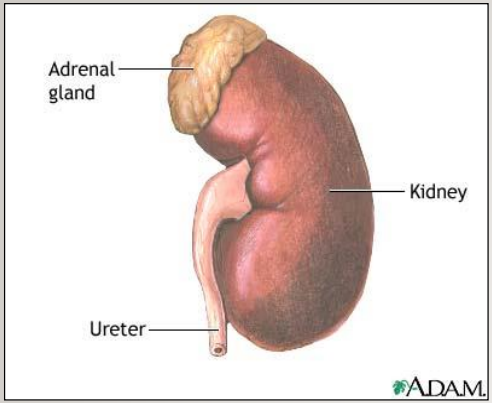
BIOCHEMISTRY OF CUSHING SYNDROME

ENDOCRINE BLOCK

OBJECTIVES

- To identify physiological and biochemical characteristics of Cortisol.
- To understand the diagnostic algorithm for Cushing's Syndrome.
- To understand the interpretation of laboratory and radiological investigations for diagnosis of Cushing's Syndrome.

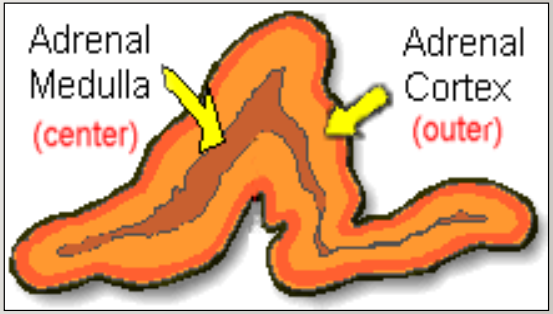


Anatomically:

The adrenal gland is situated on the anteriosuperior aspect of the kidney

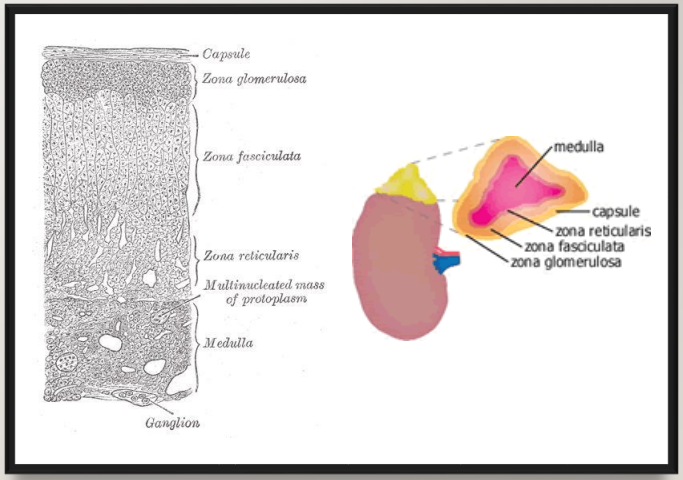
Histologically:

The adrenal gland consists of two distinct tissues of different embryological origin, the outer cortex and inner medulla.

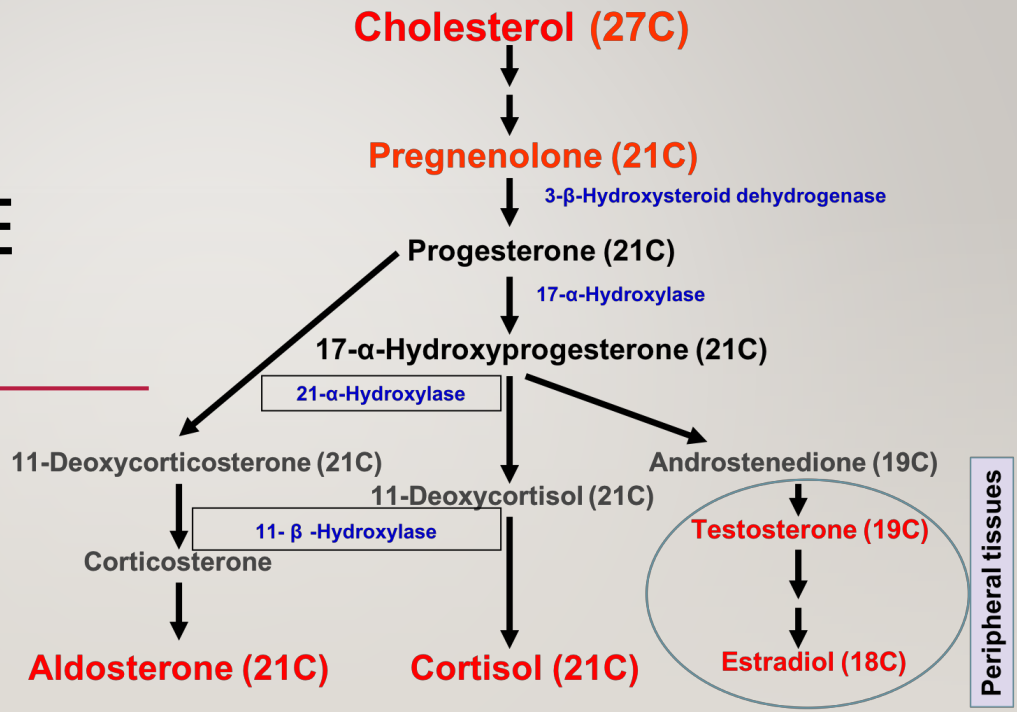


THE ADRENAL CORTEX

- Based on cell type and function, the adrenal cortex comprises three zones:
  - Zona **G**lomerulosa (the outmost zone) ➔ aldosterone, the principle mineralocorticoid.
  - Zona **F**asciculata ➔ glucocorticoids, mainly cortisol (95%).
  - Zona **R**eticularis ➔ sex hormones.



STEROID HORMONE SYNTHESIS



HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS

- The hypothalamus secretes corticotropin-releasing hormone (CRH) which stimulates the anterior pituitary gland to synthesis and release ACTH.

- ACTH acts on the zona fasciculata cells → release of glucocorticoids (*Cortisol*).

GLUCOCORTICOID FUNCTIONS

- Glucocorticoids have widespread metabolic effects on carbohydrate, fat and protein metabolism.
- Upon binding to its target, CORTISOL enhances metabolism in several ways:
  - In the liver, Cortisol is an insulin antagonist and has a weak mineralocorticoid action:
    - ↑↑ Gluconeogenesis → production of glucose from newly-released amino acids and lipids
    - ↑↑ Amino acid uptake and degradation
    - ↑↑ Ketogenesis.
  - In the adipose tissue: Cortisol → ↑↑ Lipolysis through breakdown of fat.
  - In the muscles: Cortisol →↑↑ proteolysis and amino acid release.
  - Conserving glucose: by inhibiting uptake into muscle and fat cells.

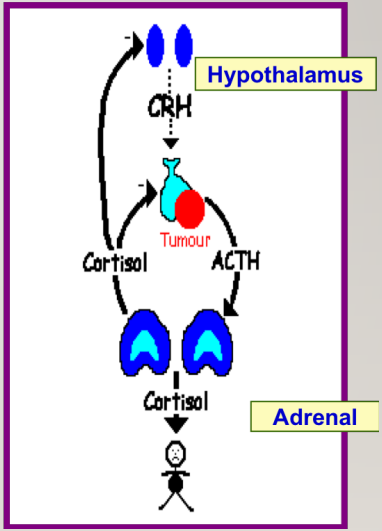
REGULATION OF ACTH AND CORTISOL SECRETION

1. Negative feedback control:

- ACTH release from the anterior pituitary is stimulated by hypothalamic secretion of corticotrophin releasing hormone (CRH).
- CRH → ↑ ACTH → ↑[Cortisol]
- ↑[Cortisol] or synthetic steroid suppress CRH & ACTH secretion

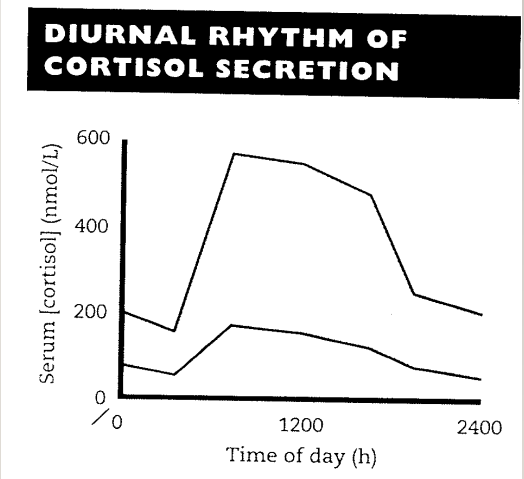
2. Stress:

(e.g. major surgery, emotional stress) Stress → ↑↑ CRH & ACTH → ↑↑ Cortisol



3. The diurnal rhythm of serum cortisol:

- Highest Cortisol level in the morning (8 - 9 AM).
- Lowest Cortisol level in the late afternoon and evening (8 - 9 PM).



The diurnal rhythm of cortisol secretion; the area between the curves represents values that lie within the reference range

PLASMA CORTISOL-BINDING GLOBULIN (CBG)

- In the circulation, glucocorticoids are mainly protein-bound (about 90%), chiefly to CBG (transcortin).
  - ↑↑ in pregnancy and with estrogen treatment (e.g. oral contraceptives).
  - ↓↓ in hypoproteinemic states (e.g. nephrotic syndrome).
- The biologically active fraction of cortisol in plasma is the free (unbound) component.

CORTISOL AND ACTH MEASUREMENTS

Serum (Cortisol) and plasma (ACTH):

- Samples must be collected (without venous stasis) between 8 a.m. and 9 a.m. and between 10 p.m. and 12 p.m. because of the diurnal rhythm.
- Temporary ↑↑ in these hormones may be observed as a response to emotional stress.

Urinary Cortisol excretion:

- Cortisol is removed from plasma by the liver → metabolically inactive compounds → excreted in urine mainly as conjugated metabolites (e.g. glucuronides).
- A small amount of cortisol is excreted unchanged in the urine (UFC).
- In normal individuals:
  - Urinary free cortisol (UFC) is < 250 nmol/24 h.

CAUSES OF ELEVATED SERUM CORTISOL

Increased cortisol secretion:

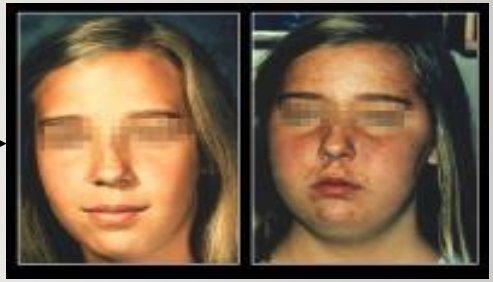
- Cushing's syndrome
- Exercise
- Stress, Anxiety, Depression
- Obesity
- Alcohol abuse
- Chronic renal failure

Increased CBG:

- Congenital
- Estrogen therapy
- Pregnancy

SYMPTOMS OF CUSHING'S SYNDROME

- Weight gain, central obesity.
- Buffalo's hump.
- Moon face
- Excessive sweating
- Atrophy of the skin and mucous membranes
- Purple striae on the trunk and legs
- Proximal muscle weakness (hips, shoulders)
- Hirsutism
- The excess cortisol may also affect other endocrine systems → ↓ libido, amenorrhoea and infertility
- Patients frequently suffer various psychological disturbances ranging from euphoria to frank psychosis.



SIGNS

- Loss of diurnal rhythm of cortisol and ACTH.
- Hypertension (due to the aldosterone - like effects)
- Hyperglycemia or diabetes due to insulin resistance.
- Hypokalemic alkalosis
- ↑ protein metabolism.
- Impaired immunity.

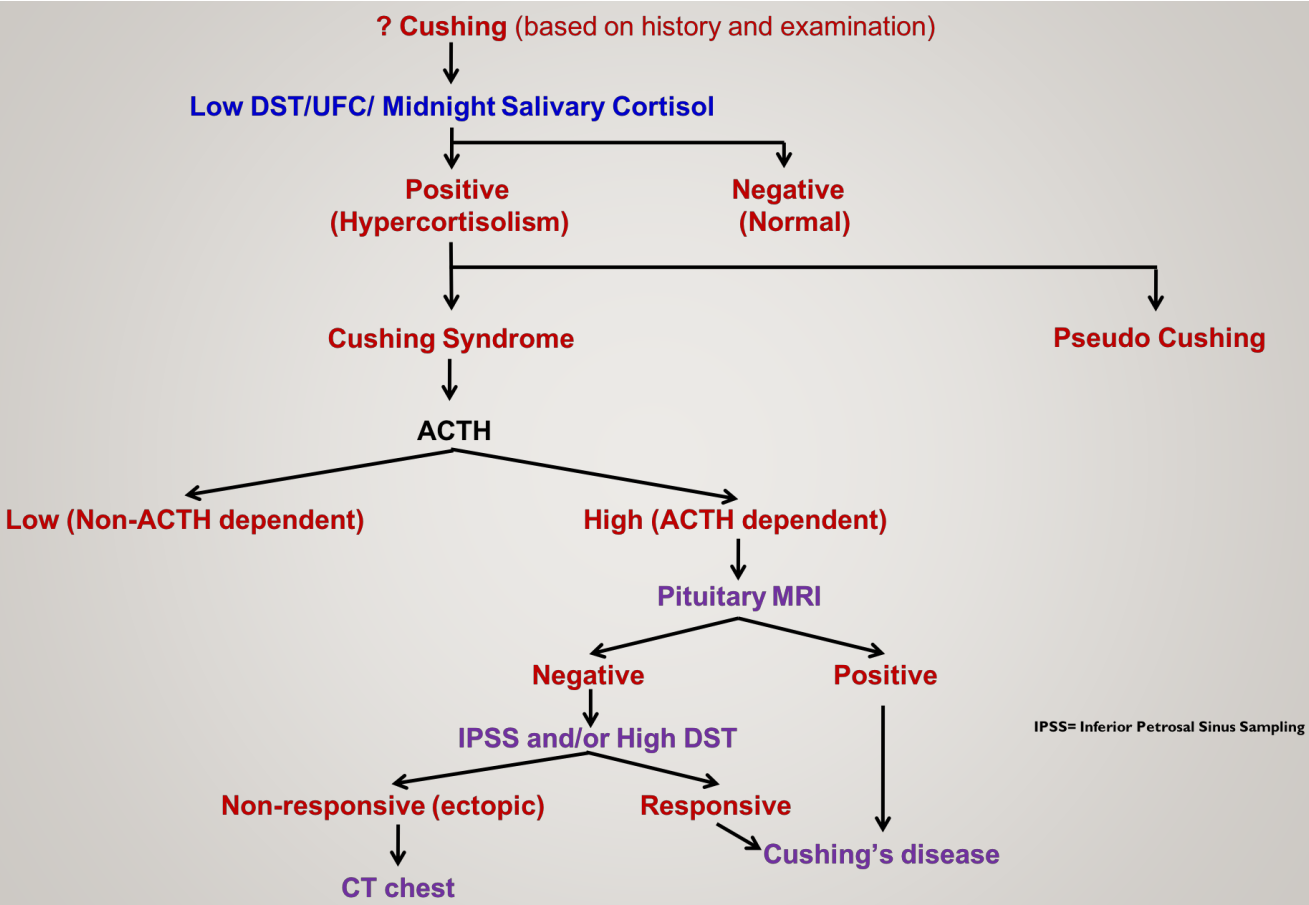
INVESTIGATIONS OF SUSPECTED ADRENOCORTICAL HYPERFUNCTION

A. Screening and confirmatory tests: to assess the clinical diagnosis of adrenocortical hyperfunction.

B. Tests to determine the cause: to ascertain:

(a) The site of the pathological lesion (adrenal cortex, pituitary or elsewhere?)

(b) The nature of the pathological lesion.


? Cushing (based on history and examination)
Low DST/UFC/ Midnight Salivary Cortisol
Positive (Hypercortisolism)
Negative (Normal)
Cushing Syndrome
Pseudo Cushing
ACTH
Low (Non-ACTH dependent)
High (ACTH dependent)
Pituitary MRI
Negative
Positive
IPSS and/or High DST
Non-responsive (ectopic)
Responsive
Cushing's disease
CT chest
IPSS= Inferior Petrosal Sinus Sampling

SCREENING TESTS

1. **Low-dose DST:**

Procedure:

1 mg dexamethasone (DXM) administered at 11-12 PM the night before attending the clinic. Serum cortisol is measured at 8-9 AM.

Result:

Cortisol < 50 nmol/L (suppression)→ exclude hypercortisolemia (Cushing Syndrome)

Precautions:

Drugs that induce hepatic microsomal enzymes (Phenobarbitone & phenytoin) → ↑ DXM metabolism and ↓ DXM blood level to achieve CRH suppression (false diagnosis of Cushing)

2. 24- hour urinary free cortisol:

Result: Cortisol < 250 nmol/day → exclude Cushing Syndrome.

Disadvantage: incomplete collection of urine → a false-negative result.

3. Midnight Salivary Cortisol.

Result: Cortisol < 100 ng/dL → exclude Cushing Syndrome.

CONFIRMATORY TESTS

- Positive results of at least two screening tests would confirm the clinical diagnosis.
- Further investigations are required.

TESTS USED TO DETERMINE THE CAUSE OF CUSHING'S SYNDROME

1. To differentiate ACTH-dependent from ACTH-independent:

   Plasma ACTH (Diurnal rhythm)

2. To distinguish between ACTH-dependent causes (Pitutary Vs Lung):
   a) High-dose DST.
   b) Inferior Petrosal Sinus Sampling.

3. Radiological tests: MRI of pituitary and ultrasound or CT of adrenals.

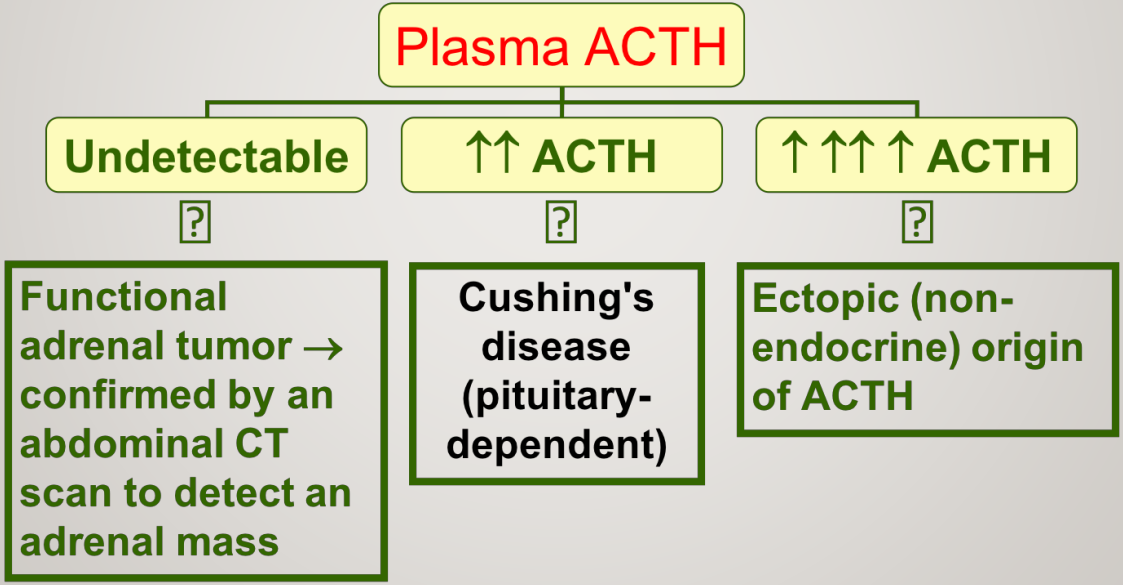
CAUSES OF ADRENOCORTICAL HYPERFUNCTION (CUSHING'S SYNDROME)

- **ACTH - dependent:**
  1. ↑ Pituitary ACTH >90% (Cushing's disease).
  2. Ectopic ACTH by neoplasms <10%.

- **ACTH - independent:**
  1. Glucocorticoid therapy.
  2. Adrenal tumor <20% (adenoma or carcinoma).

PLASMA ACTH

It should be measured on blood specimens collected at 8-9 a.m. and 8-9 p.m.



HIGH-DOSE DST

It is used to distinguish Cushing's disease from ectopic ACTH secretion.

- 2 mg DXM six-hourly for 48 hours to suppress cortisol secretion.

- Basal (pre-DXM) serum cortisol or 24-hour urine free cortisol is compared with the results at the end of the 48-hour period.
- Suppression is defined as a fall to less than 50 % of basal value.

- About 90 % of patients with Cushing's disease show suppression of cortisol output.

- In contrast, only 10% of patients with ectopic ACTH production (or with adrenal tumors) show suppression.

OTHER BLOOD TEST

The following blood tests are commonly performed for patients suspected to have Cushing's syndrome:

- Full blood count
- Blood glucose
- Blood electrolytes and pH
- Renal function tests
- Liver function tests

CASE STUDY

58 years old man was admitted with weight loss and respiratory distress. He had increased pigmentation and BP was 140/80.

Lab tests

Urea	8.6	(2.5-7 mmol/L)
Sodium	144	(135-145 mmol/L)
Potassium	2.0	(3.5-4.5 mmol/L)
Cortisol	1650	(150-550 nmol/L)
Post overnight DMX	1530	(<50nmol/L)

Further investigation revealed the following

DMX suppression test	Basal	after 48 h 0.5 mg qid	after 48h 2.0 mg qid	
Serum cortisol	1350	1420	1100	No suppression
	8 am	22.00 pm		
Plasma ACTH (ng/L)	220	180		Ref. range: 7-51

TAKE HOME MESSAGES

- Initial screening for Cushing by 24 h urine free cortisol, low-dose dexamethasone suppression test or midnight Salivary Cortisol.
- Confirmatory tests for Cushing by getting positive results of at least two of the screening tests.
- Tests to determine the cause of Cushing: Plasma ACTH, high-dose dexamethasone suppression test, Inferior Petrosal Sinus Sampling and radiological investigations.
- ACTH-dependent Cushing: due to pituitary causes (Cushing's disease) and due to ectopic production of ACTH.
- ACTH-independent Cushing: due to adrenal adenoma or carcinoma and due to steroid therapy (iatrogenic).

REFERENCES

- Lecture notes, Clinical Biochemistry, Wiley BlackWell, 9th edition, 2013, chapter 9, page 116-133.
- Clinical Chemistry, Principles, Procedures, Correlations, Lippincott Williams & Wilkins, 7th edition, 2013, chapter 21, page 453-471.
- Lippincott's Illustrated Reviews: Biochemistry 6th edition, Unit III, Chapter 18, Pages 219-244.
- https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/84225