiologically, inadequate HGH secretion is nearly universal. In adults, HGH deficiency may be clinically silent; growth has already ceased, and the episodes of spontaneous hypoglycemia associated with HGH deficiency in children are extremely rare. Nevertheless, inadequate response of serum HGH to provocative stimuli in the right clinical circumstances provides an early and sensitive indicator of developing hypopituitarism.

Evaluation of Prolactin Secretion

**Basal Prolactin Secretion**

Since the number of prolactin cells in the anterior pituitary varies in different physiological states, it is not remarkable that basal secretion and serum concentrations of this hormone similarly vary. The mean and normal range for different groups is not yet established, but there is agreement that basal serum levels of prolactin are higher in women than in men, increase geometrically during pregnancy and vary only slightly during the menstrual cycle.

Using heterologous radioimmunoassays Buckman et al found that normal men had a mean basal prolactin level of 34 ng per ml ± 25.5 S.D., and normal women a level of 45 ng per ml ± 31.5 S.D. They also noted that most patients with functional galactorrhea had serum prolactins that fell within two standard deviations of the mean for normal females, though their mean serum concentration (73.5 ng per ml) was significantly higher than normal. In addition, patients with prolactin-secreting pituitary tumors had serum prolactins that occasionally overlapped the higher levels of the functional galactorrhea group, despite a mean concentration more than 100 times greater than this group. Friesen et al, using a homologous radioimmunoassay, reported a mean basal serum prolactin level in men of 7 ng per ml, with a range up to 28, with a corresponding mean of 10 and range up to 20 in women during the follicular phase, and mean of 11 and range up to 42 in women during the luteal phase of the menstrual cycle. The mean level of 30 ng per ml in the first trimester of pregnancy doubled in the second trimester, redoubled in the third and nearly redoubled again at term.

**Prolactin Stimulation**

*Thyroid-releasing hormone (TRH).* This hypothalamic hormone appears to release prolactin by direct action on the anterior pituitary (figure 3). A simple tripeptide, \((\text{pyro})\text{Glu-His-Pro}(\text{NH}_2)\), TRH has been synthesized and the synthetic form used to
test for both thyrotropin (TSH) and prolactin responsiveness. Doses of 100, 200 and 800 micrograms have been given by bolus intravenous injection in 10 cc saline to patients who have fasted overnight and had blood samples taken in the basal state. Definite increases in serum prolactin have been noted with all three dosages. A striking and consistent elevation has been noted five minutes after intravenous TRH, with peak values (two to 15 times higher than basal) at 15 to 20 minutes, and a slow decline to the baseline at three hours. For clinical purposes, blood can be collected at 15 and 30 minutes after injection, and then at half-hour intervals until the third hour. Though the dose-response function is not yet clear, and no serious side effects have been reported in TRH doses up to two mg intravenously (mild nausea without vomiting, a mild urge to urinate and a flush over the body may be transiently noted), 200 micrograms of TRH appears to be an adequate stimulus for prolactin secretion when this test is indicated.

Chlorpromazine stimulation. Phenothiazine derivatives, including chlorpromazine, interfere with availability of brain catecholamines by blocking attachment of these amines to their receptor sites. Presumably chlorpromazine acts in the hypothalamus to block the effect of prolactin-inhibiting factor (PIF), which is under catecholamine control (figure 3). This leads to an increase in prolactin secretion by the anterior pituitary, and a rise in serum prolactin concentration. A single intramuscular injection of 25 to 50 mg of chlorpromazine is given to patients who have fasted overnight and had blood samples taken in the basal state. Further samples are collected at half-hour intervals for three to six hours. A doubling of the fasting prolactin concentration is normally found in the first 30 to 60 minutes, with peak values found at two to three hours. This peak is as high as 20 times the basal value when the higher dose is used. Elevations in serum prolactin may continue for six hours or more.

Prolactin Inhibition

Water-loading test. Prolactin may play a role in regulating serum osmolality by facilitating water retention (figure 3). Water loading is normally followed by a fall in serum prolactin concentration, which may then contribute to the diuresis. After overnight abstinence from food and water, subjects are given an oral load of 20 cc water per kg of body weight over a half-hour period; blood samples are obtained before the water ingestion and at half-hour intervals afterward for three hours. The normal response is a decrease of at least 50 percent from the baseline serum prolactin concentration at any of the times after the water load. The nadir of serum prolactin is usually reached at one to two hours.

A similar marked decrease, but one more promptly noted, occurs when a hypotonic load (0.45 percent saline, 20 mg per kg) is infused intravenously over a one hour period. With intravenous infusions, serum prolactin reaches its lowest point within 30 minutes of the start of the infusion. The maximum decrease in serum prolactin (to nonmeasurable levels) correlates with a drop in serum osmolality to less than 274 mOsm per Kg induced by the hypotonic load.

L-dopa administration. Oral ingestion of L-dopa is followed by a decrease in serum prolactin concentration. This effect is probably related to the ability of L-dopa to cross the blood-brain barrier and be decarboxylated to dopamine. This catecholamine (and possible norepinephrine) may act at two sites to inhibit prolactin secretion: on the anterior pituitary to suppress prolactin secretion, and on the hypothalamus to stimulate secretion of prolactin-inhibiting factor (PIF). After an over-
night fast, blood samples are obtained before an oral dose of 500 mg of L-dopa, and at half-hour intervals afterward for three to four hours. In normal subjects serum prolactin declines to less than 4 ng per ml by two to three hours. It is important to distinguish patients with tumor-induced galactorrhea from patients with these various types of functional galactorrheas for several reasons. A most important one is the clinical fact that long term follow-up of patients with these “functional” states has sometimes finally revealed the presence of a pituitary adenoma. Thus, a prolactin-secreting chromophobe adenoma may evolve from, or be the hidden cause of, a clinical picture originally consistent with Chiari-Frommel syndrome, Ahumada-Del Castillo syndrome or secondary amenorrhea caused by cessation of oral contraceptives.

The laboratory aids most useful in distinguishing these various galactorrheic states are X-rays of the skull with special views of the sella turcica, basal serum prolactin levels, and the water-load test for prolactin suppression. Prolactin-secreting chromophobe adenomas can be small, and it may be months or years before they become manifest on skull X-rays. However, basal serum prolactin concentrations in patients with these tumors are usually recorded in the microgram per ml level, contrasted with the millimicrogram (nanogram) per ml level found in normal subjects and patients with functional galactorrhea. In rare cases, serum prolactin levels as “low” as 200 to 300 ng per ml have been found in patients with these tumors, and prolactin levels as high as this have been found in patients with functional galactorrhea. In such problem cases depression of the serum prolactin level by oral water loading is useful. In normal subjects and subjects with functional galactorrhea, the water load usually decreases serum prolactin to undetectable levels; in all cases so far reported serum prolactin is reduced by at least 50 percent. In patients with prolactin-secreting pituitary tumors serum prolactin is minimally depressed (less than 50 percent reduction) by the water load. L-Dopa suppression has also been at-
TABLE I

CAUSES OF HUMAN HYPERPROLACTINEMIA

I. **Physiologic**

A. Pregnancy (Serum concentration doubles each trimester and peaks just before delivery)

B. Post-partum
   1. Non-nursing mothers, up to 4 weeks
   2. Nursing mothers, same; suckling induces marked but transient increases during next 3 months

C. Breast (or breast and nipple) stimulation in non-post-partum women; nipple stimulation in men

D. Fetal life

E. Early infancy (first week, with decline to adult levels at 3 to 6 months)

F. Stress: anesthesia, surgery, exercise, acute anxiety

G. Sexual intercourse in women (10 to 30 minutes after completion, perhaps earlier)

H. Hypoglycemia

II. **Pathologic**

A. Hypothalamic disorders
   1. Chiari-Frommel syndrome: pathologically prolonged post-partum lactation
   2. Ahumada-Del Castillo syndrome: idiopathic galactorrhea
   3. Hypothalamic tumors: craniopharyngioma, ectopic pinealoma, metastatic tumors
   4. Nontumorous hypothalamic infiltration
      (a) Disseminated sarcoidosis
      (b) Histiocytosis X
   5. Post-resection of craniopharyngioma (in children with normal or accelerated growth rates despite decreased growth hormone)

B. Prolactin-secreting pituitary tumors
   1. Forbes-Albright syndrome: pituitary tumor with galactorrhea and (usually) amenorrhea

   2. Acromegaly (infrequent)
   3. Nelson’s syndrome: hyperpigmentation often with pituitary tumor after bilateral adrenalectomy for Cushing’s syndrome
   4. “Nonfunctioning” chromophobe adenoma (30 percent of cases)

C. Surgical transection of pituitary stalk

D. Primary hypothyroidism, with pituitary prolactin secretion apparently stimulated by increased secretion of thyrotropin-releasing hormone (TRH) of hypothalamus

E. Chronic renal failure (20 percent of cases)

F. Ectopic prolactin production by tumors
   1. Bronchogenic carcinoma
   2. Hypernephroma

G. Irritative lesions of the chest wall
   1. Herpes zoster
   2. Chest surgery
   3. Trauma to the intercostal nerves

III. **Pharmacologic**

A. Psychotropic drugs
   1. Phenothiazines: chlorpromazine, fluphenazine, promazine, perphenazine
   2. Tricyclic antidepressants: amitriptyline, nortriptyline, imipramine, desipramine
   3. Reserpine
   4. Sulpiride (an antiemetic and tranquilizer)
   5. Butyrophenones: haloperidol (Note: psychotropic drugs without effect on serum prolactin include lithium carbonate and chlordiazepoxide)

B. Anti-hypertensive drugs
   1. Reserpine
   2. Alpha-methyldopa

C. After discontinuance of oral contraceptives

D. Estrogen therapy

E. Injection of thyrotropin-releasing hormone (TRH)

Chromophobe Adenoma

Until very recently the inclusion of a major discussion of chromophobe adenomas as part of a section on prolactin would have been unthinkable; today, the authors of such a discussion deserve at worst a rebuke for slight exaggeration. All still agree that chromophobe adenomas may slowly destroy the function of the anterior lobe, and may also cause headache and visual field defects by their propensity to...
enlarge the sella turcica and grow out of it. Classically, these most common of all pituitary tumors were considered hormonally inactive. Now, however, it is clear from electron microscopic studies of the anterior pituitary in various species including man, that the term chromophobe, "connoting an inactive cell, is a misnomer. . . . Secretory granules have been present in every chromophobe adenoma that we have studied with the electron microscope although they may be few in number and lack the electron density of granules in normal cells."14 Indeed, when appropriate stains are used the chromophilic nature of the cells of such adenomas becomes so apparent that the very existence of nonsecretory, agranular "chromophobe" cells in the human pituitary is now doubted.16 Moreover, it seems that prolactin-secreting "chromophobe" adenomas are the commonest hormone-secreting tumors of the pituitary.8 9 Because most of these prolactin-secreting tumors do not produce galactorrhea despite chronically elevated serum prolactin levels, they were classified as "nonfunctional" chromophobe adenomas until sensitive and specific assays for prolactin became available. Now it is recognized that about 30 percent of chromophobe adenomas secrete prolactin, with more than two-thirds of these remaining hormonally silent (i.e., not producing the Forbes-Albright syndrome with galactorrhea and amenorrhea).9 Why this hormonal silence in some patients? The answer is not known, except that other hormones (estrogens and glucocorticoids) are needed in the right environmental "mix" for lactation to occur. Despite the absence of galactorrhea, serum prolactin levels are very high in patients with these prolactin-secreting chromophobe adenomas (higher than 200 ng per ml). Lesser elevations of prolactin may be due to infiltrative or other diseases involving the hypothalamus (table I), or to extension to the hypothalamus of any type of pituitary tumor (or craniopharyngioma). The water-load test may be helpful in the uncommon patient with serum prolactin levels of 200 to 300 ng per ml. On the other hand, when clinical findings are nonspecific (e.g., persistent headache), there is no evidence of extrasellar disease, and there is no certain increase in the size of the sella turcica; markedly elevated serum prolactin levels indicate the presence of chromophobe adenoma of the anterior pituitary.

Secretory or not, chromophobe adenomas can produce hypopituitarism. This ability to destroy the hormone-producing capacity of the anterior pituitary is shared by many other diseases that may thus mimic chromophobe adenoma. These include intra­sellar cysts, granulomas, and aneurysms, postpartum pituitary necrosis, and necrosis due to diabetes mellitus, shock, increased intracranial pressure and head trauma—to recount some of the more celebrated causes. The hypopituitarism induced by chromophobe adenoma may be partial or complete. Rabkin and Frantz,19 estimating the frequency of hormone loss in 25 patients with hypopituitarism, found abnormally low growth hormone responses in 25 percent of patients, abnormally low urinary gonadotropins in 88 percent, and abnormally low ACTH and TSH in 56 percent and 52 percent respectively. The clinically evident extent of the hypopituitarism is usually far less than the deficiency revealed by testing hormonal function. It is therefore necessary to assess each function, taking particular care to use provocative tests to best elicit the individual hormonal deficiency.

Evaluation of ACTH Secretion

Basal ACTH Secretion

A radioimmunoassay for ACTH has been developed, but is not yet readily available. Moreover, the hormone is measured in micromicrogram (picogram, pg) per ml quantities, and reproducibility of results
based on such miniscule concentrations is still difficult. At 8 AM, following an overnight fast of at least eight hours, the basal plasma ACTH level is 10 to 70 pg per ml.

More useful clinically are plasma cortisol levels. Biosynthesis of cortisol in the zona fasciculata of the adrenal cortex is directly dependent on circulating ACTH. It is well established that ACTH and cortisol are secreted cyclically throughout each 24 hour period, with their maximum concentrations occurring at about 8 AM, and their lowest levels between 8 PM and midnight. This normal diurnal variation in plasma cortisol concentration is tested by obtaining blood from resting subjects at 8 AM and again at 8 PM. The normal plasma cortisol level is 5 to 30 micrograms per 100 ml at 8 AM, with a decline of at least 50 percent to less than 10 microgram per 100 ml 12 hours later. In Cushing’s syndrome, this diurnal variation is absent. There is no significant decrease from the morning cortisol level even though the morning concentration, considered by itself, is normal or high-normal. This loss of diurnal variation of plasma cortisol is the sine qua non for the diagnosis of Cushing’s syndrome.

**ACTH Stimulation**

Many stimuli can provoke ACTH secretion. This review will be limited to the two most important provocative agents, metyrapone and pyrogen.

**Metyrapone test (figure 4).** Metyrapone (Metopirone) is an inhibitor of the adrenal cortical enzyme, 11-beta-hydroxylase. In figure 4 A and B are depicted the usual sequence of events following normal cortisol secretion and after metyrapone has effected a substantial block in cortisol synthesis. In essence, metyrapone produces a marked rise in serum ACTH consequent to the fall in serum cortisol and the excess production of the metabolic intermediary, 11-deoxycortisol (compound S), which is not a glucocorticoid and, therefore, is without suppressive effect on the hypothalamic hormone that stimulates ACTH release, corticotropin releasing factor (CRF). How-
ever, 11-deoxycortisol, like cortisol, has a 17,21-dihydroxyacetone side chain, and therefore reacts with phenylhydrazine to form the Porter-Silber chromagen in the standard test for 17-hydroxycorticosteroids. Thus, there is normally an increase in urinary 17-hydroxycorticosteroids (17-OHCS) after metyrapone administration, secondary to the increased secretion of ACTH and 11-deoxycortisol. To perform a metyrapone challenge correctly, three consecutive separate 24 hour urine specimens must be accurately collected. The first specimen is a baseline study. The second is collected on the day of metyrapone administration (750 mg orally every four hours for 24 hours). The third urine specimen is collected the day after drug administration, since some patients demonstrate the maximum response at this time. In normal subjects a doubling or tripling of urinary 17-OHCS is usual. There is no response when there is complete ACTH deficiency. An attenuated response suggests a partial defect in ACTH secretion. Either of these responses may be found in patients with pituitary tumors (figure 4C, D).

It must be emphasized that the adrenal cortex must be capable of responding to endogenous ACTH in order for the physician to assess the metyrapone test. Patients with primary adrenal failure cannot respond to metyrapone because their adrenals are incapable of producing steroids even under maximum stimulation by circulating ACTH.

Pyrogen test. This test demonstrates the ability of the hypothalamic-pituitary system to respond to a singular form of stress-fever. The pyrogen test has been reported to be less sensitive than the metyrapone test and more distressing to the patient. The test is easily performed. A basal blood sample is drawn at 8 AM, 0.30 μgm of bacterial pyrogen injected intravenously and another blood specimen obtained 4 hours later. A rise in body temperature must be documented. Plasma 17-OHCS are determined on each specimen. At least a doubling of serum cortisol levels occurs in normal subjects.

ACTH INHIBITION

Dexamethasone test. A decrease in 24-hour excretion of 17-OHCS reflects suppression of ACTH secretion when certain testing maneuvers are used; normal urinary excretion is 3 to 10 mg per 24 hours for men, 2 to 8 mg per 24 hours for women. A fall in serum ACTH and urine 17-OHCS excretion occurs after the administration of small doses of exogenous glucocorticoids in normal subjects. This observation forms the basis of the dexamethasone test (figure 5), which assists the clinician in differentiating the normal but "Cushingoid" patient from patients with benign adrenal hyperplasia or adrenal tumor (benign or malignant) associated with hypercorticism.

Dexamethasone is an extremely potent synthetic glucocorticoid which is not detected by the usual clinical assays of urinary 17-OHCS. The test is carried out at two dose levels, two mg per day (0.5 mg every six hours), which suppresses the 24 hour urinary 17-OHCS excretion below five mg per 24 hours in normal subjects (figure 4A), but is usually without effect in patients with benign adrenal hyperplasia or adrenal tumor (figure 5B); and eight mg per day (two mg every six hours), which reduces the excretion of 17-OHCS by 50 percent or more in patients with benign adrenal hyperplasia (figure 5C). The eight mg dose level is without effect in patients with adrenal tumors or hypercorticism secondary to ectopic ACTH production by carcinomas.

The dexamethasone suppression test is easily performed on in-patients and carefully instructed out-patients. A baseline 24-hour urine is collected, dexamethasone is administered in four equal six hourly doses for 48 hours at the two mg and then at
eight mg per day dose levels. A 24-hour urine is collected on the second day of drug administration at each dosage level. Urinary 17-OHCS are determined on each specimen.

A single midnight-dose (1 or 1.5 mg) dexamethasone test utilizing morning serum cortisol determinations has been reported. However, it has proven less reliable than the urinary studies, and we cannot recommend its use at this time.

Cushing's Syndrome

Chronic hypersecretion of adrenal glucocorticoids causes Cushing's syndrome. The clinical expression is highly variable. When advanced, characteristic abnormalities include hypertension, moon facies, redistribution of body fat, easy bruisability, diabetes mellitus, and osteoporosis. The syndrome is most commonly caused by bilateral adrenal hyperplasia (80 to 85 percent), but can be produced by an adrenal adenoma or carcinoma. Bilateral adrenal hyperplasia is associated with significant elevations of serum ACTH and is of hypothalamic-pituitary origin ("Cushing's disease"). A pituitary tumor may or may not be present at the time of diagnosis. Adrenal adenomas and carcinomas are primary adrenal disorders associated with suppression of hypothalamic-pituitary ACTH release. This suppression is a normal hypothalamic-pituitary response to the elevated levels of circulating cortisol resulting from tumor production.

The basic differences in the pathophysiology of the three types of Cushing's syndrome make it reasonable to expect that the adrenal cortex in bilateral hyperplasia is responsive to maneuvers that alter ACTH levels, while adrenal tumors (benign or malignant) are less responsive. Although tests designed to distinguish hyperplasia from tumor have been of great assistance to the clinician, there are times when the results are not consistent with the proven pathological diagnosis. The clinician must not ignore his suspicions of hypercorticism and eliminate this diagnosis because of a single normal study. Multiple studies and repeated observations of the patient's clinical appearance and course may be required to make the diagnosis.

In table II are shown the usual responses to be expected in Cushing's syndrome to
TABLE II

RESULTS OF BASAL SERUM ACTH LEVELS AND VARIOUS STIMULATORY AND INHIBITORY TESTS
OF ADRENOCORTICAL FUNCTION IN DIFFERENT CAUSES OF CUSHING'S SYNDROME

<table>
<thead>
<tr>
<th></th>
<th>Bilateral Adrenal Hyperplasia</th>
<th>Adrenal Adenoma</th>
<th>Adrenal Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ACTH</td>
<td>High with loss of diurnal variation</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Dexamethasone Suppression (2 mg per d)</td>
<td>Usually no suppression</td>
<td>No suppression</td>
<td>No suppression</td>
</tr>
<tr>
<td>Dexamethasone Suppression (8 mg per d)</td>
<td>More than 50% fall in urinary 17-hydroxy corticosteroids</td>
<td>No suppression</td>
<td>No suppression</td>
</tr>
<tr>
<td>ACTH Stimulation</td>
<td>Brisk response (increased plasma cortisol and 17-hydroxy-corticosteroids)</td>
<td>May or may not respond</td>
<td>No response</td>
</tr>
<tr>
<td>Metyrapone Test</td>
<td>Brisk response (increased 17-hydroxy-corticosteroids)</td>
<td>Usually no response</td>
<td>No response</td>
</tr>
</tbody>
</table>

the provocative and inhibitory stimuli already discussed. Some changes in the hypothalamic-pituitary-adrenocortical relationships are found in figures 4D and 5B and C. It should also be noted that patients with adrenal carcinoma may be virilized as well as Cushingoid. Marked elevations in 24 hour urinary 17-ketosteroids (over 30 mg) are very suggestive of malignant adrenal disease. The normal circadian rhythm of serum ACTH and cortisol is absent in all forms of Cushing's syndrome.
Postadrenalectomy Syndrome
(Nelson’s Syndrome)

In 1958 Nelson and coworkers described a woman who developed marked cutaneous and buccal pigmentation and a chromophobe adenoma several years after total adrenalectomy for Cushing’s syndrome due to bilateral adrenal hyperplasia. The authors noted that “it was not possible to be sure whether the pituitary tumor antedated the adrenalectomy,” an uncertainty that persists to this day in such cases. Hyperpigmentation following adrenalectomy for Cushing’s syndrome, often (but not always) associated with development of pituitary tumor, is now called the “postadrenalectomy syndrome,” or Nelson’s syndrome. The syndrome has been found only in patients who have had Cushing’s syndrome, not in those who have had adrenalectomies for other reasons. Corticoid replacement therapy in the postadrenalectomy period has no effect on the development of the clinical picture.

The pituitary tumors, when they occur, are usually chromophobe adenomas that tend to invade surrounding neural or vascular structures, and sometimes metastasize to extracranial areas. Despite uncertainty concerning time of onset of these tumors, present evidence favors the idea that their development is somehow provoked by adrenalectomy, and is not a part of the natural history of Cushing’s syndrome. The important histologic, secretory, and clinical differences between pituitary tumors that cause Cushing’s syndrome and those arising in the postadrenalectomy state are summarized in table III.

The intense hyperpigmentation of Nelson’s syndrome appears to be caused by increased secretion of the anterior pituitary hormone, β-melanocyte stimulating hormone (β-MSH). The plasma concentration of β-MSH in normal subjects is 10 to 100 pg per ml, and in patients who have had adrenalectomy for Cushing’s syndrome without further complications the mean value is 300 to 400 pg per ml. In patients with Nelson’s syndrome the mean value is 4,300 pg per ml, more than 40 times the highest normal concentration. Although ACTH, which is also hypersecreted in Nelson’s syndrome, has intrinsic melanotropic activity, it is not a major contributor to the hyperpigmentation. The reason is apparent: the plasma concentrations of ACTH and β-MSH are approximately equal, but the melanotropic activity of β-MSH is 25 times greater than that of ACTH.

ACTH Deficiency Due to Pituitary Tumor

ACTH deficiency results in secondary adrenocortical failure. Chronic weight loss, anorexia, weakness, irritability, and occasionally hypoglycemia may dominate the clinical picture. Although the adrenal cortex is atrophied for lack of normal ACTH stimulation, it is still responsive to exogenous ACTH. This characteristic is the key to proper testing for secondary adrenocortical failure. ACTH must be administered at rates of 40 to 80 units each day for three to five days to stimulate the atrophied adrenal cortex and enhance steroid output. A steady rise in the 24 hour excretion of
<table>
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<th>Pituitary Tumor Causing Cushing's Syndrome</th>
<th>Pituitary Tumor Associated with Nelson's Syndrome</th>
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<tr>
<td><strong>Histologic Characteristics</strong></td>
<td><strong>Histologic Characteristics</strong></td>
</tr>
<tr>
<td>Basophil, eosinophil, chromophobe or mixed tumors</td>
<td>Usually chromophobe adenomas</td>
</tr>
<tr>
<td><strong>Secretory Characteristics</strong></td>
<td><strong>Secretory Characteristics</strong></td>
</tr>
<tr>
<td>Increased β-MSH secretion; plasma levels 100 to 600 pg per ml</td>
<td>Markedly increased β-MSH secretion; plasma levels 900 to 10,000 pg per ml</td>
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<tr>
<td>Increased ACTH secretion; plasma levels up to 200 pg per ml</td>
<td>Markedly increased ACTH secretion; plasma levels up to 12,000 pg per ml</td>
</tr>
<tr>
<td><strong>Clinical Characteristics</strong></td>
<td><strong>Clinical Characteristics</strong></td>
</tr>
<tr>
<td>Slow-growing tumors</td>
<td>More rapidly-growing tumors; locally invasive, sometimes metastasizing extracranially</td>
</tr>
<tr>
<td>No dermal hyperpigmentation</td>
<td>Intense dermal hyperpigmentation</td>
</tr>
</tbody>
</table>

17-OHCS from an initially low baseline level after administration of ACTH by intravenous drip for six hours each day, or after intramuscular injection of ACTH gel twice daily, indicates normally responsive adrenal glands. However, the patient with secondary adrenal failure responds abnormally to metyrapone stimulation because there is no ACTH response to the presumably lower levels of serum cortisol induced by metyrapone-inhibition of adrenal cortisol synthesis.

Aldosterone function in secondary adrenal failure is adequate, for ACTH plays only a small role in control of its secretion. Therefore, hyponatremia and hyperkalemia, which are produced by aldosterone deficiency, are much more likely to be found in primary adrenal failure (Addison's disease) than in secondary adrenal failure caused by ACTH deficiency. In primary adrenal insufficiency, exogenous ACTH is without effect since little or no responsive adrenal tissue exists. The history, physical findings (field defects, pigmentation), serum electrolytes, skull films for changes in sella turcica size or evidence of erosion of clinoid processes all enable the physician
to pursue the most logical course in differentiating primary from secondary adrenal failure.

Other Hormonal Deficiencies Due to Pituitary Tumors

TSH Deficiency

Having made the diagnosis of hypothyroidism on the basis of clinical findings, low serum thyroxine concentration, and low 24 hour thyroidal radioactive iodine (RAI) uptake, the clinician must differentiate primary thyroidal failure (primary hypothyroidism) from pituitary failure (secondary hypothyroidism) or hypothalamic failure (tertiary hypothyroidism). This is especially important in the relatively few patients whose hypothyroid symptoms are clinically apparent but who have unprepossessing evidence of underlying hypopituitarism. The unknowing initiation of thyroid replacement therapy in these patients without simultaneous glucocorticoid replacement can precipitate acute adrenal failure leading to shock and death.

Until recently, there was only one way to distinguish primary hypothyroidism from the other, and rarer, forms. That method, which is still useful, consisted of giving 10 units of TSH intramuscularly daily for three days after performing a baseline 24 hour RAI uptake, and repeating the 24 hour RAI uptake on the fourth day. A significant rise in uptake indicates a thyroid gland responsive to the stimulatory effects of TSH, thus eliminating primary thyroidal failure and placing the defect somewhere in the hypothalamic-pituitary area controlling thyroidal function. Failure to respond to the TSH injections indicates the presence of primary thyroidal failure.

A simpler approach is now possible because of the availability of an immunoassay method for measuring serum TSH concentration (figure 6). In primary hypothyroidism, serum TSH levels are considerably higher than the normal upper limit for basal concentration (6 to 8 microunits per ml). This constitutes a normal response by hypothalamic TRH and pituitary TSH to the absent hormonal components (thyrox-
Figure 7. Mechanisms controlling secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the anterior pituitary and their alteration in pituitary failure and primary gonadal failure. FSHRF, follicle stimulating hormone releasing factor; LHRF, luteinizing hormone releasing factor.

Thyroid function regulation and triiodothyronine (T3) of the negative feedback loop (figure 6C). In pituitary or hypothalamic disease producing hypothyroidism, serum TSH is low despite the inadequate negative feedback. The situation pertaining to hypothyroidism induced by pituitary failure is depicted in figure 6B.

Synthesis of the hypothalamic tripeptide, TRH, now allows differentiation of hypothalamic and pituitary hypothyroidism. In a hypothyroid patient who has low serum TSH levels, the intravenous administration of 500 micrograms of synthetic TRH may or may not stimulate the secretion of pituitary TSH and increase the serum TSH level. If serum TSH increases, pituitary secretion is intact and the defect is in the hypothalamus. If serum TSH does not increase, pituitary failure is the cause of the hypothyroidism.

Hypothyroidism in association with pituitary tumor is well-documented; hyperthyroidism caused by a TSH-secreting pituitary tumor is extremely rare. In only three cases of the tens of thousands of cases of thyrotoxicosis has there been any evidence of a pituitary tumor secreting excessive amounts of TSH and thereby producing hyperthyroidism. A single well-documented case of a TSH-producing chromophobe adenoma causing hyperthyroidism is on record.\\n
Gonadotropin Deficiency

Deficiency of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), is common in patients with pituitary tumors. Symptoms due to altered gonadal function are among the most common complaints in these patients. Post-pubertal women experience secondary amenorrhea, and men lose libido and become functionally impotent. Both sexes experience an insidious loss of secondary sexual characteristics.

The most frequently used gonadotropin assay is the 24 hour total gonadotropin (both LH and FSH) urinary excretion. This bioassay lacks the sensitivity of the radioimmunoassay method, which can be
used for measuring blood levels of each gonadotropin. The distinction between primary gonadal failure and pituitary failure causing gonadal hormone deficiency can be made by utilizing the pathophysiological changes shown in figure 7. Patients who have signs and symptoms of hypogonadism with significantly reduced estrogen or testosterone production, and low total urinary gonadotropins (or low serum levels of FSH and LH), have hypogonadotropic hypogonadism (figure 7B). Pituitary tumor is one of many pituitary or hypothalamic diseases that can interfere with gonadotropin production and cause this form of hypogonadism.

**Antidiuretic Hormone Deficiency**  
(Diabetes Insipidus)

Diabetes insipidus, which results from a lack of the antidiuretic hormone, arginine vasopressin, is characterized clinically by persistent polyuria and polydipsia. Vasopressin is normally synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and reaches its storage site, the neurohypophysis, by axonal flow (figure 8). In primary diabetes insipidus there is a marked reduction in the number of neurons in these hypothalamic nuclei. In the less common secondary form, tumors of the pituitary itself (usually chromophobe adenomas) or craniopharyngiomas interfere with transmission of the hormone down the pituitary stalk or interfere with its orderly storage and secretion by the neurohypophysis. The physiological changes to be expected in normal subjects and those with diabetes insipidus are shown in figure 8.

It is a good general rule that patients with secondary forms of diabetes insipidus also show the clinical features of the underlying disease. Thus patients with pituitary tumors causing vasopressin deficiency can be expected to show the usual local and endocrine manifestations of their disease, in addition to polyuria and polydipsia. Two points must be remembered. First, other endocrinopathies can cause polyuria and polydipsia; these include diabetes mel-
itary tumors have panhypopituitarism and diabetes insipidus; in these patients, polyuria and polydypsia may become evident only after the patient has received glucocorticoid and thyroid replacement.

Radioimmunoassays for antidiuretic hormone are not readily available, and still cannot reliably detect the hormone at its normal plasma concentration of about 2.5 micrograms per ml. Therefore a diagnosis of diabetes insipidus is best established by demonstrating an elevated serum osmolality in the presence of an inappropriately dilute urine. A normal but dehydrated subject can concentrate his urine to more than 1,200 mOsm per kg (figure 8B). In contrast, the patient with diabetes insipidus may have urine osmolalities less than 300 mOsm per kg despite dehydration and a high serum osmolality (figure 8C). The more complete the vasopressin deficiency, the less able is the patient to raise his urine osmolality as required by a dehydration test.

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References


