Biochemistry Bandits 434

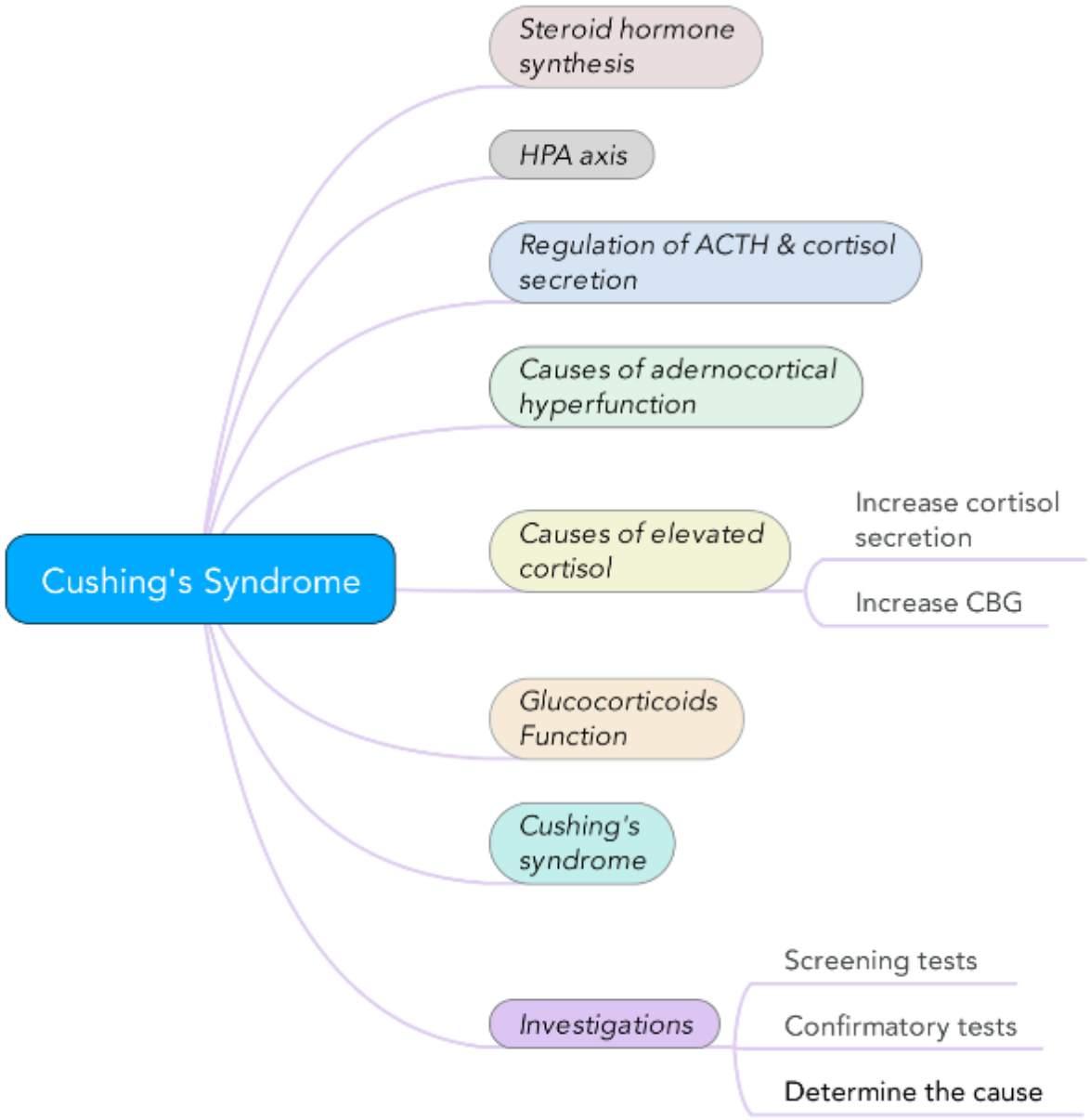
CUSHING'S SYNDROME

OBJECTIVES:

- To identify different causes of Cushing's syndrome
- To understand the diagnostic algorithm for Cushing's syndrome
- To understand the interpretation of laboratory and radiological tests of Cushing's syndrome

❖ **Important** ❖ Extra ❖ Biochemistry Edit

MIND MAP


Cushing's Syndrome
Steroid hormone synthesis
HPA axis
Regulation of ACTH & cortisol secretion
Causes of adernocortical hyperfunction
Causes of elevated cortisol
Increase cortisol secretion
Increase CBG
Glucocorticoids Function
Cushing's syndrome
Investigations
Screening tests
Confirmatory tests
Determine the cause

ANATOMY & HISTOLOGY

- The adrenal gland is situated on the **anteriosuperior** aspect of the kidney.
- The adrenal gland consists of two distinct tissues of different embryological origin, the **outer cortex** and **inner medulla**.

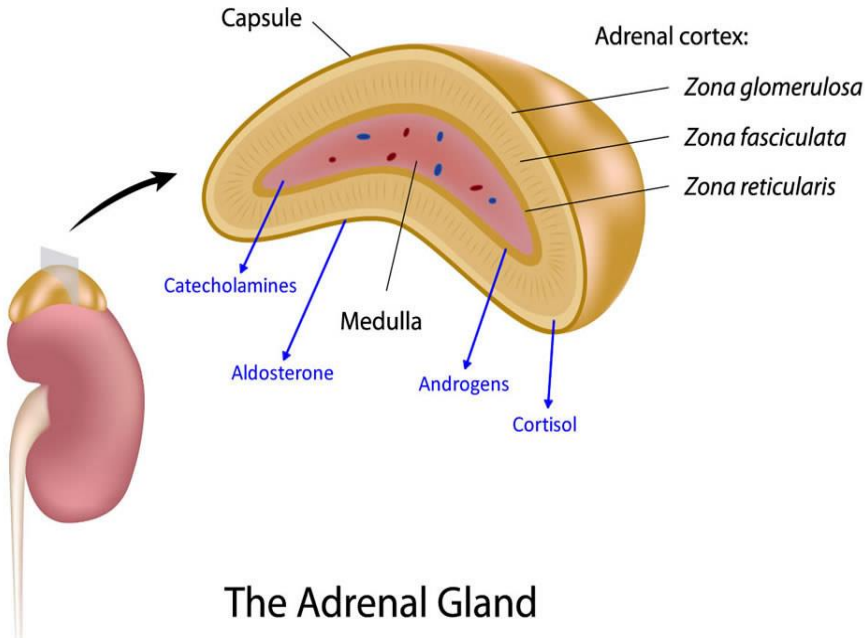
❖ **The adrenal cortex comprises three zones based on cell type and function:**

1. <u>Zona glomerulosa</u>
The outermost zone **aldosterone (**the principal mineralocorticoid**).**

➢ The deeper layers of the cortex**:**

2. <u>Zona fasciculata</u>
glucocorticoids – mainly cortisol (95%)

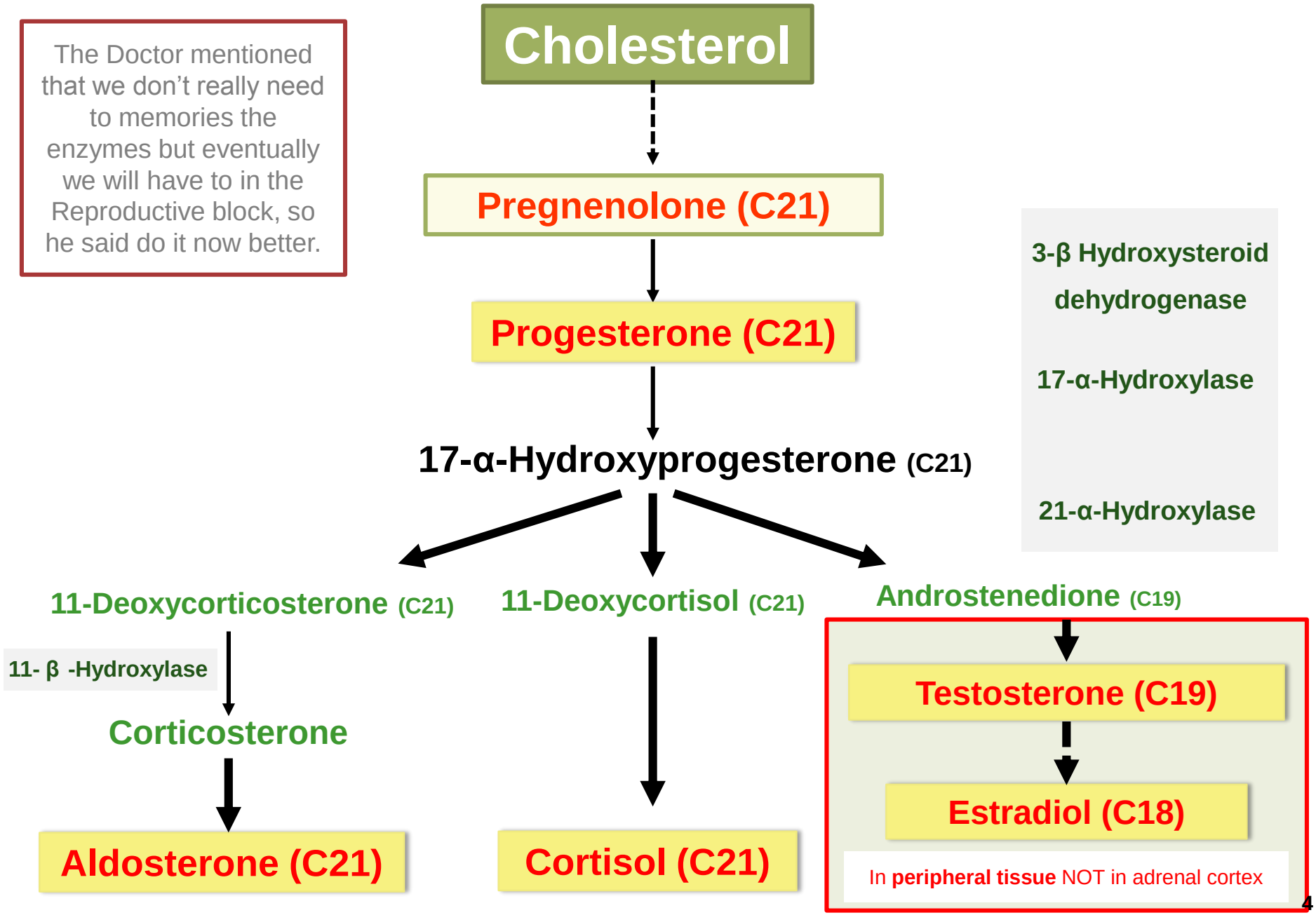
3. <u>Zona reticularis</u>
Sex hormones



The Adrenal Gland

STEROID HORMONE SYNTHESIS

The Doctor mentioned that we don't really need to memories the enzymes but eventually we will have to in the Reproductive block, so he said do it now better.



HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS

- The **hypothalamus** secretes **corticotrophin-releasing hormone** (**CRH**) which **stimulates** the anterior pituitary gland to **release ACTH**.

→

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

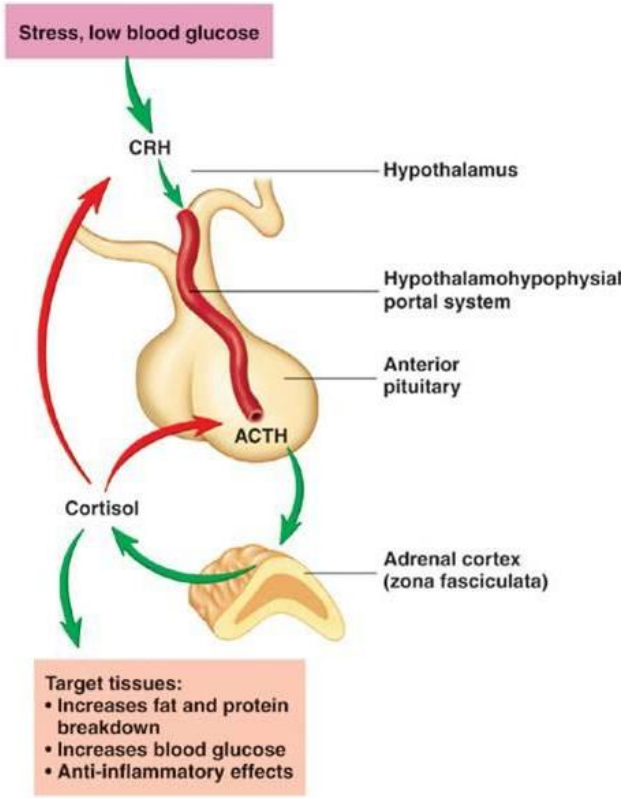
REGULATION of ACTH and Cortisol Secretion:

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[1] suppress CRH & ACTH secretion



1.The basis for dexamethasone suppression test (discussed later)

CONT.

2. Stress:

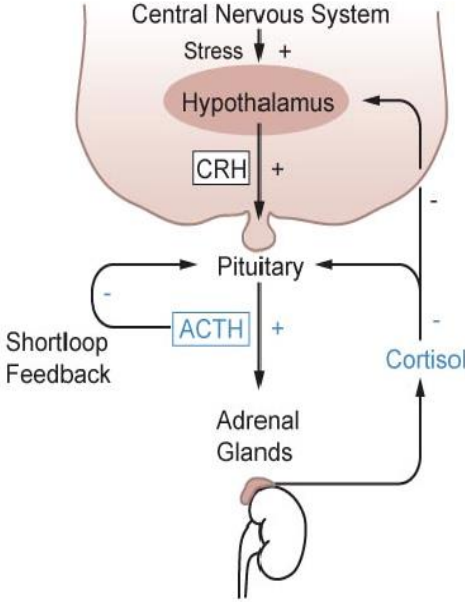
(e.g. major surgery, emotional stress)

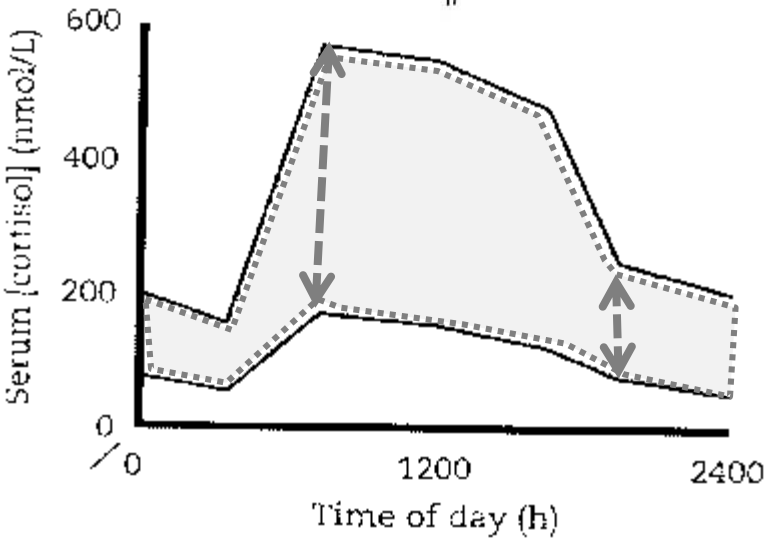
Stress → ↑↑ CRH & ACTH → ↑↑ Cortisol

3. The diurnal* rhythm of plasma 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

* Means the all day activity of cortisol specially beginning of morning and night

CORTISOL BINDING

Plasma [CBG] :

In the circulation, glucocorticoids are mainly protein-bound (about 90%), chiefly to cortisol-binding globulin (CBG or 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.

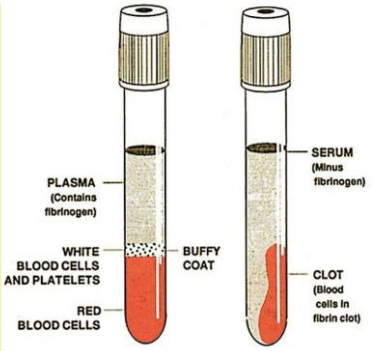
CORTISOL AND ACTH MEASUREMENTS

Serum [cortisol] and plasma [ACTH]:

Serum* → **cortisol**

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.



* Serum means collecting blood in empty tube but plasma in tube filled with anticoagulation.

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:

1. **Urinary free cortisol* (UFC):** is < 250 nmol/24 h.

2. **Cortisol / Creatinine ratio*:** in an early morning specimen of urine is < 25 µmol cortisol / mol creatinine.

- For the first test, it is difficult on patient to do b/c it requires 24h collecting urine, meanwhile in the second test it is easy and more frequently in-use b/c only one morning sample.

GLUCOCORTICOID FUNCTIONS

Glucocorticoids have widespread metabolic effects on carbohydrate, fat and protein metabolism.

Upon binding to its target, CORTISOL enhances metabolism in several ways:

Cortisol in love with liver

In the liver, Cortisol is an insulin antagonist and has a weak mineralocorticoid action:

- **Gluconeogenesis***
- **Amino acid uptake and degradation**
- **Ketogenesis**

Lipolysis through breakdown of fat

Proteolysis and amino acid release

Catabolic effect in muscles

Conserving glucose:

by inhibiting uptake into muscle and fat cells.

* production of glucose from newly-released amino acids and lipids

CAUSES OF ADRENOCORTICAL HYPERFUNCTION

CUSHING'S SYNDROME

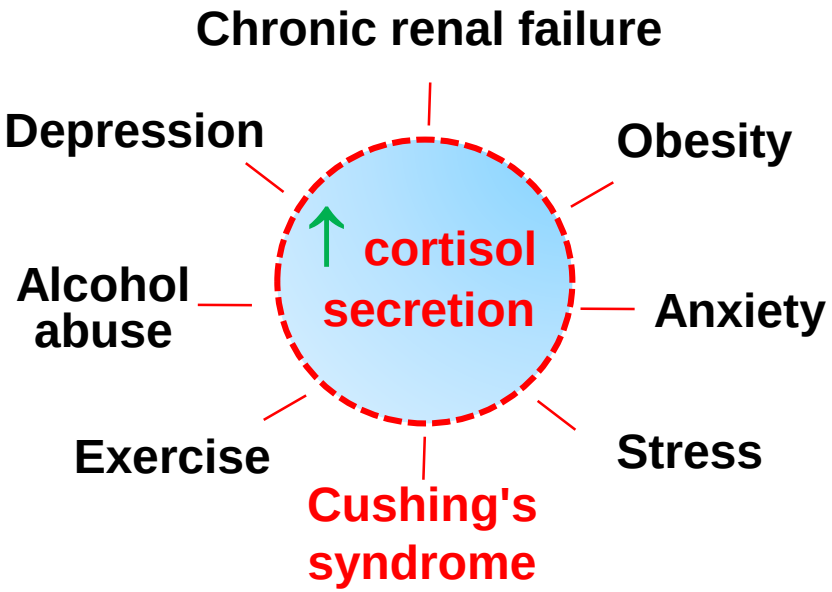
ACTH - dependent :

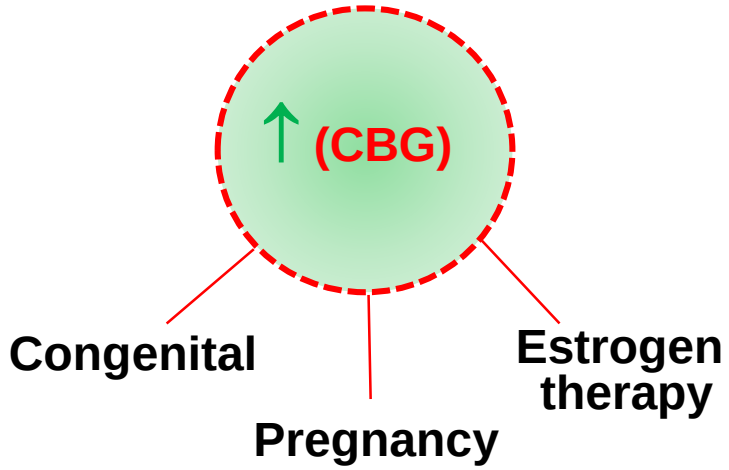
1. **Pituitary ACTH** 70% (Cushing's disease).
2. **Ectopic ACTH** by neoplasms 10%.
3. **ACTH therapy**.

ACTH - independent :

1. **Adrenal tumor** 20% (adenoma or carcinoma)
2. **Glucocorticoid therapy.**

❖ CAUSES OF ELEVATED SERUM CORTISOL CONCENTRATIONS:





CUSHING'S SYNDROME

SYMPTOMS

- Purple striae
- Proximal muscle weakness
- Hirsutism
- Weight gain — **trunk** and **face** with sparing of the limbs (central obesity)
- Moon face
- Buffalo's hump
- ↓ libido
- psychological disturbances — ranging from **euphoria** to frank **psychosis**.
- Excessive sweating
- Skin Atrophy
- amenorrhea
- infertility

(a)

(b)

SIGNS

- Hypo-kalemic (alkalosis)
- Loss of diurnal rhythm
- Hyper-tension
- ↑ protein metabolism
- Hyper-glycemia
- Impaired immunity

INVESTIGATIONS OF ADRENOCORTICAL HYPERFUNCTION

out-patient

Screening tests

to assess the clinical diagnosis of adrenocortical hyperfunction.

Low DXM/UFC

in-patient

Confirmatory tests

to confirm or exclude the provisional[1] diagnosis

Insulin hypoglycemia

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.

ACTH
High DXM
CRH Test
MRI pituitary
CT chest
ULS/CT adrenals

1. A provisional diagnosis is either the first considered diagnosis, or a subsequent diagnosis after the previous one has been found wanting.

INVESTIGATIONS OF CUSHING'S

Cushing ?

Screening — **Low DXM/UFC**

The aim of this test is to determine whether if there is hypercortisolism Or not

- **Pesudo-Cushing**
- **True Cushing**

Confirmatory — **Insulin hypoglycemia**

The aim of this test is to determine whether the hypercortisolism is due to Cushing or not
Insulin will induce hypoglycemia then we will wait for ACTH response to the hypoglycemia

Cause

- **Normal response**
  - **Alcoholism**
  - **Severe illness**
  - **Depression**
- **No response** → **ACTH/High DXM**
  - **ACTH-dependent** → **CRH Test**
    - **Pituitary** → **MRI pituitary**
    - **Ectopic** → **CT chest**
  - **Adrenal** → **ULS/CT adrenals**

If it is true Cushing then there will be no ACTH response b/c it is already elevated and can't be more than that, meanwhile if it isn't; that will elevate ACTH which is the normal response.

STEP ONE: SCREENING TESTS

- **Screening tests need to be sensitive but do not have to be highly specific.** (used to determine if there is hyperfunctioning regardless the cause)
- **out-patient procedure.**
- **E.g. distinguish simple non-endocrine obesity from obesity due to Cushing's syndrome.**

❖ **There are two screening tests:**

1. Low-dose dexamethasone suppression test: (Overnight suppression test)

Dexamethasone[1] → ↓ CRH → ↓ ACTH → ↓ cortisol (negative feedback)

- **Procedure:** One mg DXM administered at 11-12 PM the night before attending the clinic (that's why it's called Overnight suppression test), serum cortisol is measured at 8-9 AM.
- **Result:** Cortisol **< 50 nmol/L** (suppression)→ **exclude Cushing's disease**

- **Precautions:** DXM is metabolized by hepatic microsomal enzymes so if we induce those enzymes using drugs such as (Phenobarbitone & phenytoin) DXM metabolism will increase causing low DXM blood level to achieve CRH suppression. (false diagnosis of Cushing's)

2. 24-hour urinary free cortisol:

- **Result:** Cortisol **< 250 nmol/day** → **exclude Cushing's disease**.

- **Disadvantage:** incomplete collection of urine → a false-negative result. (it's hard for the patient to carry his urine collection bottle all day)
  - ✓ **An alternative to avoid this disadvantage is to determine the urinary cortisol: creatinine ratio on an early morning urine specimen.**

1) A Synthetic Glucocorticoids

STEP TWO: CONFIRMATORY TEST

Insulin-induced hypoglycemia test:

- ✓ **Used to test the integrity of the hypothalamic-pituitary-adrenal (HPA) axis, Hypoglycemia →↑ CRH →↑ ACTH →↑ cortisol**
- ✓ **To distinguish true Cushing's syndrome from pseudo-Cushing's syndrome, (to rule out pseudo-Cushing's syndrome)**
- ✓ **In-patient procedure.**
- ✓ **Contraindicated in: epilepsy or heart disease.** (hypoglycemia can induce shock)

➢ **Procedure:** Insulin I.V. (0.15 U/kg) to lower blood glucose to 2.2 mmol/L or less .

Samples for simultaneous measurement of serum glucose and cortisol levels are taken basally (**before insulin injection**) and at 30, 45, 60 and 90 min after I.V. insulin injection.

➢ **Failure to achieve a glucose level of 2.2 mmol/L invalidates the test and should be repeated with increment in step of 0.05U/kg** "i.e. we must lower the blood glucose until it reaches 2.2 mmol/L or less"

EXs of Pseudo-Cushing's syndrome causes:

- **Depressed or extremely anxious patients**
- **Severe intercurrent illness**
- **Alcoholism**

STEP TWO: CONFIRMATORY TEST

normal	• Basal serum cortisol: **at least 145 nmol/L** • At 60 - 90 minutes: **the level > 425 nmol/L**
Pseudo-Cushing	• **show abnormal diurnal rhythm of S. cortisol**, but with Insulin-induced hypoglycemia → ↑ **CRH, ACTH** and **cortisol** blood levels
Cushing's syndrome	• **do not respond normally to insulin-induced hypoglycemia**. • **High basal serum cortisol than normal** . • At 60 - 90 minutes: **no increase in S. cortisol**, despite the production of an adequate degree of hypoglycemia. (do not show any increase because cortisol is already secreted at maximum rate)

STEP THREE: TESTS USED TO DETERMINE THE CAUSE OF CUSHING'S SYNDROME

1. **Plasma ACTH. (Diurnal rhythm)**
2. **High-dose dexamethasone suppression test.**
3. **CRH stimulation test.**
4. **Radiological tests: MRI of pituitary and ultrasound or CT of adrenals.**

At this step we already know it's Cushing's syndrome.

STEP THREE: TESTS USED TO DETERMINE THE CAUSE OF CUSHING'S SYNDROME

1. Plasma ACTH (Diurnal rhythm):

- **Procedure**: Plasma [ACTH] should be measured on blood specimens collected at 8-9 a.m. and 8-9 p.m..
- **Results:**
  - Undetectable ACTH: Functional adrenal tumor (ACTH-independent) → confirmed by an abdominal CT scan to detect an adrenal mass.
  - High ACTH: Cushing's disease. (pituitary dependent) (ACTH-dependent)
  - Extremely high ACTH: Ectopic (non-endocrine) origin of ACTH. (ACTH-dependent)

2. High-dose dexamethasone suppression test:

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

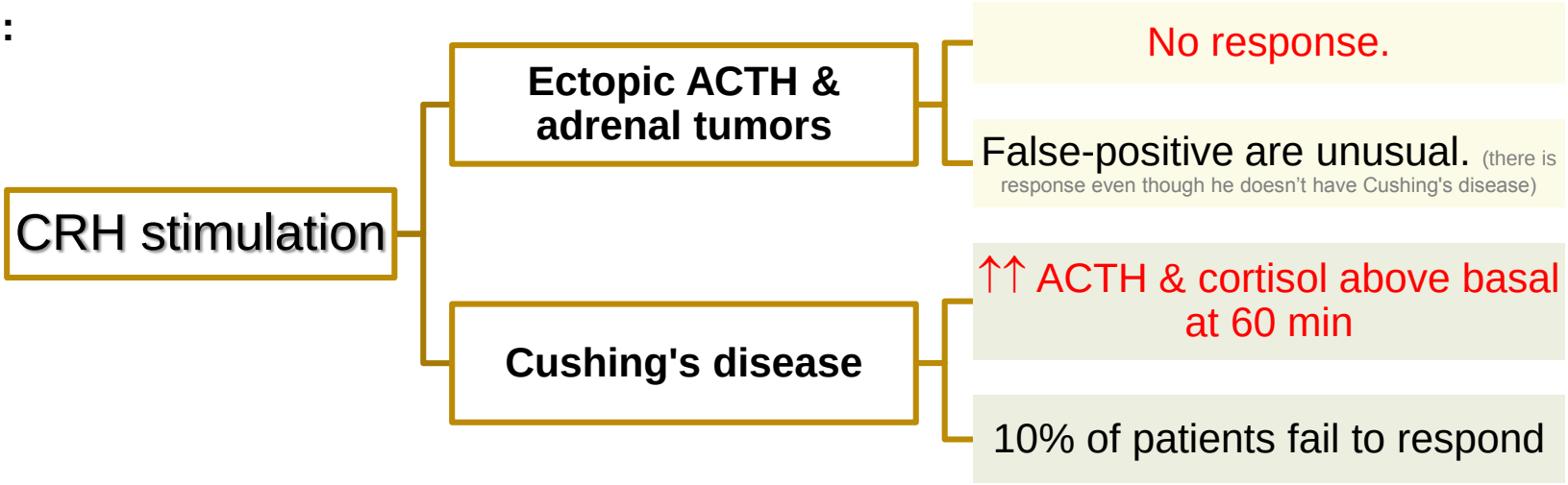
- **Procedure:** 2 mg dexamethasone six-hourly for 48 hours to suppress cortisol secretion. Basal (pre-dexamethasone) serum cortisol or 24-hour urine free cortisol is compared with the results at the end of the 48-hour period.
- **Results:**
  - Suppression is defined as a fall to less than 50% of basal 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.

STEP THREE: TESTS USED TO DETERMINE THE CAUSE OF CUSHING'S SYNDROME

3. CRH stimulation test:

Procedure: Measures the **ACTH** and **cortisol** levels basally and 60 minutes after injection of 100 µg **CRH**.

Results:



4. Radiological Investigations:

- **Ultrasound or CT scanning of the adrenal glands.**
- **MRI of the pituitary gland. (contrast-enhanced)**

❖ Other blood tests commonly performed for patients suspected to have Cushing's syndrome:

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

In Cushing's disease:

High-dose **dexamethasone suppression test** + the **CRH** test → 100 % specificity and sensitivity.

SUMMARY

Test	Pseudo-Cushing	Cushing's disease	Adrenal tumor	Ectopic ACTH secreting tumor
S. cortisol	↑	↑	↑	↑
Dexamethasone Low dose test	Not suppressed	Not suppressed	Not suppressed	Not suppressed
Urinary cortisol	↑	↑	↑	↑
Diurnal rhythm	Lost	Lost	Lost	Lost
Insulin-induced hypoglycemia	Will ↑ CRH, ACTH and cortisol blood levels	No response	No response	No response
Plasma [ACTH]	-	Normal or ↑	Not detectable	↑↑↑
Dexamethasone High dose test	-	suppressed	Not suppressed	Not suppressed
CRH test	-	↑	No response	No response

SUMMARY

Investigations	Tests	Notes
Screening tests **(out-patient)**	Low dose of DOX	Administered at night and measure it at morning < 50 nmol/L
	24 h urine sample	• Cortisol <250 nmol/L • Alternative way is urinary cortisol:creatinine ratio
Confirmatory tests **(in-patient)**	Insulin-induced hypoglycemia	• Distinguish between Cushing's syndrome & Pseudo-Cushing's syndrome. • C.I. in Epilepsy & heart disease. • Normal → increase in serum cortisol. • Cushing's syndrome → no respond.
Determine the cause of Cushing's syndrome	Plasma ACTH	• Adrenal tumor → Undetectable. • Cushing disease → little increase. • Ectopic ACTH tumor → high increase.
	High-dose of DOX	• Cushing disease → Suppression of cortisol. • Ectopic ACTH tumor → no respond.
	CRH stimulation test	• Cushing's disease → increased. • Ectopic ACTH tumor → no respond.
	Radiological tests	Ultrasound or CT-scan of MRI

MCQs & SAQs

1) **Testosterone is a hormone derive it from?**
   - A. Androstenedione.
   - B. 11-Deoxycortisol.
   - C. 11-Deoxycorticosterone.

2) **Distinguish non-endocrine obesity from obesity due to Cushing's syndrome, is define ?**
   - A. Confirmatory tests.
   - B. Screening tests.
   - C. Urinary cortisol: creatinine ratio .
   - D. B & C

3) **Screening tests should be?**
   - A. Sensitive.
   - B. Specific.
   - C. Both.

4) **Insulin-induced hypoglycemia**
   - A. To distinguish Cushing's syndrome from pseudo-cushing syndrome.
   - B. Contraindicated in epilepsy & heart disease.
   - C. Should lower blood glucose to 2.2 mmol/L.
   - D. All of them.

5) **Which of the followings is used to distinguish Cushing's disease from Ectopic ACTH secretion?**
   - A. Insulin-inducer hypoglycemia.
   - B. High dose DOX .
   - C. Plasma ACTH.
   - D. Non of them .

1.A 2.D 3.A 4.D 5.B

❖ **Define each layer of adrenal cortex ? and it's manly secretion ?**

Zona glomerlosa → Aldosterone, Zona fasciculata → Cortisol, Zona reticularis → sex hermone.

❖ **Define diurnal rhythm of plasma cortisol ?**

Mean during the day and it's:

highest cortisol level → morning (8-9 AM).
lowest cortisol level → late afternoon (8-9PM)

❖ **Mention the causes for elevated cortisol level ?**

✓ Increased cortisol secretion .. [Cushing's syndrome, exercise, stress, anxiety, obesity …]

✓ Increased (CBG) .. [Congenital, Estrogen therapy, Pregnancy]

Biochemistry Bandits 434

اللهم إني استودعتك ما قرأت وما حفظت وما تعلمت فرده لي عند حاجتي إليه إنك على كل شيء قدير

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