Biochemistry of Cushing Syndrome

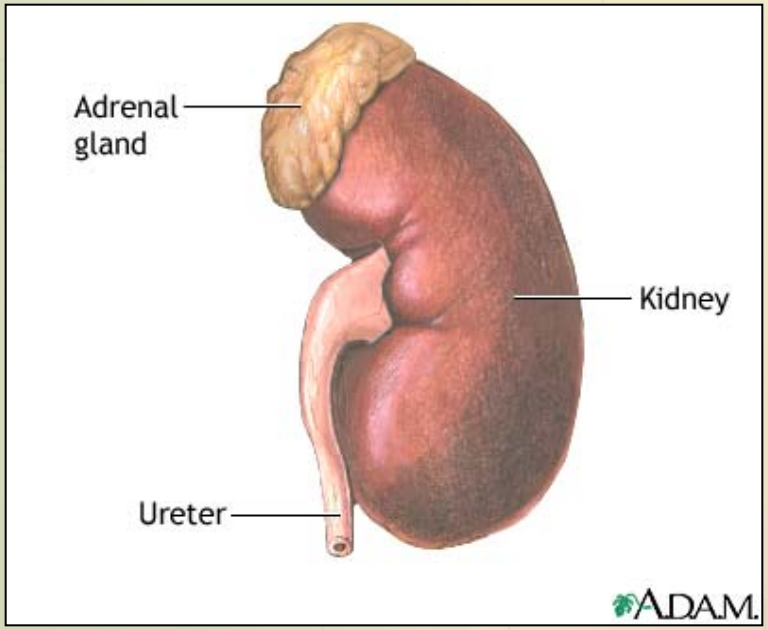
Endocrine Block

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
- To identify the importance of radiological investigations for diagnosis of Cushing's syndrome.

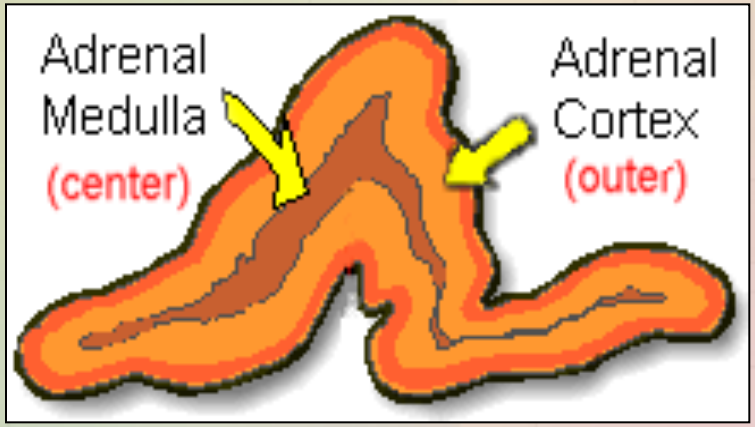
ANATOMICALLY:

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



HISTOLOGICALLY:

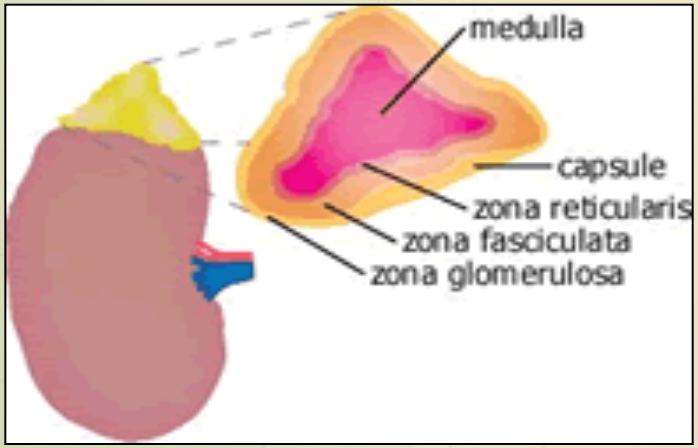
- The adrenal gland consists of two distinct tissues of different embryological origin, the outer cortex and inner medulla.



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

- **Zona Glomerulosa**

  The outermost zone → aldosterone (the principal mineralocorticoid).



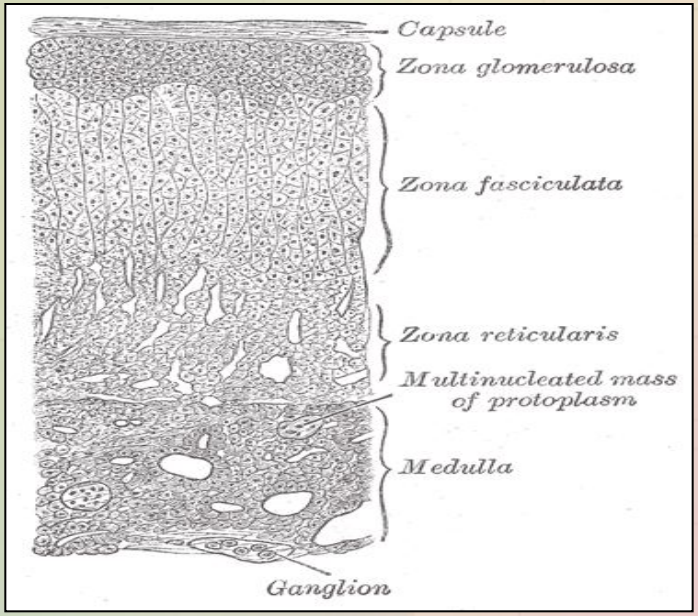
The deeper layers of the cortex:

- **Zona Fasciculata**

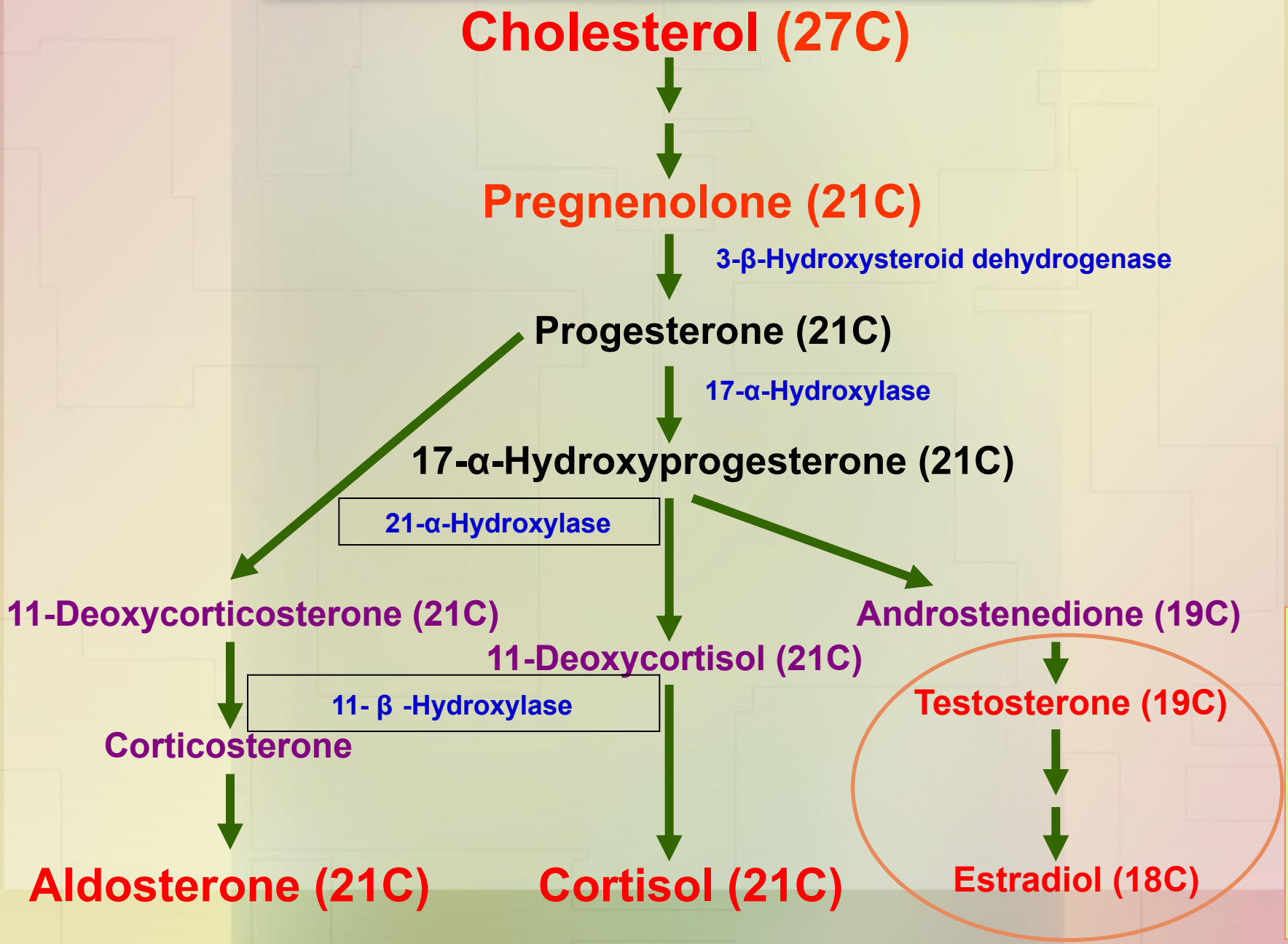
→ glucocorticoids – mainly cortisol (95%)

- **Zona Reticularis**

→ Sex hormones



Steroid Hormone Synthesis



Hypothalamic-Pituitary-Adrenal (HPA) Axis

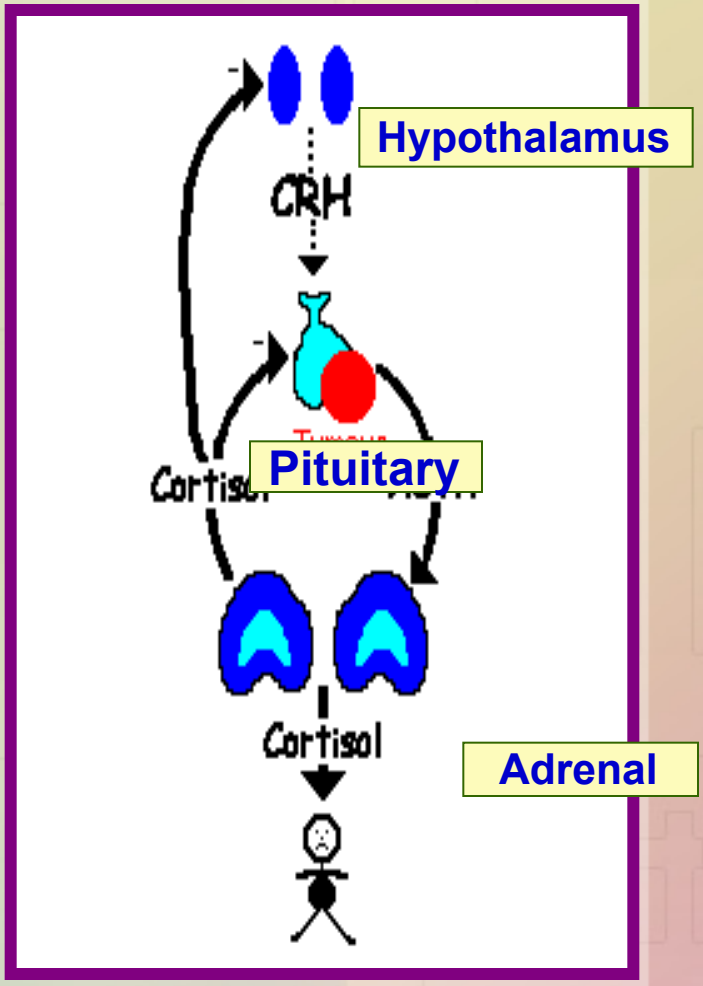
- The hypothalamus secretes corticotropin-releasing hormone (CRH) which stimulates the anterior pituitary gland to synthesis and release ACTH.

- ACTH acts on the zona 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 suppress CRH & ACTH secretion

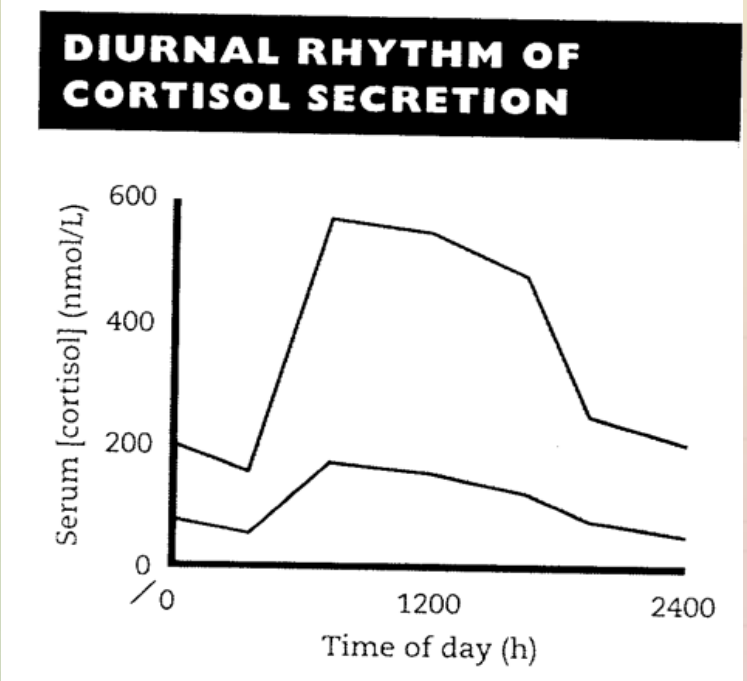


2. Stress (e.g. major surgery, emotional stress)

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

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 measurement is preferred for cortisol and Plasma for 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.
  - Cortisol / Creatinine ratio in an early morning specimen of urine is < 25 µmol cortisol / mol creatinine.

CAUSES OF ADRENOCORTICAL HYPERFUNCTION: CUSHING'S SYNDROME

- **ACTH - dependent :**

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

- **ACTH - independent :**

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

Causes of elevated serum cortisol concentrations:

1. Increased cortisol secretion:

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

2. Increased cortisol binding globulin (CBG):

- Congenital
- Estrogen therapy
- Pregnancy

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 .

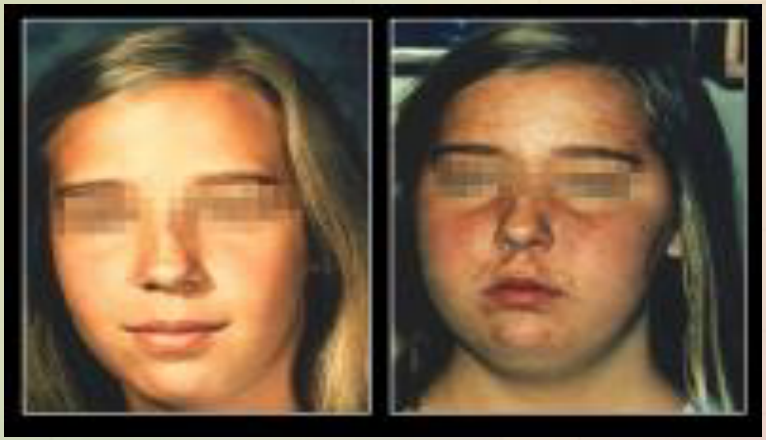
- In the muscles: Cortisol →↑↑ proteolysis and amino acid release.

- Conserving glucose: by inhibiting uptake into muscle and fat cells.

Cushing's Syndrome

Symptoms:

- **Weight gain:**
  trunk and face with sparing of the limbs (central obesity)
- **Buffalo's hump.**
- **Moon face**
- **Excessive sweating**



Symptoms .... (contd)

- Atrophy of the skin and mucous membranes
- Purple striae on the trunk and legs
- Proximal muscle weakness (hips, shoulders)
- Hirsuitism
- 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

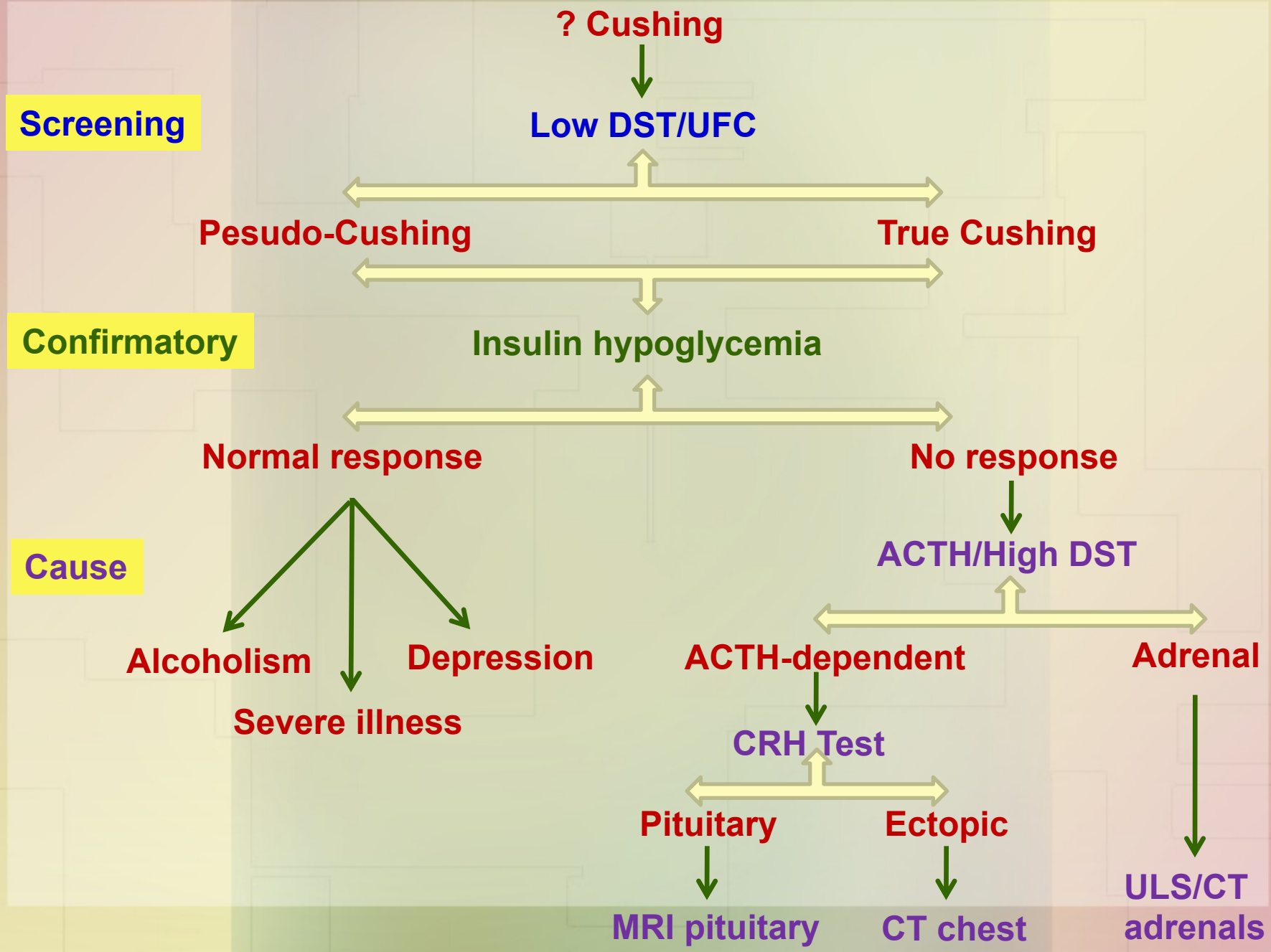
A. **Screening tests** (out-patient): to assess the clinical diagnosis of adrenocortical hyperfunction.

B. **Confirmatory tests** (in-patient): to confirm or exclude the provisional diagnosis

C. **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
Screening
Low DST/UFC
Pesudo-Cushing
True Cushing
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Insulin hypoglycemia
Normal response
No response
Cause
ACTH/High DST
Alcoholism
Depression
Severe illness
ACTH-dependent
Adrenal
CRH Test
Pituitary
Ectopic
MRI pituitary
CT chest
ULS/CT
adrenals

A. Screening tests:

Effective screening tests need to be sensitive but do not have to be highly specific.

It includes:

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

   DXM → ↓ CRH → ↓ ACTH → ↓ cortisol

2. 24-hour urinary free cortisol

A. Screening tests:

1. Low-dose DST: (outpatient procedure)

Procedure:

1 mg 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 hypercortisolnemia (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)**

A. Screening tests: .... Cont'D

2. 24- hour urinary free cortisol:

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

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

- An alternative is to determine the urinary cortisol : creatinine ratio on an early morning specimen

Interpretation of screening tests:

The screening tests serve to:

- distinguish simple non-endocrine obesity from obesity due to Cushing's syndrome.

- **Confirmatory tests (in-patient basis)** are required to rule out pseudo-Cushing's syndrome

- **Pseudo-Cushing's syndrome:**
  - Depressed or extremely anxious patients
  - Severe intercurrent illness
  - Alcoholism

B. Confirmatory tests: (Inpatient)

Insulin-induced hypoglycemia

Pseudo-Cushing patients show abnormal diurnal rhythm of S. cortisol, but, with Insulin-induced hypoglycemia → ↑ CRH, ACTH and cortisol blood levels

True Cushing patients:

No response to hypoglycemia

B. Confirmatory tests: ... Cont'd

Insulin-induced hypoglycemia:

- Hypoglycemia →↑ CRH →↑ ACTH →↑ cortisol
- To test the integrity of the hypothalamic-pituitary-adrenal (HPA) axis.
- To distinguish true Cushing's syndrome from pseudo-Cushing's syndrome
- Contraindicated in: epilepsy or heart disease.

Insulin hypoglycemia test .... Cont'd

- **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.**

Insulin hypoglycemia test …. Cont'd

Interpretation of the results:

Normally:

- **Basal serum cortisol:** at least 145 nmol/L
- **At 60 - 90 minutes:** the level > 425 nmol/L

Patients with Cushing's syndrome:

- Whatever the cause, 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.

C. Tests used to determine the cause of Cushing's syndrome:

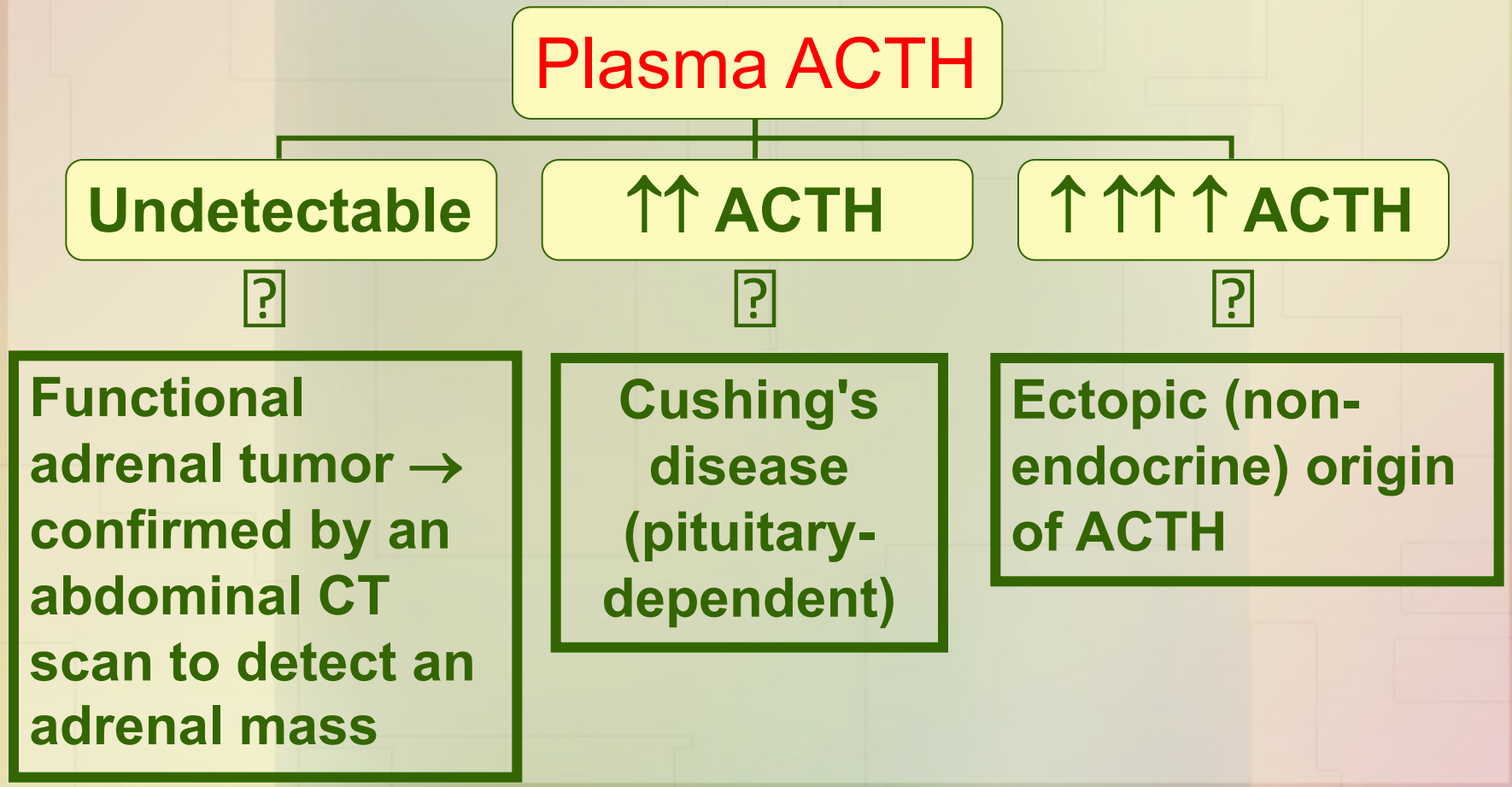
1. To differentiate ACTH-dependant from ACTH-independent: Plasma ACTH (Diurnal rhythm)

2. To distinguish between ACTH-dependent causes (Pitutary Vs Lung):
   a) High-dose DST.
   b) CRH stimulation test

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

1. Plasma [ACTH]:

Plasma [ACTH] should be measured on blood specimens collected at 8-9 a.m. and 8-9 p.m.



2 (a). High-dose DST:

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

- 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.

2 (a). High-dose DST ..... *Continued*

- 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.

2 (b). CRH stimulation test:

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

Ectopic ACTH & adrenal tumors	Cushing's disease
• No response • False-positive responses are unusual	• ↑↑ ACTH & cortisol above basal at 60 min • 10% of patients fail to respond

In Cushing's disease: High-dose dexamethasone suppression test + the CRH test → 100 % specificity and sensitivity.

3. Radiological Investigations:

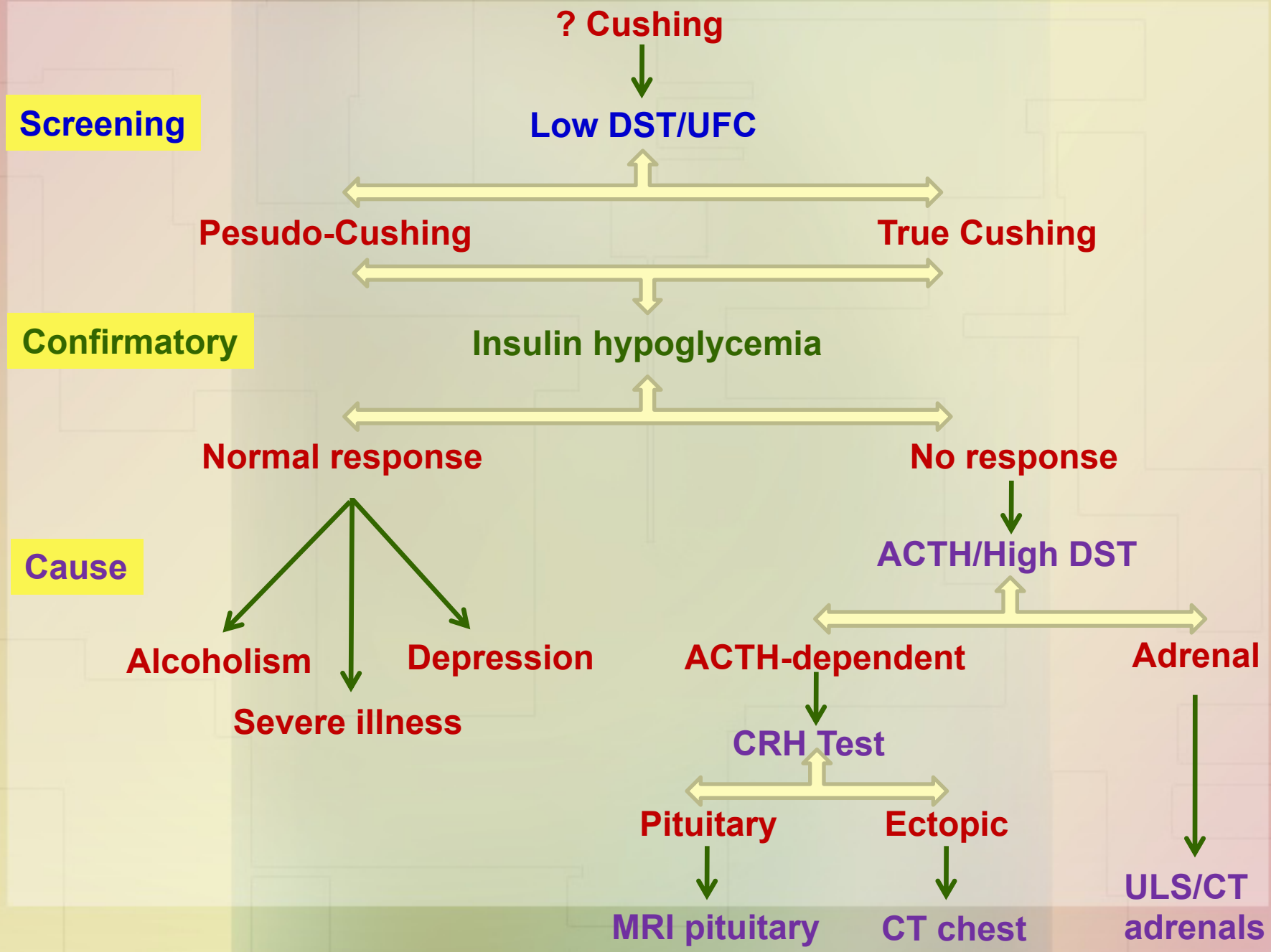
- CT scanning of the adrenal glands/

  Lungs

- MRI of the pituitary gland

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

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

Summary of Biochemical Tests

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

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

CRH showed flat response for cortisol and ACTH

Take Home Message

- 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).
- Initial screening for Cushing by 24 h urine free cortisol or low-dose dexamethasone suppression test
- Confirmatory tests for Cushing by diurnal rhythm of plasma cortisol and insulin-induced hypoglycemia
- Tests to determine the cause of Cushing: Plasma ACTH, high-dose dexamethasone suppression test, CRH stimulation test and radiological investigations

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.