Adrenal Cortex

Lecture Goal(s): To introduce normal adrenal cortex function and to describe the normal effects of adrenal cortical hormones on the body. Pathologic conditions of the cortex will be discussed and laboratory evaluation of cortical function will be considered. Correlation of laboratory data with disease will be emphasized.

Lecture Objectives: Upon completion of this class material each student will be able to do the following:

1. Cog/I Provide the anatomical location of the adrenal gland and the distinct zones which make up to adrenal cortex. List the major hormone classes produced by each division of the cortex.

2. Cog/II Describe the importance of glucocorticosteriods (e.g., cortisol) during periods of stress.

3. Cog/II Describe the negative feedback regulation of glucocorticosteroid production.

4. Cog/II List and explain factors that can override the negative feedback regulation of cortisol production, such as circadian rhythm.

5. Cog/I Outline the synthesis of glucocorticosteroids noting the following points.
   a. the starting point
   b. the C-19 products
   c. the C-21 products
   d. zona glomerulosa specific enzymes
   e. zona fasiculata enzymes
   f. zona reticularis enzymes

6. Cog/I Recognize the chemical structure of the following.
   a. the cylcopentanoperhydrophenanthrene nucleus
   b. cholesterol
   c. cortisol
   d. 17-ketosteroids
   e. 17-hydroxycorticosteroids

7. Cog/I List the transport forms of cortisol once it is released into the blood stream. Note the physiologically active form of cortisol.

8. Cog/II Describe the mechanism of action of cortisol and the effects that result from its action.

9. Cog/II Describe the metabolism of cortisol.

10. Cog/II Describe the following disorders of the adrenal cortex.
    a. 1° and 2° Cushing's Syndrome
    b. hyperaldosteronism
    c. congenital adrenal hyperplasia
    d. hypoadrenalism (Addison's Disease)

11. Cog/II Describe the following laboratory evaluations of adrenal function.
    a. Porter-Silber Reaction
    b. Zimmerman Reaction
    c. plasma cortisol
    d. overnight dexamethasone suppression test
    e. 24-hour urine free cortisol
    f. low-dose dexamethasone suppression test
    g. plasma ACTH
    h. high-dose dexamethasone suppression test

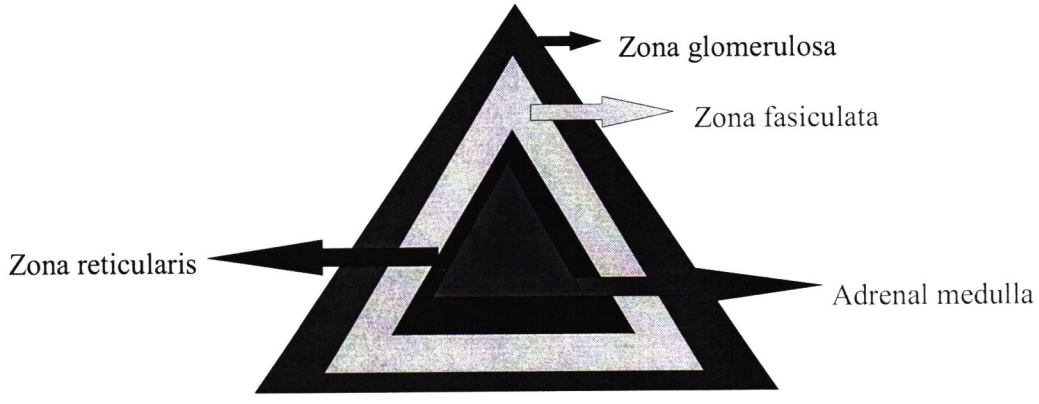
12. Cog/III Evaluate the results of the above laboratory tests (#11) in cases of the above disorders of the adrenal cortex (#10).

Reading: Suggested Reading- Chapter 46.

I. The Adrenal Gland - The adrenal gland sits on top of the kidney (one on each kidney) and it is roughly in the shape of a pyramid. It is divided into two functionally distinct areas:

A. the adrenal cortex, or the outer portion - The adrenal cortex is made up of distinct zones: (See Fig. 46-1, page 878 in Kaplan)
1. zona glomerulosa (outer)
2. zona fasiculata (middle)
3. zona reticularis (inner)

B. the adrenal medulla, or the inner portion.



C. Functions of Adrenal Cortex

1. The zona glomerulosa - produces mineralocorticosteroids (also known as mineralocorticoids) which regulate salt and water balance in the body, primarily affecting the kidney.
a. The primary mineralocorticoid of interest is aldosterone.

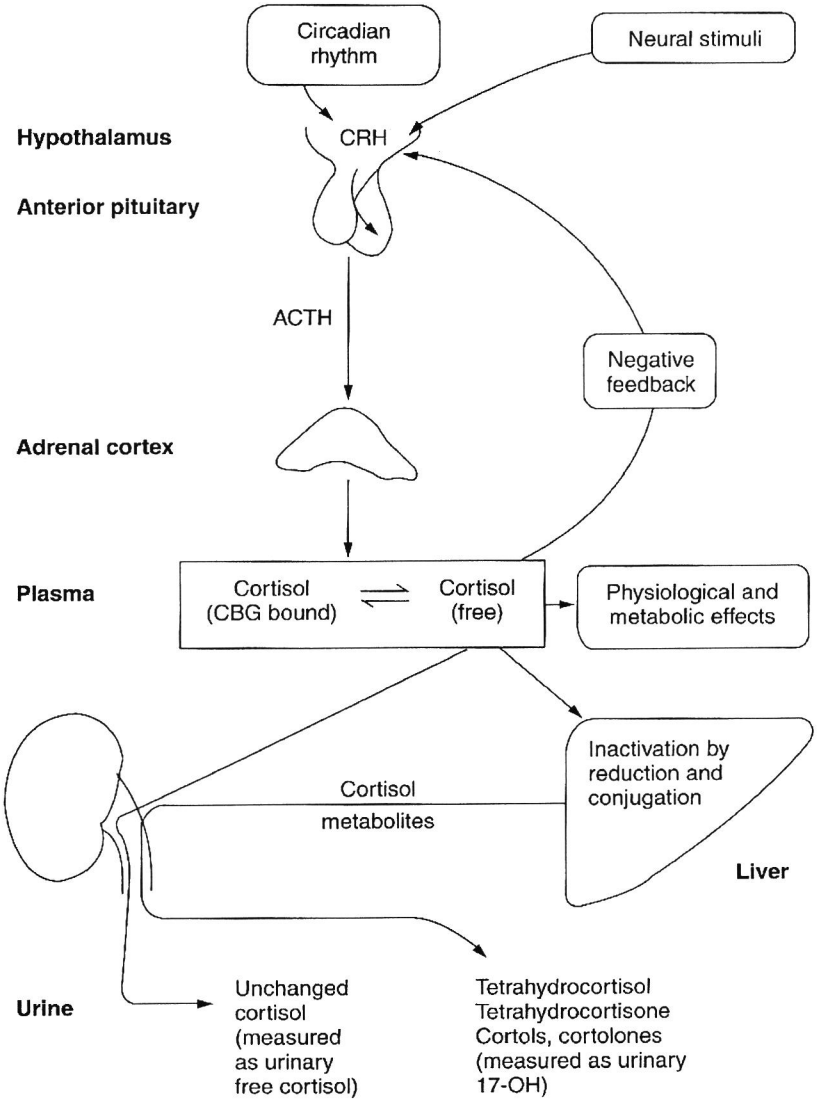
2. **The zona fasiculata and zona reticularis** - are both involved in the production of some sex hormones (i.e. some androgens and some estrogens) as well as the production of glucocorticosteroids (also known as glucocorticoids).

b. The major glucocorticoid is cortisol.

1) Glucocorticoids are essential for life, especially when the human body is subject to stress such as surgery, major illness, or severe trauma. Cortisol concentrations increase greatly during these stresses, with output of cortisol from the adrenal glands increasing from 25 mg/day to 200 to 300 mg/day. If steroids, such as cortisol, are not present in sufficient quantities in times of major stress, the patient may die of vascular collapse.

2) Large quantities of glucocorticoids also have anti-inflammatory and immunosuppressive properties.

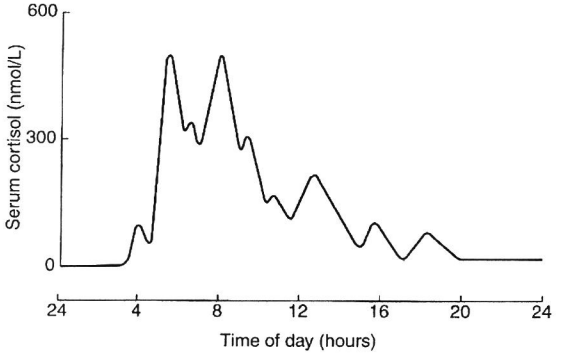
II. Control and Metabolism of Glucocorticoids and Mineralocorticoids



A. **Negative Feedback mechanism.** - In a similar manner to the thyroid gland,

1. The hypothalamus will release corticotropin releasing hormone (CRH) which will stimulate the adenohypophysis to release adrenocorticotropin hormone (ACTH).
2. ACTH will enter the blood stream and pass to the adrenal cortex where it will stimulate the production of glucocorticoids, such as cortisol.
3. Once cortisol levels become adequate in the blood stream, the free form of cortisol will then exert a negative feedback action on the adenohypophysis suppressing the release of additional ACTH.

B. **Overriding this system of negative feedback control** - are the higher centers of the brain, which establish the normal diurnal variation of cortisol. The diagram below illustrates the variation of serum cortisol concentration during a 24-hour period in a normal individual.



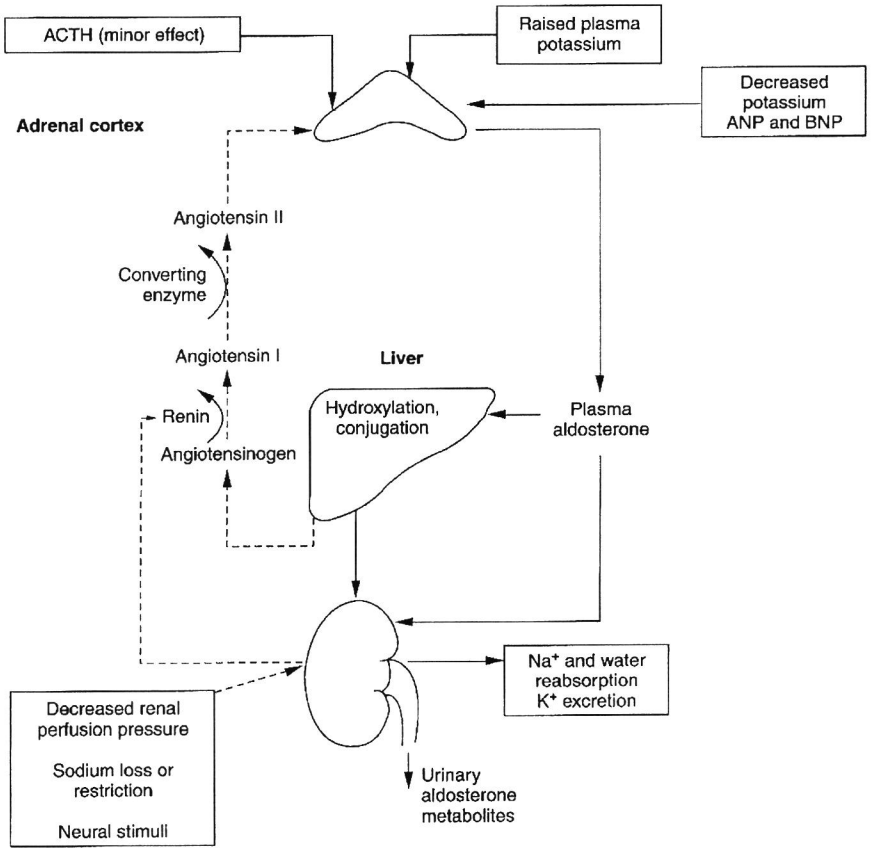
1. **This pattern is mainly affected by the sleep-wake cycle of an individual**. Serum cortisol levels are normally highest in the morning on waking and lowest in the late evening. . If the sleep-wake cycle is altered, several days are required for the pattern to change.
   a. The circadian pattern of ACTH release is controlled by CRH secretion from the hypothalamus. Short term release of cortisol is episodic, following the pattern of ACTH pulses by about 2 to 3 minutes.
2. **Stress** is another factor that can override the negative feedback of cortisol on ACTH release.
   a. Stress stimulates release of neurogenic amines (epinephrine and norepinephrine), which in turn stimulates the release of CRH.
   b. The inflammatory cytokines, tumor necrosis factor α, interleukin-1, and interleukin-6 also stimulate the release of ACTH. (These actions are better illustrated in the feedback system in Figure 46-6, page 885 in Kaplan.)
   c. ACTH levels can increase up to tenfold in times of stress, resulting in high levels of cortisol.
   d. Hypoglycemia, which is a form of chemical stress, can also increase CRH release, ultimately leading to an increase in cortisol. The effect is mediated by glucose receptors in the hypothalamus.

C. While glucocorticoids, in particular cortisol, are the main topic of this lecture, a few characteristics of mineralocorticoids, in particular aldosterone, will be reviewed.

1. Focusing on aldosterone regulation, in normal individuals, three main factors control the secretion of aldosterone from the zona glomerulosa:

a. The renin-angiotensin system
b. Potassium levels in the plasma
c. ACTH

2. Under normal circumstances, the renin-angiotensin system predominates. Also, recently described A-type and B-type natriuretic peptides (i.e., ANP and BNP) exert part of their physiological function through inhibition of aldosterone release. The diagram below illustrates normal aldosterone regulation.

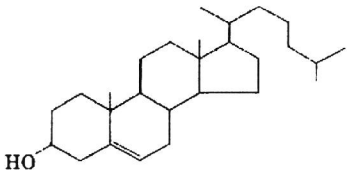


III. Synthesis of Adrenocortical Hormones

A. **All adrenocortical hormones are classified as steroids** - it is important to note that all of these hormones are derived from cholesterol (specifically LDL-cholesterol) and are therefore all classified as steroids.

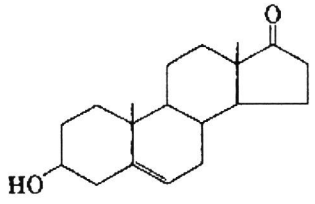
B. All adrenocortical hormones contain the cyclopentanoperhydrophenanthrene nucleus

C. There are two basic structural types of adrenocortical hormones. These are illustrated below beginning with the chemical structure of cholesterol.



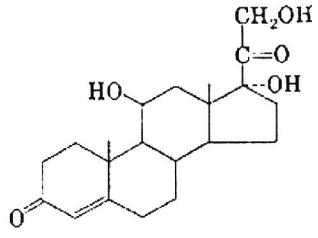
CHOLESTEROL

1. **The first structural type of adrenocortical hormones is known as a "C-19 steroid."** Most of the C-19 steroids have a keto group at position 17 in the cyclopentanoperhydrophenanthrene nucleus.
   a. **C-19 steroids are also known as 17-ketosteroids.** An example of C-19 steroids is dehydroepiandrosterone.



DEHYDROEPIANDROSTERONE

2. **The second structural type of adrenocortical hormones is known as a "C-21 steroid."** Most of the C-21 steroids have a hydroxy group at position 17 in the cyclopentanoperhydrophenanthrene nucleus.
   a. **Thus, C-21 steroids are also known as 17-hydroxy corticosteroids**. An example of C-21 steroids is cortisol.



CORTISOL

D. The diagram on the following page illustrates the synthetic pathway of the important adrenocortical steroids. You are not responsible for learning this pathway. As you review this pathway, note the following points:

1. Only the zona glomerulosa contains the 18-hydroxylase and 18-hydroxyl dehydrogenase which are necessary for the production of aldosterone. Therefore, only the glomerulosa produces aldosterone.

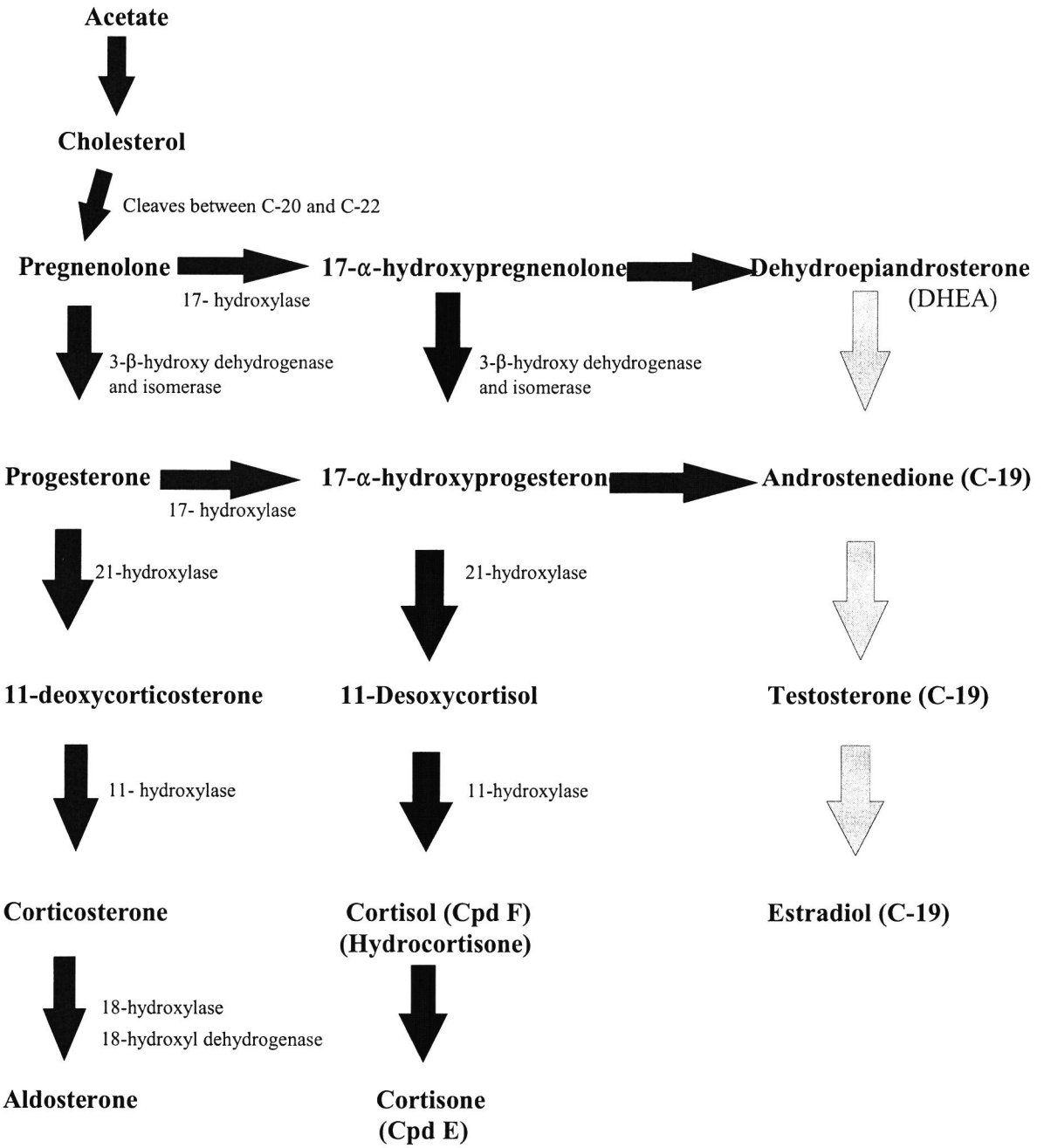
2. The zona fasiculata and reticularis contain the enzyme 17-α-hydroxylase. Therefore, both of these zones may produce cortisol. This is not found in the zona glomerulosa.

3. Pregnenolone and progesterone are considered important precursors in the synthesis of adrenocortical steroids and all pathways are possible only through one of these two precursors.

4. Pregnanolone and progesterone are important in the biosynthesis not only of glucocorticoids and mineralocorticoids, but also of androgens and estrogens. The androgens (male sex hormones) synthesized and secreted by the adrenal cortex are dehydroepiandrosterone, androstenedione, testosterone, and 11-hydroxyandrostenedione. Estrogens and progesterone (female sex hormones) are also secreted by the adrenal cortex. Therefore, patients with certain types of adrenocortical hyperfunction may secrete abnormal quantities of androgens and estrogens.

5. Correlate the names Compound F and Compound E with cortisol and cortisone, respectively.

Pathway For Synthesis of Steroids



IV. Cortisol - Returning to the focus on cortisol,

A. Once cortisol is secreted into the blood stream it is transported as follows:

1. Approximatley 90 percent of the cortisol is bound to corticosteroid-binding-globulin (CBG, also known as transcortin)
2. Approximately 5 percent is transported by albumin.
3. Approximately 4-5 percent is free. It is the free form that is the metabolically active form.

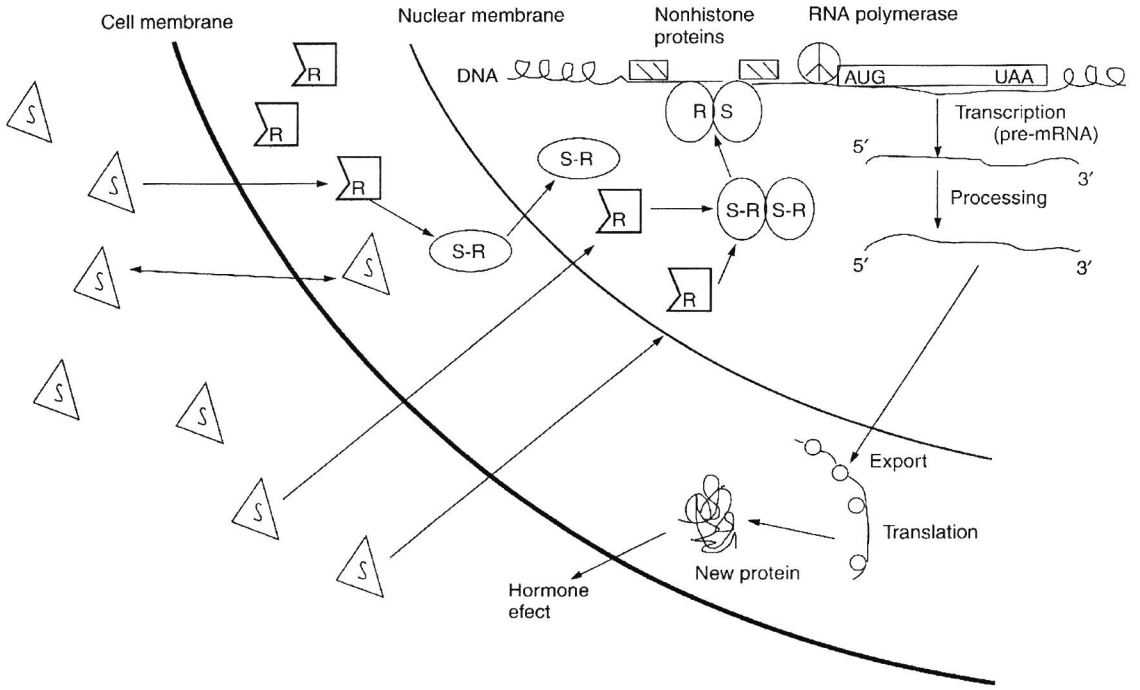
B. Cortisol and other steroid hormones act on target cells by penetrating the cell membrane. 1. Once inside the target cell, these hormones make their way to the nucleus. Here it binds to DNA and alters the transcription of RNA.

a. One of the results of this action occurs on metabolic processes. Here, an acceleration of the enzymatic breakdown of muscle proteins and conversion of their amino acids into glucose occurs. Also, fat in adipose tissue is mobilized for energy purposes.

b. Another effect is the uptake of glucose by muscle is inhibited which is why cortisol is referred to as an antagonist to insulin.

c. Yet another effect of cortisol is it reduces cellular reaction to inflammatory agents and lessens the immune response by inhibiting antibody formation.

The diagram below summarizes the action of cortisol and related steroid hormones.



Current proposed mechanism of action of steroid hormones (estrogens, androgens, progesterone, glucocorticoids, aldosterone). Steroids, *S*, diffuse across the plasma membrane and bind to a cytosolic protein receptor, *R*. Steroid binding activates the receptor complex, which then translocates to the nucleus of the cell, where it interacts with chromatin at a specific binding site on DNA called the *steroid-response element*. This binding activates the transcription of specific genes involved in steroid hormone action. Transcription of messenger RNA then takes place with the eventual synthesis of specific proteins by the cells that are linked to steroid hormone action.

C. **Cortisol is metabolized by the liver.** In considering the following process, please refer to the diagram on the previous page for the control and metabolism of glucocorticoids.

1. Cortisol metabolism begins with a reduction of the double bond between C4 and C5 producing dihydrocortisol. (The "dihydro" refers to the 2 hydrogen atoms added as the double bond was reduced.)
2. This is followed by the reduction of the ketone group at C3 producing tetrahydrocortisol. (Once again two hydrogen atoms were added in the reduction of the ketone. The total number of hydrogens added is now 4.) Tetrahydrocortisol is the major excretory metabolite of cortisol. Also, there may be some hydrogenation of the C20 ketone function of cortisol to form hexa-hydro products known as cortols.
3. These products are conjugated primarily with glucuronic acid for urinary excretion.
4. The same process occurs for cortisone (the oxidation product of cortisol).Also, there may be some free cortisol excreted unchanged in the urine.

V. Disease States Associated with the Adrenal Cortex

A. **Hyperadrenalism** - There are three basic conditions associated with hyperadrenalism, each of which may have more than one cause. These are Cushing's syndrome, resulting from excess cortisol production; hyperaldosteronism; and congenital adrenal hyperplasia.

1. **Cushing's Syndrome** - Cushing's syndrome is the term used to describe any condition resulting from an increased concentration of circulating glucocorticoid, usually cortisol.
   a. Excess cortisol can be caused by primary adrenal disorders (primary hyperadrenalism), for example, benign adrenal adenoma or carcinoma. (An adenoma refers to abnormal tissue growth that is primarily glandular epithelium and is not associated with malignancy. Carcinoma is associated with malignancy.) These are autonomous (i.e., not from external influences) secreting tumors that are independent of control by ACTH. The most common cause of hyperadrenalism in children is adrenal carcinoma.
   b. Another cause of excess cortisol may be bilateral adrenal hyperplasia resulting from autonomous secretion of ACTH by a benign pituitary adenoma (Cushing's disease) or from excess ectopic ACTH produced by a malignant tumor (secondary hyperadrenalism).
      1) The most common source of ectopic ACTH is bronchogenic carcinoma of the lung.
   c. The most common cause of Cushing's syndrome is iatrogenic, the chronic administration of exogenous glucocorticoid used in the treatment or management of a wide variety of disorders (such as cortisone shots).
   d. The most common noniatrogenic causes of hyperadrenalism are, in order:
      1) pituitary tumor (60%),
      2) ectopic ACTH (20%)
      3) adrenal adenoma and adrenal carcinoma (combined 20%).

4) The most common cause of hyperadrenalism in children is adrenal carcinoma. Adrenal carcinoma has particularly bad prognosis with a mean survival time following diagnosis of only 6 months.

B. **Hyperaldosteronism**. - Primary autonomous hypersecretion of aldosterone by the zona glomerulosa can be caused by two disorders:

1. an adrenal adenoma producing aldosterone
2. idiopathic hyperadrenalism caused by bilateral hyperplasia.
    a. The major clinical feature of these disorders is hypertension. The hypertension associated with primary aldosteronism can be explained by the actions of aldosterone that result in elevated plasma sodium levels and decreased plasma potassium levels.
3. Hyperaldosteronism may also result from secondary causes.
    a. In these situations the adrenal gland is not autonomously secreting aldosterone, but is responding to enhanced production and release of renin from the kidney which may be triggered by sodium loss, decreased renal perfusion, or volume depletion. In contrast to primary aldosteronism where renin is decreased, plasma renin is elevated in secondary aldosteronism.

C. **Adrenogenital Syndrome (Congenital Adrenal Hyperplasia)**. This syndrome results in a masculinization effect in women and children.

1. It is associated with the overproduction of adrenal steroids and it may be produced by a single enzyme defect.
2. Congenital adrenal hyperplasia or adrenogenital syndrome describes a group of inborn errors of metabolism involving deficiency of enzymes in the biosynthetic pathways leading to cortisol and aldosterone production. There are at least six distinct inheritable defects in this pathway,
    a. the most common of which is a 21-hydroxylase deficiency.
        1) These enzyme defects lead to diminished production of cortisol, which results in increased levels of ACTH.
        2) Increased ACTH in turn stimulates the adrenal gland. Referring back to the metabolic pathway considered above, increased stimulation of the adrenal gland coupled with a deficiency of enzymes such as 21 hydroxylase, the synthetic pathway will shunt over to steroid sex hormone production. The block is usually partial, and the patient may be capable of maintaining normal levels of cortisol and aldosterone under normal circumstances at the expense of accumulation of steroid precursors that are diverted down other metabolic pathways. This results in an increased production or excess of sex hormones and masculinization.

    b. The second most common enzyme deficiency associated with adrenogenital syndrome is a11-hydroxylase deficiency. Again, depending on the severity of the deficiency, cortisol production may or may not be adequate. A unique feature of this enzyme deficiency is the accumulation of 11-deoxycorticosterone, a precursor in the aldosterone pathway. This steroid promotes sodium reabsorption and therefore can cause hypertension. Again, one would see the excess production of sex hormones and masculinization.

c. The third most commonly encountered enzyme deficiency is an absence of 3-β hydroxy dehydrogenase and isomerase. Here, sex hormones are increased and there is no cortisol production.

In all of the deficiencies, there is a decreased cortisol. As a result, the feedback mechanisms are absent. Therefore, there is the increased production of ACTH which leads to the increased production of sex hormones in most cases.

Treatment is to give cortisol in order to stimulate the feedback mechanism.

D. **Hypoadrenalism** - Adrenal hypofunction or insufficiency can be caused by:

1. primary disease at the adrenal level involving the entire adrenal cortex;
2. secondary adrenal insufficiency caused by decreased levels of CRH or ACTH, a result of pituitary or hypothalamic disease;
3. long-term suppression of hypothalamic pituitary adrenal axis by glucocorticoids, which leads to adrenal atrophy.

4. Primary adrenal hypofunction or insufficiency - also known as Addison's disease, is a relatively rare (estimated prevalence 1:50,000).

a. The major cause is autoimmune adrenalitis with circulating adrenal antibodies. This disorder, accounting for 70% of Addison's disease, may be associated with other autoimmune disorders such as Hashimoto's thyroiditis, hypoparathyroidism, diabetes mellitus, pernicious anemia, and primary ovarian failure.

b. Other causes of primary adrenal failure include granulomatous diseases such as tuberculosis, histoplasmosis, or sarcidosis; hemorrhagic disorders; neoplasms; amyloidosis; hemochromatosis; infections; and surgery.

c. Symptoms of Addison's disease begin to appear after about 90% of the adrenal cortex has been destroyed. The disease usually develops slowly with progressive loss of cortisol and increasing ACTH levels, resulting in hyperpigmentation of the patient because of the melanocyte-stimulating hormone properties of ACTH. Because of coincident aldosterone deficiency, there is sodium loss and potassium retention. Hypoglycemia may be present because of cortisol deficiency. An Addisonian crisis is a result of an acute deficiency of both mineralocorticoids and glucocorticoids. An Addisonian crisis is likely to appear when a patient experiences a stressful situation such as illness, surgery, or trauma. Hyperkalemia and hyponatremia are common laboratory findings along with hemoconcentration and elevated urea levels resulting from fluid loss.

VI. Laboratory Tests for the Detection of Adrenal Hormones

A. Screening Tests

There are two urine screening tests that may be used in the evaluation of adrenocortical hormones. These tests differ by the type of adrenocortical steroid detected (i.e., C-21 steroids or C-19 steroids). These tests are typically run on 24-hour urine samples to indicate hormone levels throughout the day.

1. **Porter-Silber Reaction**. The Porter-Silber Reaction is used to measure C-21 steroids that contain a hydroxy group at C-17. Thus, the Porter-Silber Reaction is used to measure 17-hydroxycorticosteroids. Specifically this test measures cortisol, cortisone, and the tetra-hydro derivatives. An elevated test result is suggestive Cushing's syndrome and a decreased test result is suggestive of Addison's disease. The basic steps of the Porter-Silber Reaction include the following:

   a. Hydrolysis of the conjugates either by acid hydrolysis or the enzyme β-glucuronidase. This step must be timed very closely. If time is too short, the conjugates will not completely be broken. If the time is too long, the corticosteroid may break up.
   b. Extraction with chloroform (to isolate hormone and metabolite).
   c. Wash the extract with alkali to remove estrogens, bile acids, and interfering chromagens.
   d. Carry out a colorimetric reaction with an alcoholic phenyl-hydrazine-sulfuric acid reagent.
   e. A yellow color will be produced that is measured spectrophotometrically at 410 nm.

B. **Zimmermann Reaction**. The Zimmerman Reaction is used to measure C-19 steroids that contain a ketone group at C-17. Thus, the Zimmerman Reaction is used to measure 17-ketosteroids. The main purpose for measuring 17-ketosteroids is to assess adrenal androgen function. The bulk of urinary 17-ketosteroids arise from the adrenal gland. An elevated test result is suggestive Cushing's syndrome and a decreased test result is suggestive of Addison's disease. The basic steps of the Zimmermann Reaction include the following:

   1. Hydrolysis of the conjugates either by acid hydrolysis or the enzyme β-glucuronidase. This step must be timed very closely. If time is too short, the conjugates will not completely be broken. If the time is too long, the corticosteroid may break up.
   2. Extraction with methylene chloride (to isolate hormone and metabolite).
   3. Wash the extract with alkali to remove estrogens, bile acids, and interfering chromagens.
   4. Carry out a colorimetric reaction with meta-dinitrobenzene.
   5. A purple color will be produced that is measured spectrophotometrically at 520 nm.

It has been observed that the serum levels of many of these hormones can vary minute-by-minute depending on a large number of factors. Therefore, the level of the hormone the moment the blood is being drawn may not fairly represent the secretory activity of the adrenals. As a result, the two screening tests describe above may provide important information about adrenocortical hormone production.

B. Laboratory Tests for Hyperadrenalism - In the laboratory investigation of Cushing's syndrome, it must first be established that the patient actually has autonomous cortisol production. Once this is established, the laboratory investigation is used to differentiate the cause of the Cushing's syndrome.

1. **Cortisol**. Serum/Plasma cortisol levels are probably the first laboratory test performed in the evaluation of adrenocortical function. Cortisol values normally display diurnal variation with the highest levels occurring in the morning and the lowest levels in the early evening. Evening values are less than 50% of the early morning concentrations. Classically, samples are drawn at 8:00 a.m. (normal range 140 to 660 nmol/L) and 4:00 p.m. (normal range 50 - 330 nmol/L). Many patients with Cushing's syndrome will not show this diurnal variation and will have elevated concentrations at both times. However, the release of cortisol is episodic, and there is considerable overlap between normal patients and patients with Cushing's syndrome. Circadian variation is not always lost in Cushing's syndrome. To differentiate patients with Cushing's syndrome from normal patients it is best to take the sample at the time when cortisol is normally at its lowest concentration in the circulation. This time happens to be at midnight, and because it is not always practical to draw blood at this time, the more practical time of 8:00 p.m. is often chosen. Plasma cortisol levels greater than 420 nmol/L at this time are highly suggestive of hypercortisolism (i.e. primary hyperadrenalism or Cushing's syndrome).

2. **Overnight Dexamethasone Suppression Test**. Once autonomous cortisol production has been established, the second test that may be performed is the overnight dexamethasone suppression test. Dexamethasone is a synthetic glucocorticoid that is approximately 30 times as potent as cortisol. The patient is given a tablet containing 1 mg of dexamethasone and is instructed to take this at 11:00 p.m. and come to the laboratory for plasma cortisol determination at 8:00 a.m. the following morning. The action of dexamethasone is to suppress ACTH release from the adenohypophysis resulting in a decreased stimulation of the adrenal gland to produce cortisol. A morning cortisol level less than 140 nmol/L usually excludes any cause of primary hypercortisolism. This is a normal response (normal 8:00 a.m. cortisol range is 140 to 660 nmol/L). Levels greater than 280 nmol/L indicate primary hypercortisolism, (i.e., Cushing's syndrome) because the adrenal gland is producing cortisol without stimulation from ACTH. False positive results can be seen in patients with some forms of mental depression, stress-induced hypercortisolism, or pseudo-Cushing's syndrome resulting from chronic alcoholism. This test has a reported sensitivity of 98% For Cushing's syndrome. However, the specificity is not as good, but it is an ideal test to screen for Cushing's syndrome.

3. **24-hour urine Free Cortisol**. Another useful initial investigative test is the measurement of a 24-hour urine free cortisol level. This is in essence a direct measure of the amount of cortisol that is not bound to plasma protein that is excreted unmetabolized in the urine over a 24-hour period. This test also shows good sensitivity (95%) for Cushing's syndrome. A 24-hour urine cortisol requires a simultaneous urine creatinine determination to ensure the adequacy of collection

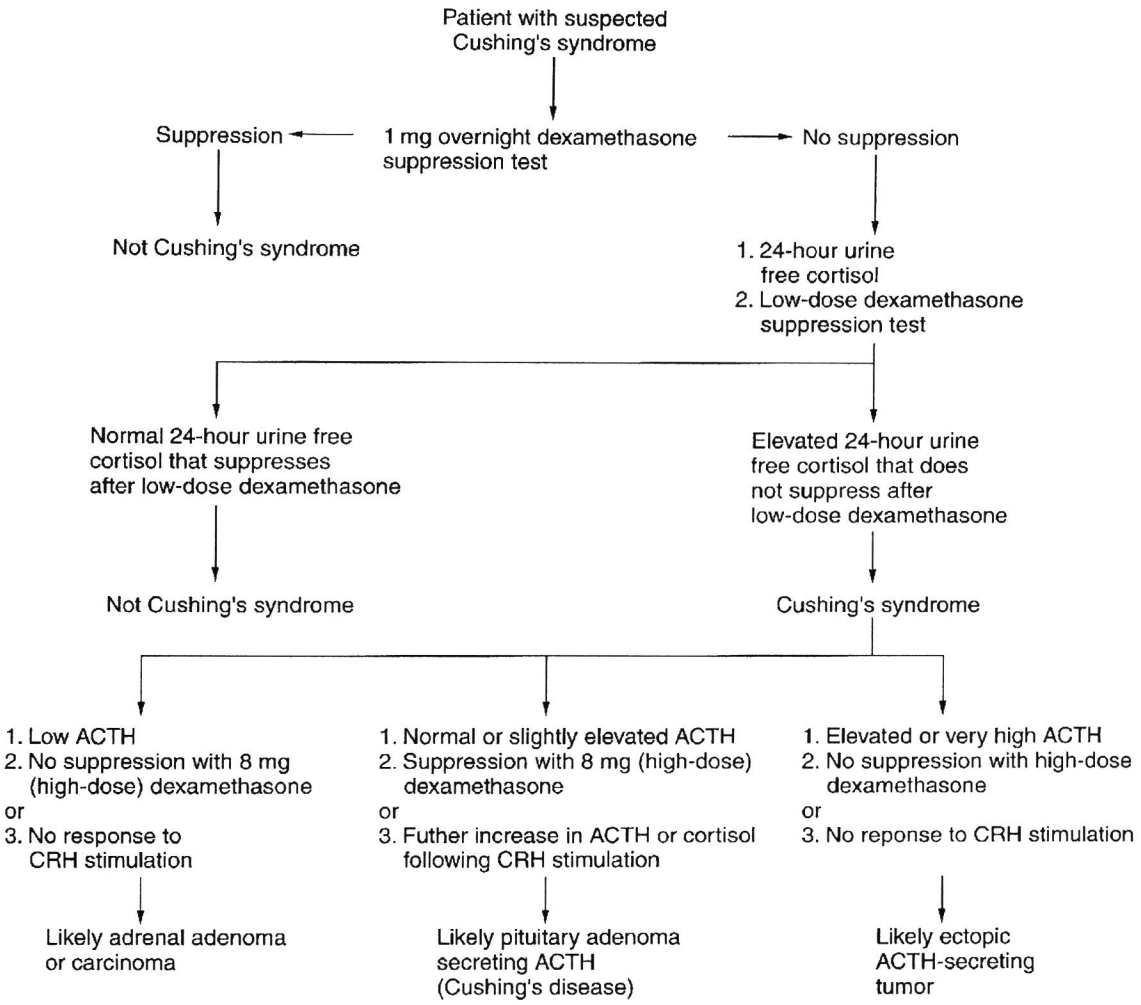
4. **Low-Dose Dexamethasone Suppression Test**. Another variation of the overnight dexamethasone suppression test is the low-dose dexamethasone suppression test, which can also be used to establish that the patient has Cushing's syndrome. However this test is more time consuming. The patient is given a total of 2 mg of dexamethasone per day for two days. The dose is given in 0.5 mg doses every 6 hours. During the second day, a 24-hour urine specimen is collected for urine-free cortisol. Plasma cortisol measurements may also be performed. Patients with Cushing's syndrome generally will not show significant suppression of cortisol output with this dose of dexamethasone. Normal patients should show greater than 50 percent suppression.

The above laboratory tests serve to detect hyperadrenalism and Cushing's syndrome. There are several possible approaches that can now be used to differentiate the cause of Cushing's syndrome.

5. **Plasma ACTH**. Plasma ACTH levels, which can be measured by radioimmunoassay, are very useful in differentiating the cause of Cushing's syndrome. ACTH is a labile polypeptide hormone, and special precautions are required in its handling. Plasma samples should be collected on ice and the plasma separated in a refrigerated centrifuge and stored frozen. ACTH levels in the upper normal range (normal range, 0 to 22 pmol/L) and up to twice the upper limit of normal are consistent with pituitary cause of Cushing's syndrome. Non-detectable levels of ACTH (less than 2 pmol/L) suggest an adrenal tumor. Very high levels of ACTH (greater than 50 pmol/L) are suggestive of an ectopic source such as a malignant tumor.

6. **High-Dose Dexamethasone Suppression Test**. A high-dose dexamethasone suppression test may also be useful in differentiating the cause of Cushing's syndrome. This can be done as an overnight procedure using 8 mg of dexamethasone, or it can be carried out over 2 days giving a total of 8 mg of dexamethasone per day divided into four aliquots of 2 mg every 6 hours. The response can be determined by measurements of plasma cortisol or 24-hour urine free cortisol. Patients who have pituitary tumors secreting excess ACTH (Cushing's disease) will show greater than 50% suppression of their glucocorticoid output following the high-dose dexamethasone suppression test. Pituitary tumors remain responsive to negative feedback, however, requiring higher levels of corticosteroid than normal. Patients with adrenal tumors or ectopic sources of ACTH will fail to show suppression of glucocorticoids. Anomalous responses do occur.

The following diagram illustrates the laboratory protocol for the investigation of Cushing's syndrome.


Patient with suspected Cushing's syndrome
1 mg overnight dexamethasone suppression test
Suppression
No suppression
Not Cushing's syndrome
1. 24-hour urine free cortisol
2. Low-dose dexamethasone suppression test
Normal 24-hour urine free cortisol that suppresses after low-dose dexamethasone
Elevated 24-hour urine free cortisol that does not suppress after low-dose dexamethasone
Not Cushing's syndrome
Cushing's syndrome
1. Low ACTH
2. No suppression with 8 mg (high-dose) dexamethasone
or
3. No response to CRH stimulation
Likely adrenal adenoma or carcinoma
1. Normal or slightly elevated ACTH
2. Suppression with 8 mg (high-dose) dexamethasone
or
3. Futher increase in ACTH or cortisol following CRH stimulation
Likely pituitary adenoma secreting ACTH (Cushing's disease)
1. Elevated or very high ACTH
2. No suppression with high-dose dexamethasone
or
3. No reponse to CRH stimulation
Likely ectopic ACTH-secreting tumor

C. **Laboratory Tests for Hypoadrenalism** - A patient with postural hypotension, low serum sodium, and increased serum potassium should suggest the possibility of primary adrenal insufficiency or Addison's disease. Samples should be drawn immediately for ACTH and baseline cortisol determinations. A synthetic form of ACTH (cortrosyn), consisting of the first 24 amino acids of ACTH, can then be injected intravenously or intramuscularly. Blood samples for serum cortisol should then be drawn at 30 to 60 minutes following injection. A normal response to the cortrosyn (ACTH) stimulation test consists of a rise of the serum cortisol by at least 280 nmol/L unless the baseline is already above 550 nmol/L. A low baseline cortisol result with a failure to respond to ACTH may suggest primary adrenal failure or may be a result of atrophy caused by long-term steroid therapy or pituitary insufficiency. In primary adrenal insufficiency the ACTH levels will be greatly elevated (greater than 50 pmol/L) and in fact may be clinically apparent because of pigmentation of the patient. In secondary adrenal insufficiency or atrophy resulting from exogenous steroids, the ACTH levels will be suppressed (less than 10 pmol/L).

The following diagram illustrates the laboratory protocol for the investigation of Addison's disease. Secondary adrenal insufficiency is unlikely to occur with hyperkalemia.