144 Abbreviated Tests of Endocrine Function

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Definition

An abbreviated test consists of the use of one or two hormonal assays to confirm or exclude a clinically suspected endocrine diagnosis. For example, a high serum luteinizing hormone (LH) value with a low serum testosterone in a male is an abbreviated endocrine test diagnostic of primary hypogonadism. The identical testosterone value in a woman with an elevated LH value may be consistent with ovulation or the steady-state syndrome. Thus interpretation of abbreviated endocrine testing requires knowledge of the basic science of the hormones involved, the clinical setting, and the differential diagnosis of abnormal results. In this chapter, ten abbreviated tests of pituitary reproductive and metabolic function are discussed. The test for chorionic gonadotropin is included because of the hormone's structural similarities to pituitary gonadotropins. The emphasis on pathophysiology of hormonal secretion is the basis for understanding how each simple test may be applied to multiple clinical problems.

Technique

The development of radioimmunoassay (RIA) by Yalow and Berson (1959) has made abbreviated endocrine function tests possible.

Recent advances in RIA technology have increased the sensitivity and specificity of RIA. New assays utilize two antibodies, one of which is radioactively labeled, and each reacts with different sites on the hormone. Thus, the hormone is measured in a "sandwich reaction." These methods may be called immunoradiometric assays (IRMA) or simply high sensitivity (HS) RIA. For a review of the rationale and application of RIA, the reader is referred to the references. RIA can routinely and accurately measure hormones in the serum in the picogram (pg) to nanogram (ng) per ml range. Units commonly used to measure hormones will be cited. The units in parentheses following the normal range indicate (the conversion to) SI units which will eventually become the standard method to report laboratory information. With regard to most peptide hormones, SI units merely express the traditional units in terms of liters.

In general the normal hormone values cited in this chapter apply to the morning fasting condition. Sleep has many effects on hormonal secretion patterns, but sleep testing is not routinely obtained in clinical medicine. Hormonal elevations caused by pathologic conditions such as primary gonadal failure are not affected by time of day or the postprandial state. Therefore, when such conditions are clinically suspected, sampling may be done at any time of day. Finally, clinical stress has important effects on hormonal secretion. Stress must be considered in assessing the clinical significance of many results discussed here.

References

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Serum Prolactin

Prolactin stimulates the synthesis and secretion of breast milk. The fasting serum prolactin level varies between 1 and 20 ng/ml (1 and 20 μg/L) in normal men and women. The hormone is normally elevated during pregnancy and lactation. An abnormal elevation of serum prolactin is associated with sexual dysfunction. Serum prolactin values greater than 200 ng/ml are consistent with prolactin-secreting pituitary macroadenomas greater than 1 cm. Lesser elevations are caused by functional disorders or microadenomas. Serum prolactin acutely increases following injection of thyrotropin releasing hormone (TRH). This maneuver is useful in evaluating patients for panhypopituitarism.

Basic Science

Prolactin is a pituitary polypeptide containing 198 amino acids. Prolactin secretion is regulated primarily by the inhibitory action of dopamine, a major neurotransmitter secreted into the hypothalamic–pituitary portal system. Agents that decrease the dopamine content of the central nervous system (CNS) (such as reserpine and alpha-methyldopa) or dopaminergic blocking agents (such as phenothiazines and haloperidol) stimulate prolactin secretion. Lesions that destroy dopaminergic nerves in the hypothalamus or interrupt the hypothalamic pituitary portal system also stimulate pro-

lactin release. The only prolactin releasing hormone known is TRH, which also stimulates synthesis and release of thyroid stimulating hormone (TSH).

The contribution of TRH in the regulation of prolactin is not fully understood. Stimuli such as infant suckling acutely increase serum prolactin in women, but do not affect serum TSH. But a depression of serum thyroxin caused by primary hypothyroidism elevates both serum TSH and prolactin. Estrogen stimulates prolactin release, but has no effect on basal TSH. Numerous other factors increase serum prolactin selectively. When no anatomic cause is found, the increase in serum prolactin is considered to be "functional" or secondary to a change in hypothalamic chemistry.

Hyperprolactinemia interferes with sexual function in women by inhibiting the normal cyclic release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the gonadotropin-releasing hormone. The result is a loss of the ovulatory surge of LH, amenorrhea, and infertility. Usually the serum estrogen level is depressed but still sufficient for a permissive effect on the prolactin mediated production of breast milk. With marked estrogen deficiency, however, hyperprolactinemia may not be sufficient in itself to stimulate milk production. Prolactin-secreting tumors can also compromise the function of FSH and LH producing cells by direct compression of the pituitary in the sella turcica.

The relationship of functional hyperprolactinemia to the development of prolactin-secreting microadenomas is not known. Moreover, it is unclear how often a microadenoma evolves into a macroadenoma. The fact that these tumors often recur after successful transphenoidal adenomectomy suggests that in these patients the normal hypothalamic inhibitory mechanism is chronically disturbed and promotes tumor formation.

Clinical Significance

Pituitary Tumors

As many as 85% of the space-occupying lesions in the hypothalamic–pituitary axis may be associated with elevation of the serum prolactin. Twenty percent of women with secondary amenorrhea may have hyperprolactinemia. Prolactin is so important that reproductive disorders can be classified into normal and hyperprolactinemic states, as shown in Table 144.1. The serum prolactin value determined at the time of the initial evaluation will often predict whether the etiology of the prolactin abnormality is functional or tumorous. Serum prolactin values greater than 200 ng/ml are almost always associated with a prolactin-secreting pituitary tumor. In general, the higher the serum prolactin, the greater the size of the tumor. The major diagnostic problem is the patient with a serum prolactin between 30 and 200 ng/ml. This may be caused by functional factors or a microadenoma. At present there are no biochemical provocative or suppressive tests that will consistently differentiate functional from tumorous hyperprolactinemia. Imaging the hypothalamus and pituitary by coronal CT after injection of a contrast agent is the most commonly used way of differentiating between these diagnostic possibilities. Recently, radiologists have noted a high prevalence of false positive diagnosis of microadenoma by CT, especially if the suspected tumor is smaller than 6 mm. Magnetic

Table 144.1
Abbreviated Tests of Pituitary Reproductive Function

	Appropriate tests	
Normal serum prolactin states (1–20 ng/ml)	Serum FSH/LH[a] (mIU/ml)	Serum testosterone[a] (ng/dl)
Women		
Cyclic ovulatory menses	5–35/5–200	30–100
Menopause	> 50/> 50	—
Steady-state syndrome	5–20/25–50	100–200
Functional amenorrhea	0–10/0–10	—
Men		
Normal sexual function with or without idiopathic hypospermia	5–25/5–25	300–1200
Kallmann's syndrome	0–10/0–10	30–100
Women and men		
Primary gonadal failure	> 50/> 50	30–100 (W) 30–300 (M)
Hypopituitarism (no prolactin response with TRH test)	0–10/0–10	30–100
Hyperprolactinemia states	**Serum prolactin[a]**	**Serum testosterone**
Women and men		
Pituitary microadenoma or Hypothalamic lesion	20–200	30–100 (W) 30–300 (M)
Pituitary macroadenoma	> 200	30–100

[a]Values indicate typical results for the suspected clinical diagnosis.

resonance imaging (MRI) is a superior imaging technique for the diagnosis of pituitary microadenoma.

Bromocriptine

The decline in serum prolactin in response to treatment with the dopaminergic agonist bromocriptine is usually dramatic in both functional or tumorous hyperprolactinemia. Restoration of ovulation indicated by a return of cyclic menses in women, or normalization of sexual function in men, occurs concomitantly with the drop in prolactin. If sexual symptoms do not improve, serial serum prolactin determinations and repeat imaging procedures are necessary to check for tumor growth.

Hypopituitarism

Prolactin deficiency may occur with destructive lesions of the pituitary such as vascular insults, inflammatory diseases, or nonprolactin-secreting macroadenomas. These conditions also compromise the release of other pituitary hormones, causing symptoms and signs of panhypopituitarism such as loss of sexual function and secondary sexual characteristics, fatigue, pallor, and disordered water metabolism. Pituitary injury occurring in the postpartum period often presents as a failure of lactation due to prolactin deficiency. The presence and severity of the pituitary injury can be assessed by measuring the serum prolactin and TSH response to TRH. Failure to stimulate both hormones is consistent with panhypopituitarism (Table 144.2). The details and limitation of the TRH test for the diagnosis of hypopituitarism are discussed with TSH.

Table 144.2
Abbreviated Tests of Pituitary or Target Gland Dysfunction

Suspected diagnosis	Appropriate test	Interpretation
Acromegaly	Serum GH/SmC	Nonstressed state: GH > 30 ng/ml SmC > 0.2 μg/ml diagnostic
Cushing's syndrome	Overnight 1 mg dexamethasone	Cortisol <5 μg/dl rules out diagnosis
Hyperthyroidism (nondiagnostic thyroxin)	Serum HS–TSH	<0.3 μU/ml consistent with diagnosis
Panhypopituitarism	Serum prolactin and TSH after TRH	Failure of both to double consistent with diagnosis
	Serum cortisol after cosyntropin	Failure to exceed 18 μg/dl consistent with diagnosis
	Serum deoxycortisol after metyrapone	<10 μg/dl consistent with diagnosis

References

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Serum Follicle-Stimulating Hormone, Luteinizing Hormone, Testosterone, and Estradiol

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) control reproductive function and the serum levels of testosterone and estradiol, which stimulate secondary sexual characteristics in men and women. FSH and LH are commonly referred to as the *pituitary gonadotropins*. In women in the reproductive age group the normal serum values of the gonadotropins vary depending on the phase of the menstrual cycle. Values fluctuate between 5 and 25 mIU/ml (5–25 IU/L) except at the time of ovulation when LH rises to as high as 200 and FSH to 35 mIU/ml. The diagnosis of primary or secondary ovarian failure can be made by considering the clinical presentation in the face of elevated, normal or reduced FSH and LH values (Table 144.1). Serum values greater than 50 mIU/ml indicate menopause or primary ovarian failure.

In men, serum FSH and LH values are relatively constant from puberty to old age and range between 5 and 25 mIU/ml. Testosterone is secreted by the interstitial cells or Leydig cells of the testes, primarily in response to stimulation by LH. Thus LH is also called *interstitial-cell stimulating hormone*. Since a low serum FSH and LH may be found normally, the values must be interpreted relative to the total serum testosterone level, which ranges between 300 and 1200 ng/dl (10.5–42.0 nmol/L).

Basic Science

FSH and LH are glycoproteins consisting of 204 amino acids and containing alpha and beta subunits. The alpha chain is identical in FSH, LH, human chorionic gonadotropin (HCG), and TSH. These hormones differ by virtue of their beta chain and carbohydrate content. FSH and LH secretion is regulated by gonadotropin-releasing hormone (GnRH) whose synthesis and release into the hypothalamic portal system are mediated by a feedback system involving peripheral sex steroids. In women, the feedback loop has positive and negative components. The decline in estradiol levels associated with menses activates the secretion of FSH, which stimulates the maturation of an ovarian follicle. During the follicular phase of the cycle, FSH and estradiol rise. At a critical estradiol concentration, LH is released in a surge and induces ovulation. This begins the luteal phase of the cycle in which LH and FSH levels rapidly fall as progesterone and estradiol levels produced from the corpus luteum rise. If conception does not occur, sex steroid levels decline, initiating menses and completing the cycle.

Many functional factors affect hypothalamic regulation of pituitary FSH and LH release, resulting in loss of the midcycle LH surge and subsequent amenorrhea secondary

to anovulation. These factors include stress, nutritional changes, and exercise associated with significant reduction in adipose mass. The mechanism by which physical and emotional stress impair FSH and LH release in women is not understood.

In men, the hypothalamic–pituitary–testicular axis is regulated as a classical negative feedback loop. FSH stimulates sperm maturation, which is associated with the production of inhibin. This peptide modulates and suppresses FSH release. Many other polypeptides and hormones, such as androgen-binding globulin and epidermal growth factor, are synthesized in the testes, but it is unclear how they affect hypothalamic–pituitary regulation. Testosterone secreted locally stimulates sperm maturation. In the systemic circulation, testosterone stimulates the development of secondary sexual characteristics. Defects in the testes that impair spermatogenesis or Leydig cell function increase FSH and LH release.

Clinical Significance

Primary Ovarian Failure

Elevated FSH and LH levels are diagnostic of primary ovarian failure. Clinical signs include amenorrhea, vasomotor phenomena such as hot flashes and cold sweats, and loss of breast mass. The causes of primary ovarian failure are chromosomal defects such as Turner's syndrome, ovarian dysgenesis, or autoimmune ovaritis, which is often associated with other endocrine deficiencies. Anovulatory menses or breakthrough bleeding in women, associated with elevated FSH and LH, is usually a perimenopausal phenomenon, but if the clinical setting is not appropriate, estradiol levels should be obtained. In these instances a low estradiol will confirm ovarian failure or impending menopause.

Steady-State Syndrome

Infertility with amenorrhea or anovulatory menses with hirsutism and other signs of virilization is consistent with steady-state syndrome. The name is derived from the FSH and LH secretory patterns. Classically FSH is low normal and LH is released in a noncyclic or tonic pattern characteristic of its secretion in men. This results in excessive androgen production by ovarian interstitial cells. Serum testosterone is often mildly elevated, varying from 100 to 200 ng/dl. Progressive virilization with elevated testosterone and suppressed LH values is against the diagnosis of steady-state syndrome and indicates the need to search for an autonomous ovarian or adrenal source of androgens.

Functional Disorders

The most common clinical problem of the reproductive axis in women involves the differentiation of amenorrhea caused by functional suppression of FSH and LH versus a destructive process in the pituitary. Measuring FSH and LH as an abbreviated test following GnRH injection does not reliably make this differential diagnosis in all cases. Thus the distinction is made on clinical grounds by correlating changes in serum FSH and LH to serum prolactin (Table 144.1). Functional suppression of FSH and LH in postmenopausal women is unusual, so low values in such patients would be strongly suggestive of pituitary pathology. In contrast, low values in young women without an increase in prolactin usually result from a functional disorder, often suggested by the clinical setting. For example, this is seen in women who are under stress, who induce vomiting, or who place a high priority on body weight (e.g., models, professional dancers, actresses). Women athletes may also experience functional suppression of gonadotropins.

Testicular Failure

Hypogonadism in men is suggested clinically by loss of libido, difficulties with sexual performance, and attenuation of male sexual characteristics. The cause of primary hypogonadism may be chromosomal defects in the testes (such as Klinefelter's syndrome) or acquired causes (such as vascular injury from testicular torsion) or thermal injury (such as undescended testes). A common clinical problem is the evaluation of the male with infertility secondary to hypospermia. Most often, serum FSH/LH are in the normal range with normal testosterone and thus no defect in the hormonal axis is detectable. The most common structural abnormality causing secondary testicular failure is the prolactin-producing tumor discussed above.

Sex Binding Protein Abnormalities

When symptoms of sexual dysfunction in men are associated with low testosterone and a normal FSH and LH, one should consider incidental depression of the sex steroid binding protein before proceeding with a pituitary evaluation for secondary hypogonadism. Testosterone is transported in the serum bound to this protein. Reductions in the binding protein caused by genetic factors, obesity, or thyroid or liver dysfunction will depress the total serum testosterone value. Thus the free or unbound serum testosterone test is necessary to determine if the sex hormone is adequate. The finding of a low total serum testosterone with normal free testosterone would confirm the binding protein abnormality and suggest that sexual symptoms may have a psychogenic cause.

Congenital Defects

Kallman's syndrome is a functional depression of FSH and LH release secondary to hypothalamic defects in GnRH synthesis and release. This syndrome occurs in both sexes and is recognized at puberty because of failure of sexual development associated with midline anomalies causing anosmia or hyposmia, color blindness, or cleft palate. FSH and LH release can be stimulated by GnRH given therapeutically—but formal endocrine testing, rather than an abbreviated test with GnRH, is necessary to confirm this type of hypothalamic hypogonadism. Selected defects may occur at any site in the axis. Isolated impairment of LH secretion may result in failure of Leydig cell function with a consequent failure of sperm maturation. Treatment with the LH-like gonadotropin HCG will restore testosterone production and reverse infertility.

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Serum Human Chorionic Gonadotropin

Human chorionic gonadotropin (HCG) is normally secreted by the trophoblastic tissue of the placenta. The appearance of the beta chain of HCG in the serum 8 days after conception is the earliest sign of pregnancy. Otherwise, HCG and its beta subunit should be undetectable in serum. In the nonpregnant woman or man, the finding of a significant level of HCG or the beta subunit in the serum is indicative of a malignant neoplasm of either trophoblastic, gonadal, or ectopic origin.

Basic Science

The structural similarities of HCG to the pituitary glycoproteins have been discussed above. Because of the common alpha subunit, some RIAs for HCG may cross-react with LH and FSH. For this reason, the RIA for the specific beta subunit of HCG is often used. HCG and LH are also functionally similar. HCG stimulates the corpus luteum of pregnancy until the placenta becomes the primary source of progesterone necessary to maintain the endometrium. In the male fetus, HCG stimulates testicular androgen production, which causes male sexual characteristics prior to the appearance of pituitary gonadotropins at 10 weeks.

Clinical Significance

Differential Diagnosis

The assays for HCG and its beta subunit are performed to diagnose pregnancy and the presence of abnormal trophoblastic tissue such as hydatidiform mole and choriocarcinoma in women, as well as various testicular carcinomas in men. Secretion of HCG can cause isosexual precocious puberty in children and gynecomastia in men. In both syndromes, the effect is mediated by sex steroids released by gonadal interstitial cells under HCG stimulation.

The sources of excess HCG in men may be a tumor in testicular tissue. Ultrasound is useful for diagnosis. HCG may be produced ectopically by anaplastic lung cancer, hepatoma, islet cell tumors, and other neoplasms. The presence of HCG in serum in the absence of pregnancy is a tumor marker that may be used to determine the effectiveness of treatment and recurrence during follow-up.

Reference

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Serum Growth Hormone, Somatomedin C

Growth hormone (GH), or somatotropin, promotes the growth of skeletal muscle, connective tissue, and viscera. In children, GH is essential for normal linear growth. Many of the anabolic effects of GH are mediated by insulinlike growth factors (IGF) known as *somatomedins*, produced and released by the liver in response to GH. After puberty, serum levels of total somatomedin C (SmC), also known as IGF I, generally correlate with GH levels and normally range between 0.1 and 0.2 μg/ml (0.1–0.2 mg/L).

Fasting GH levels vary from 0 to 7 ng/ml (0–7 μg/L), but physiologic stimuli can result in levels as high as 30 ng/ml. A low GH value may be normal or may represent the earliest manifestation of pituitary insufficiency or an isolated defect in GH regulation. Chronic elevations of GH caused by a GH-secreting tumor result in acromegaly. GH values in this condition range from normal to several hundred ng/ml and do not always correlate with the severity of the condition.

Basic Science

GH is a 190 amino acid polypeptide. Synthesis and release of GH from the somatotropes of the anterior pituitary are regulated by the opposing effects of somatostatin and growth-hormone-releasing factor (GRF), both of which are now fully characterized. The early phase of sleep, stress associated with catecholamine discharges, and metabolic stimuli such as hypoglycemia or arginine infusion all result in GH release. In contrast, hyperglycemia suppresses GH release.

A characteristic of pathologic GH secretion is the response to thyrotropin releasing hormone (TRH). Normally TRH does not affect pituitary GH release because somatostatin is a TRH antagonist. However, infusion of TRF into an animal in which the pituitary gland has been autotransplanted to the renal capsule causes an acute increase in GH. The same occurs in patients with acromegaly, suggesting that GH-secreting tumors may be the result of the unopposed effects of GRF on the somatotrope. GRF was recently isolated from the carcinoid tumors of two patients with acromegaly caused by somatotrophe hyperplasia. The factor is currently being investigated in children with short stature. It is expected to be a significant therapeutic advance in the treatment of children with hypothalamic causes of GH deficiency.

Pituitary-released GH has a half-life of 25 minutes. The resulting stimulation of SmC production in the liver, how-

ever, causes the peptide to enter the circulation where it becomes bound to transport proteins. This process gives SmC a circulatory half-life of 2 to 4 hours and allows serum SmC levels to correlate with an integrated GH effect rather than with acute changes in GH caused by physiologic phenomena. The present methodology measures total or bound SmC rather than the free component that may be responsible for biological activity.

Clinical Significance

Deficiency

GH and SmC levels are helpful in testing pituitary function and evaluating children with short stature. GH release, like that of pituitary gonadotropins, is readily impaired by disease processes in the sella turcica. This is best demonstrated by provocative endocrine testing with insulin-induced hypoglycemia. The finding of a low fasting serum GH with a reduced SmC also suggests a defect in GH regulation. Children with short stature without apparent systemic or congenital diseases may have hypopituitarism or isolated defects involving GH. As a screening test, these children may be exercised in the fasting state until they become diaphoretic. An acute increase in GH to greater than 10 ng/nl following exercise rules out both disorders and eliminates the necessity for further pituitary testing.

Hypersecretion

The diagnosis of acromegaly is usually obvious by clinical inspection and pituitary imaging. In such cases elevation of GH above 30 ng/ml with increased SmC is diagnostic (Table 144.2). Acromegaly may be associated with marginal elevations of GH that overlap the range for physiologic stress. In these cases the diagnosis of active disease must be confirmed by demonstrating failure of GH to suppress following glucose. This is done by measuring GH during a 2-hour standard glucose tolerance test. When the signs of acromegaly are subtle and the serum GH, SmC, and postglucose GH values are all equivocal, an increase of greater than 5 ng/ml in GH following 500 mcg of TRF is indicative of defective hypothalamic GH regulation consistent with acromegaly.

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Serum Thyroid Stimulating Hormone, Thyrotropin Releasing Hormone Test

Thyroid stimulating hormone (TSH), also known as thyrotropin, regulates the thyroidal synthesis and release of thyroxin and triiodothyronine. A supersensitive or high sensitivity (HS) TSH assay is rapidly becoming the standard of clinical practice. Normal values with the HS–TSH vary from 0.3 to 5 μU/ml (0.3–5 mU/L). This one assay may eventually replace all tests in differentiating hypo- and hyperthyroidism patients from normals. However, many laboratories have not yet employed the HS–TSH assay. They perform the routine TSH RIA with normal range less than 5 μU/ml (5 mU/L). This assay will not distinguish patients with autonomous thyroid hyperfunction.

When serum TSH is measured after injection of thyrotropin releasing hormone (TRH), the TSH response may be extremely useful in supporting the suspected clinical diagnosis. Failure of TSH to rise is consistent with hyperthyroidism, advanced age, or panhypopituitarism. This test is not indicated for primary hypothyroidism, since this diagnosis is confirmed by a single baseline elevation of serum TSH.

Basic Science

TSH is a glycoprotein with structural similarities to FSH, LH, and HCG, discussed above. Pituitary TSH secretion is under direct feedback control mediated by thyroxin from the periphery and under TRH control from the hypothalamus. The release of TRH also affects the lactotroph cells, which may be the reason for hyperprolactinemia associated with hypothyroidism.

The etiology of hyperthyroidism is autonomous function of the thyroid caused by autoimmune mechanisms of Graves' disease or thyroxin-secreting adenomas. Pituitary tumors producing TSH are rare. Thus TSH is suppressed in hyperthyroidism, as confirmed by use of the new supersensitive TSH assay or by the failure to demonstrate an acute increase in serum TSH following TRH injection.

Clinical Significance

Elevated Values

In hypothyroidism, serum TSH ranges from 10 to 200 μU/ml depending on the clinical situation. Some of the most severely myxedematous patients will demonstrate a relatively mild elevation, probably because of myxedema of the pituitary. Some patients with subtle symptoms and indolent onset of hypothyroidism such as after radioactive iodide therapy of Graves' disease will have TSH levels greater than 150 μU/ml.

Sick Euthyroid Syndrome

In severely ill but euthyroid patients, thyroxin may be depressed and TSH is normal. This profile is also consistent with secondary hypothyroidism caused by hypopituitarism. Differentiation of the sick euthyroid patient from the one with secondary hypothyroidism is often difficult by thyroid tests. In secondary hypothyroidism, other abnormalities in

FSH, LH, prolactin, and ACTH are also present. A TRH test may demonstrate hypopituitarism if serum TSH and prolactin are both determined.

TRH Test for Diagnosis of Hypopituitarism

After blood for TSH and prolactin is drawn, 500 μg of TRH is given intravenously (IV) to the patient maintained in the supine position. Repeat samples are obtained in 30 minutes. In normal persons, prolactin and TSH levels increase twofold or more. A marked elevation of prolactin at baseline would suggest significant pituitary pathology—most likely a macroadenoma, which might compress TSH-producing cells. However, the response of prolactin to TRH does not differentiate functional from tumorous causes of hyperprolactinemia. Low prolactin and TSH at baseline with failure of both hormones to rise indicate panhypopituitarism—often secondary to a vascular etiology. A normal prolactin response without change in TSH is either an equivocal result (indicating the need for more extensive pituitary studies) or is due to hyperthyroidism (see below). TRH testing does not differentiate secondary from tertiary causes of hypothyroidism associated with hypothalamic defects. Injection of TRH is occasionally associated with acute increases in blood pressure. Caution is required in patients in the older age groups or those with cardiovascular disease.

Diagnosis of Hyperthyroidism

Most patients with hyperthyroidism will have unequivocal elevation of thyroxin or triiodothyronine. These studies confirm the diagnosis, and no further studies are necessary. When thyroid hormones are borderline or marginally elevated and the clinical picture is suggestive but nondiagnostic, the HS–TSH assay should be done. The TRH test should be reserved for rare clinical circumstances when all other data are ambiguous.

The test is performed as described above. Only serum TSH need be measured. A baseline sample is taken and another sample 20 to 30 minutes after TRH injection. A doubling of the baseline or an increase of 4 μU/ml rules out autonomous thyroid function. No change in TSH levels is consistent with hyperthyroidism. False positive results may occur in nonhyperthyroid older patients who often fail to have a TSH response. In older patients the diagnosis of hyperthyroidism may require measurement of thyroidal radioiodide uptake, free thyroxine, and thyroid stimulating hormone.

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Plasma ACTH and Serum Cortisol, Overnight Dexamethasone Suppression Test, Cortrosyn Stimulation Test, Overnight Metyrapone Challenge Test

Pituitary secretion of ACTH (adrenocorticotropic hormone) regulates adrenal steroidogenesis by stimulating the synthesis and release of cortisol, the major glucocorticoid; the adrenal androgens; and aldosterone, the most potent mineralocorticoid. Of these, only cortisol exerts a negative feedback effect on ACTH release.

In the morning, under normal conditions, plasma ACTH varies between 50 and 200 pg/ml (11–44 pmol/L) and cortisol levels between 15 and 30 μg/dl (410–820 nmol/L). By nighttime, ACTH is about 20 to 50 pg/ml with cortisol levels of 3 to 10 μg/dl. The RIA for ACTH is technically difficult to perform, however, and physiologic values associated with stress vary so widely as to overlap values in the pathologic range. For these reasons, other indirect screening tests, such as the dexamethasone test for hyperadrenalism and the metyrapone and cosyntropin tests for adrenal insufficiency, have been applied to the many disorders affecting the pituitary/adrenal axis, as shown in Tables 144.2 and 144.3.

Basic Science

ACTH is a 39 amino acid polypeptide derived from proopiomelanocortin (POMC). This large protein, synthesized

Table 144.3
Abbreviated Tests of Hypothalamic–Pituitary–Adrenal Function

Suspected diagnosis	ACTH/Cortisol[a] (pg/ml)/(μg/dl)	Cosyntropin test cortisol	Metyrapone test deoxycortisol[a] (μg/dl)
Normal	20–200/5–30	Response >7 Peak >18	>10
Addison's disease	350–700/0–5	No response	<1
Secondary hypoadrenalism	0–50/0–5	Peak <18	<10
Cushing's syndrome[b]			
Cushing's disease	100–300/20–40	Marked response	>20
Adrenal tumor	0–50/20–60	No response	<1
Ectopic ACTH	300–1000/40–80	Marked to absent	—

[a]Typical hormone values observed.
[b]The diagnosis of Cushing's syndrome established by dexamethasone nonsuppressibility.

in the pituitary, also contains the amino acid sequence for beta-endorphins, the endogenous opioids, and beta-melanocyte stimulating hormone. The first 13 amino acids of ACTH also have melanocyte stimulating activity. Thus, clinical states of ACTH excess are associated with hyperpigmentation proportional to the chronicity and severity of the ACTH elevation.

ACTH secretion is characterized by acute secretory bursts conforming to a circadian rhythm. Frequent secretory bursts that occur during REM sleep (rapid extraocular muscle movements) prior to awakening in the early morning produce the highest ACTH and cortisol levels. Normal circadian periodicity involved in ACTH release is readily altered by stressful stimuli such as trauma, exercise, fever, vasoactive substances, hypoglycemia, and emotional distress. Stress may produce direct CNS stimulation of the hypothalamus, resulting in the release of corticotropin releasing hormone (CRH) into the hypothalamic–pituitary portal system that mediates ACTH synthesis and release. Overstimulation of the ACTH-secreting cells of the pituitary by CRH may result in a micro or macroadenoma demonstrable by pituitary CT. CRH is currently under clinical investigation to determine its usefulness in the diagnosis of pituitary ACTH pathology and other functional disorders of CNS, such as endogenous depression.

Cortisol is the life-sustaining principle that Thomas Addison associated with the function of the adrenal gland. Cortisol is synthesized from cholesterol taken up by the adrenal gland through the low-density lipoprotein receptor pathway. The designation *glucocorticoid* is related to cortisol's action in stimulating conversion of amino acids to glucose. Deficiency of cortisol or Addison's disease is associated with hypotension, electrolyte abnormalities (hyperkalemia and acidosis), vascular collapse, and hypoglycemia in the face of stress. Administration of cortisol (hydrocortisone) reverses this crisis and readily suppresses compensatory pituitary release of ACTH.

The clinical state of excessive glucocorticoids, known as Cushing's syndrome, is associated with obesity, loss of muscle mass (myopathy), loss of bone matrix (osteopenia), and loss of subcutaneous and perivascular collagen (thin skin, stria, and easy bruisability). At high physiologic concentrations cortisol has aldosterone-like effects on water and salt metabolism, resulting in hypertension and hypokalemia. The most common cause of Cushing's syndrome is Cushing's disease, the result of inappropriate and excessive secretion of pituitary ACTH.

The most convenient screening test to rule out Cushing's syndrome consists of administration of dexamethasone. This potent, long-acting cortisol analogue is not detected in the assay for serum cortisol. Thus serum cortisol following dexamethasone may be measured as a sign of ACTH suppression. Failure to suppress cortisol after a physiologic dosage of dexamethasone is consistent with Cushing's syndrome.

Metyrapone, which interferes with cortisol synthesis, is also used to assess the function of the hypothalamic–pituitary–adrenal axis. This agent competitively suppresses 11 beta hydroxylase enzyme, which catalyzes the conversion of 11-deoxycortisol to cortisol. A block in cortisol synthesis activates the feedback mechanisms, which, under normal conditions, causes an acute increase in 11-deoxycortisol. A failure of 11-deoxycortisol to increase after metyrapone indicates a defect in the axis. The adrenal component of the axis is quickly tested by administration of cosyntropin, a synthetic ACTH derivative with an amino acid sequence identical to the first 24 amino acids of ACTH. The normal adrenal response to cosyntropin is an acute release of all adrenal steroids.

Clinical Significance

The RIA for plasma ACTH has its greatest diagnostic power in three clinical settings: the diagnosis of Addison's disease, the diagnosis of the paraneoplastic syndrome (commonly called ectopic production of ACTH), and the differentiation of Cushing's syndrome caused by Cushing's disease from tumorous production of cortisol by the adrenal gland. Nevertheless, ACTH is readily degraded in plasma, and the process of transferring the plasma to a reference laboratory can introduce technical problems and delays. Moreover, plasma ACTH has limited usefulness in differentiating the rare patient with ACTH-secreting carcinoid tumor or the common patient with stress and obesity from patients with Cushing's disease.

Addison's Disease

This diagnosis is suspected clinically in a patient with fatigue, nonspecific abdominal complaints, hyperpigmentation, and orthostatic hypotension. A markedly elevated plasma ACTH associated with a profoundly low serum cortisol confirms the diagnosis of Addison's disease. If symptoms and signs are subtle, plasma ACTH values may not be high enough to confirm the diagnosis. Thus, when the diagnosis is not clinically obvious, a cortrosyn test of adrenal function is the appropriate screening study for Addison's disease.

Ectopic Production of ACTH

The patient with this diagnosis presents with generalized weakness associated with weight loss, nocturia, hypertension, myopathy, peripheral edema, and evidence of neoplasia. Profound hypokalemia with metabolic alkalosis is indicative of the highest cortisol values seen clinically. Plasma ACTH values in the nanogram range confirm the diagnosis and may serve as a marker of the neoplasm. The syndrome is most often caused by undifferentiated carcinomas of the lung, both large (anaplastic) cell and small (oat) cell, which are synthesizing large ACTH precursor molecules of variable biological activity. ACTH-secreting tumors have also been found in the pancreas as islet cell tumors and in the thymus. Carcinoid tumors both malignant and benign may secrete ACTH, but these tumors have a long indolent course that cause the classical stigmata of Cushing's syndrome (obesity, stria, osteopenia). Thus carcinoid tumors mimic Cushing's disease clinically; ACTH levels in both conditions may be at the nonspecific high normal range of 150 to 200 pg/ml. The differential diagnosis of carcinoid-causing Cushing's syndrome requires more extensive dexamethasone testing and imaging studies.

Differential Diagnosis of Cushing's Syndrome

When the diagnosis of Cushing's syndrome is considered after demonstrating lack of cortisol suppression by physiologic dexamethasone (overnight dexamethasone test, see below), the plasma ACTH level will differentiate a pituitary

or ectopic tumor source of ACTH from autonomous cortisol-producing adrenal tumor. In the latter, ACTH should be suppressed to less than 20 pg/ml. The presence of an adrenal adenoma or carcinoma should be readily confirmed by cross-sectional imaging.

A common clinical problem is the exclusion of Cushing's syndrome in a woman with obesity, hypertension, hirsutism, diabetes, and menstrual abnormalities. Though these are all characteristics of excess glucocorticoids, these signs are nonspecific and are typically a manifestation of exogenous obesity. In this situation ACTH and cortisol profiles are not helpful, since high values associated with circadian rhythm and stress overlap the values of Cushing's disease. The overnight dexamethasone suppression test may readily make the differentiation. However, 15% of patients without Cushing's syndrome will fail to suppress. The 24-hour urine free cortisol determination is not confounded by obesity, making it a useful second screening study.

Suppressed Pituitary–Adrenal Axis

Another common question of pituitary–adrenal function is the status of the axis after exposure to pharmacologic dosage of glucocorticoids or following surgical removal of an adrenal tumor. Usually the axis requires 6 to 9 months after withdrawal of long-term steroids to recover ACTH and cortisol responsiveness to stress. Some patients continue to have constitutional complaints suggestive of adrenal insufficiency for 1 to 2 years. ACTH levels are of no value in assessing adrenal recovery, since the pituitary recovers before the adrenal. Finding a cortisol level above 18 μg/dl would essentially exclude a prolonged adrenal suppression syndrome. If the value is lower, cosyntropin or metyrapone tests would be necessary to determine the responsiveness of the axis to stress.

Overnight Dexamethasone Suppression Test

This test measures the suppressibility of ACTH produced by the hypothalamic–pituitary axis following a physiologic dosage of dexamethasone. A normal suppression indicated by a reduced cortisol rules out Cushing's syndrome. A failure of cortisol to suppress after dexamethasone requires measuring the 24-hour urinary free cortisol and/or a 48-hour dexamethasone suppression test to rule out a false positive result.

In normal individuals 1 mg of dexamethasone taken at 11:00 P.M. suppresses early morning elevation of ACTH and results in an 8:00 A.M. serum cortisol value of less than 5 μg/dl. Patients with Cushing's syndrome show no response to dexamethasone and maintain their serum cortisol level greater than 20 μg/dl throughout the day. This loss of suppressibility and the loss of normal diurnal circadian rhythm of ACTH and cortisol secretion are pathognomonic of Cushing's syndrome.

Cortrosyn® (Cosyntropin) Stimulation Test

This test measures the increment in serum cortisol following an injection of Cortrosyn (cosyntropin). The test is extremely useful in screening for Addison's disease and secondary (pituitary dependent) adrenal insufficiency. A normal response definitely rules out Addison's disease—but an abnormal response requires further testing before the diagnosis of adrenal insufficiency can be made with certainty. Cortrosyn, 250 μg given IV or IM, normally increases baseline cortisol by at least 7 μg/dl to a value greater than 18 μg/dl. This response is observed in 30 minutes after IV or in 60 to 90 minutes after IM injection.

If both the 7 μg/dl increment in cortisol and the stimulated value of 18 μg/dl are attained, the adrenal gland is presumed to be under adequate stimulation by endogenous ACTH. Therefore these values not only rule out Addison's disease but nearly always exclude the possibility of secondary adrenal insufficiency.

The Cortrosyn test may be used under emergency conditions when the diagnosis of acute adrenal insufficiency or adrenal crisis is suspected on clinical grounds. In this setting (usually associated with the marked stress of a life-threatening infection or a bleeding diathesis) treatment could be instituted before cortisol values are known. Cortrosyn can be given IV concomitantly with dexamethasone, glucose, and saline, after a baseline blood for serum cortisol is drawn. Dexamethasone will not inhibit the cortrosyn effect. Blood for serum cortisol is again taken in 30 minutes, after which time the steroid infusion may be changed to high dosage hydrocortisone to provide an additional mineralocorticoid effect. The same criteria are used to interpret results. With normal adrenal function, however, the serum cortisol is directly proportional to the severity of the stress. A baseline value less than 18 μg/dl that fails to stimulate confirms the diagnosis of adrenal crisis.

The Cortrosyn test may also be used as an aid in the differential diagnosis of Cushing's syndrome. If Cortrosyn is given to a patient with Cushing's disease, the baseline cortisol will be high and an exaggerated stimulatory response will occur indicative of adrenal hyperplasia (e.g., 25 μg/dl baseline to 75 μg/dl). In adrenal adenoma, the baseline will be elevated, but often no response will occur because of autonomous tumor function. Remarkable elevations of serum cortisol, such as baseline values of 60 to 80 μg/dl, are suggestive of adrenal carcinoma or ectopic production of ACTH. In neither case does cortrosyn produce a further stimulation. The adrenal carcinoma, even more than the adenoma, is likely to be autonomous. In the paraneoplastic syndrome, the adrenal gland is already under maximal stimulation by extraordinarily high concentrations of ectopic ACTH.

Overnight Metyrapone Challenge Test

This test measures the hypothalamic–pituitary feedback response to an acute inhibition of cortisol synthesis. The increase in 11-deoxycortisol following metyrapone also indicates normal adrenal gland function. Thus the metyrapone test assesses all levels of adrenal gland regulation. A normal response definitively rules out both Addison's disease and secondary adrenal insufficiency. A subnormal response requires that each level of the axis be tested, starting with the adrenal response to cortrosyn.

In the normal individual 30 mg/kg of metyrapone given at bedtime is associated with an acute increase in ACTH that elevates the 0800 h 11-deoxycortisol to greater than 10 μg/dl. This value is tenfold higher than the normal baseline of less than 1 μg/dl (30 nmol/L) (which need not be measured). The metyrapone test should be used cautiously, if at all, in patients suspected of having Addison's disease. A metyrapone-induced reduction in cortisol synthesis in an

Addisonian may produce acute adrenal insufficiency. Metyrapone is also contraindicated if a patient is in acute distress, under which circumstances the cortrosyn test with dexamethasone is indicated. The metyrapone test, like the Cortrosyn test, may be done in patients with Cushing's syndrome. The 11-deoxycortisol response for the various causes of Cushing's disease is compared to the cortrosyn responses in Table 144.3.

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