UNIVERSITY OF PAPUA NEW GUINEA
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
PBL MBBS Year III; BMLS & BDS Year 3
ADRENAL HORMONES – An Overview

- Adrenal gland consist of: Outer Cortex and Inner Medulla
- Hormones secreted by Adrenal Cortex are: Glucocorticoid, Mineralocorticoid and Sex Steroids

What hormones are synthesized in the Adrenal Cortex?

- Hormones synthesized in Three Zones in Adrenal Cortex:
  - Mineralocorticoid (Aldosterone): in Zona Glomerulosa
  - Glucocorticoid (Cortisol): in Zona Fasciculata and Zona Reticularis
  - Sex Steroids: mainly in Zona Reticularis

CORTISOL (MAIN GLUCOCORTICOID)

How is Cortisol synthesized? (Fig. 1)

- Glucocorticoids are 21-Carbon steroids
  - Cortisol is main Glucocorticoid in humans
  - Natural or Synthetic steroids with Cortisol-like effects are called Glucocorticoids
- Cortisol is synthesized from Cholesterol delivered to Adrenal cortex by Low-Density Lipoprotein (LDL)
- Number of LDL receptors is increased when Adrenal cortex is stimulated by **AdrenoCorticoTrophic Hormone (ACTH, also called Corticotrophin**)
- Adrenal cortex can synthesizing Cortisol from acetate, though this appears to be of minor importance
- Steroidogenesis flow chart shows pathway for biosynthesis of Cortisol

How is biosynthesis of Cortisol regulated? (Fig. 2)

- Biosynthesis of Cortisol is regulated tightly by Hypothalamus and Anterior Pituitary Gland, with classic Feedback Inhibition
  - Hypothalamic-Pituitary-Adrenocortical Axis (**HPA- axis**):
  - Corticotrophin-Releasing Hormone (CRH) is secreted by Hypothalamus under influence of Cerebral Factors
  - Binding of CRH to Anterior Pituitary induces production of large molecular weight protein **Pro-opiomelanocortin (POMC)**
  - POMC is cleaved into fragments: **ACTH, Melanocyte-Stimulating Hormones (MSH), Beta-Lipotrophins, and Beta-Endorphins**
  - ACTH (Corticotrophin), a 39-amino-acid hormone, is released from Anterior Pituitary and enters systemic circulation
  - ACTH acts on Adrenal Cortex to stimulate biosynthesis and secretion of Cortisol
  - Hypothalamic secretion of CRH and Anterior Pituitary secretion of ACTH are modulated by Cortisol in Negative Feedback Loops

Fig. 1: STEROIDOGENESIS FLOW CHART

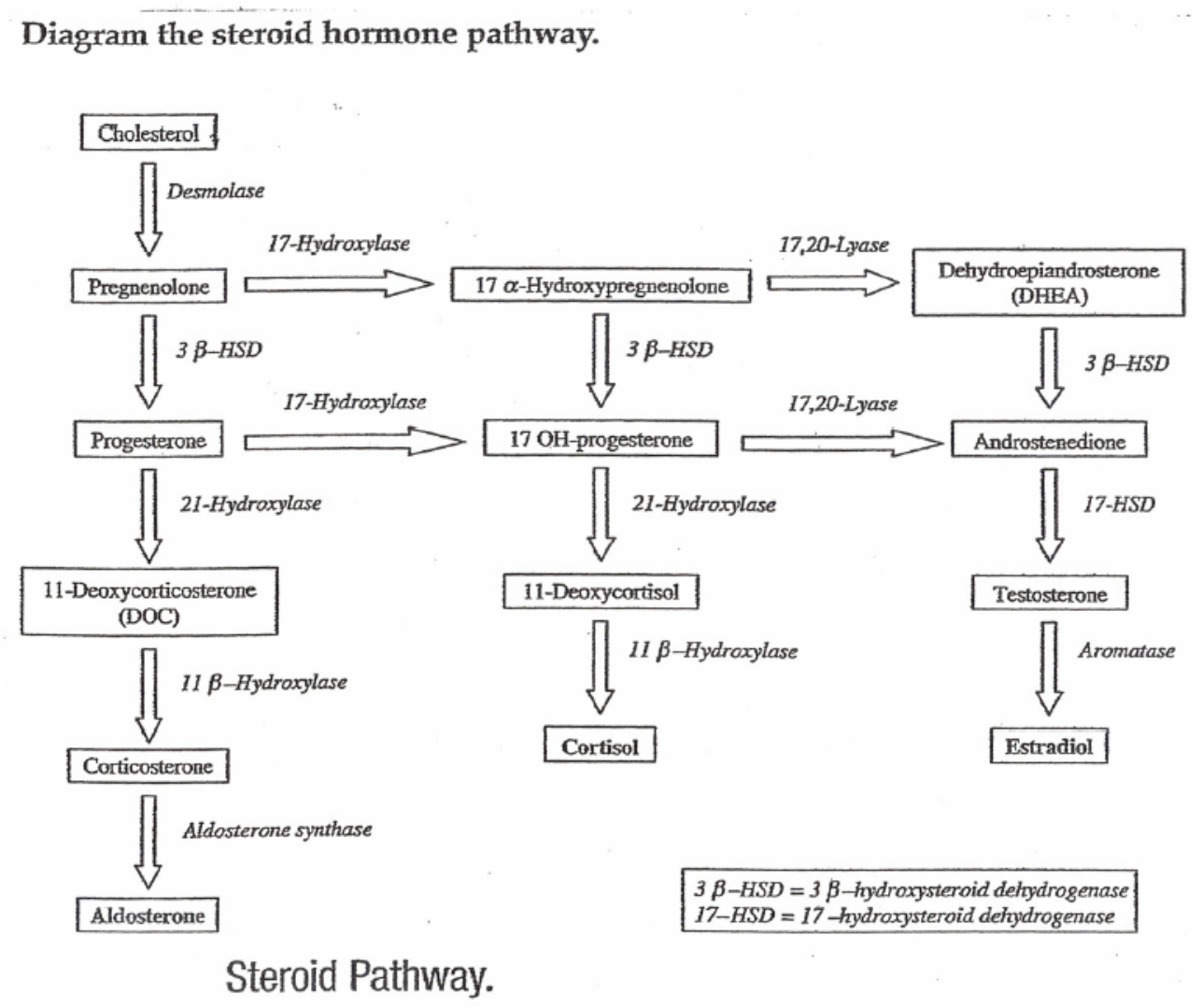
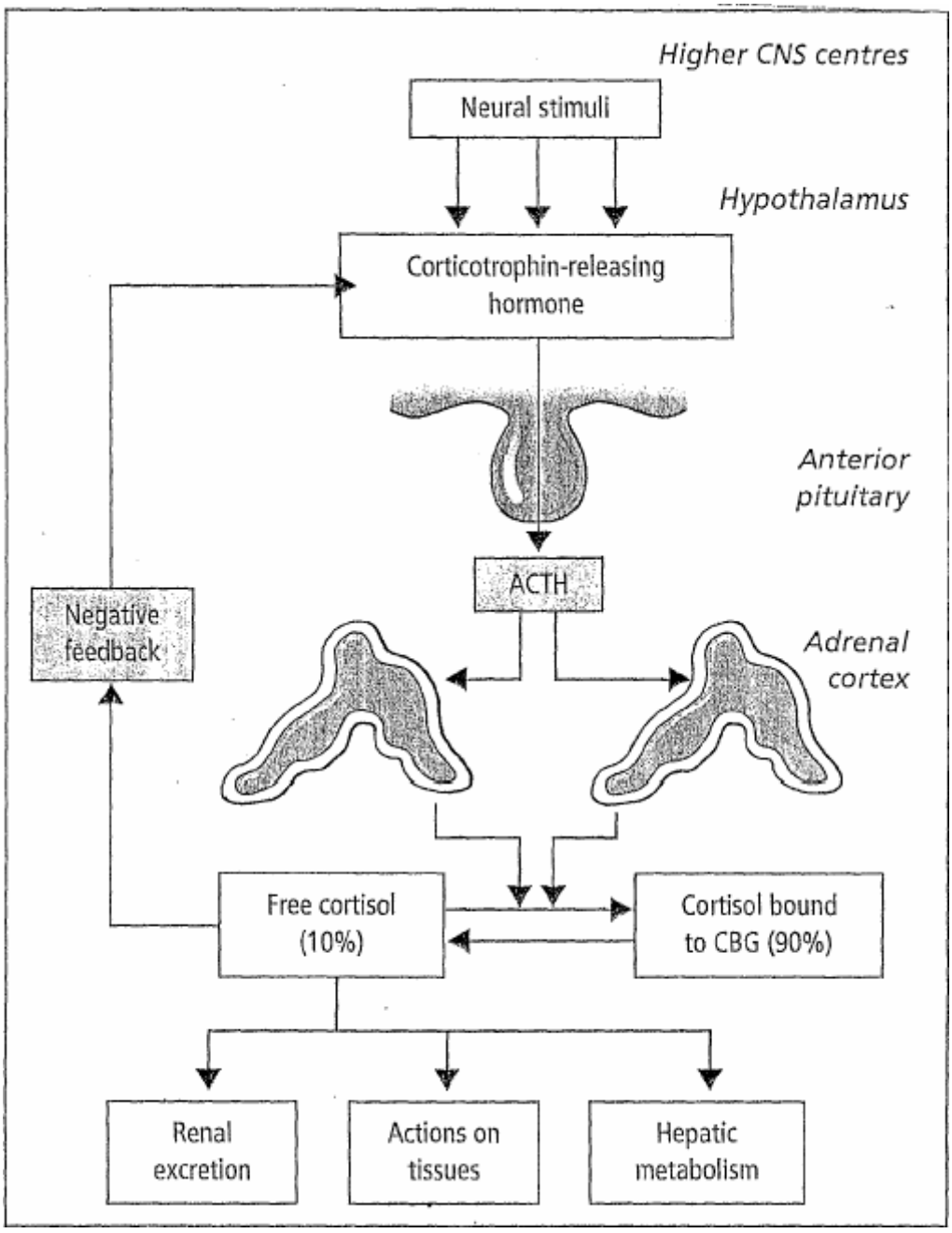


Fig. 2: Regulation of Cortisol secretion: Hypothalamic-Pituitary-Adrenocortical Axis (HPA-axis)



Give a brief outline of the negative feedback control of Cortisol (Fig. 2):

- Hypothalamus is stimulated to produce CRH by:
  - Low Plasma Cortisol level, or
  - Stress (Emotional stress, Fear, or Physical stress, Pain or Cold),
- CRH stimulates Anterior Pituitary to produce ACTH
- ACTH acts on Adrenal Cortex
  - Resultant effect is increase in Steroidogenesis leading to increased secretion of Cortisol and other Glucocorticoids in plasma
- High plasma Cortisol produces Negative Feedback Control on Hypothalamus and Anterior Pituitary (Long-Loop Feedback)
  - Resultant effect is decreased secretion of CRH and ACTH

TAKE NOTE:

- **Only** Cortisol can exerts Negative feedback on ACTH release
- Lack of Cortisol caused by enzyme deficiencies (e.g., 21-Hydroxylase), leads to failure in Feedback control of ACTH secretion,
  - High and continuous production of ACTH causes Adrenal Hyperplasia, which leads to Congenital Adrenal Hyperplasia
- Condition can be controlled by Administration of Cortisol:
  - Correcting Cortisol deficiency, and normalizing ACTH secretion via feedback inhibition of Hypothalamus and Anterior Pituitary

Does Daily rhythm affect plasma Cortisol and ACTH levels (Fig. 3)?

- Diurnal Rhythm of ACTH and Cortisol secretion present
- Cortisol levels are:
  - Highest in the morning and shortly after waking-up, and are
  - Lowest in late afternoon and evening
  - ACTH and Cortisol secretion are Minimal at Midnight
- Rhythm may be independent of sleep, but is abolished by stress and Cushing's syndrome (excessive production of ACTH)

How is Cortisol transported in plasma?

- Cortisol secreted by Adrenal cortex is transported mainly bound to plasma proteins, specifically **Corticosteroid-Binding Globulin (CBG, Transcortin)**
- Cortisol binds tightly to CBG and have a half-life in plasma of about 1.5 to 2.0 hours
- Other binding proteins in plasma are Albumin and Sex Hormone-Binding Globulin
- About 90% of Cortisol and Glucocorticoids are bound to proteins
- "**Free Fraction**," or **Non-protein-bound Cortisol** is biologically active, because it binds to specific receptors on target cells/tissues
- Level of CBG in plasma is increased in pregnancy and with Estrogen treatment (Oral Contraceptives)
- CBG level is decreased in Hypo-proteinaemic states (e.g., Nephrotic Syndrome)

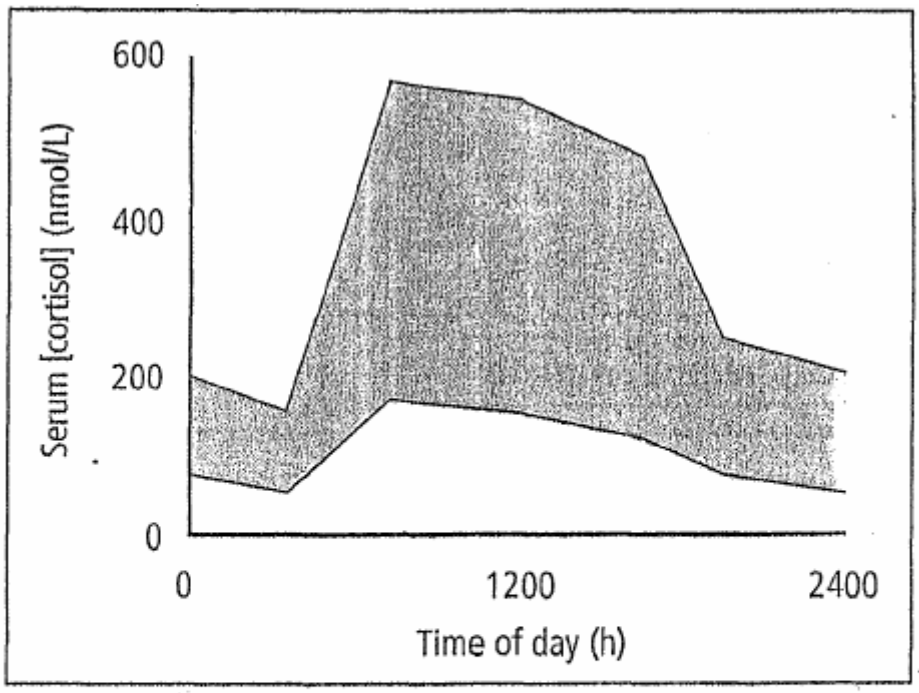


Figure The diurnal rhythm of cortisol secretion; the shaded area represents values that lie within the reference range. There is a similar rhythm for the secretion of ACTH by the anterior pituitary. Patients with Cushing's syndrome lose this diurnal variation.

How are Cortisol and other Glucocorticoids excreted from the body?
(Metabolism and Urinary Excretion of Cortisol):

- Cortisol metabolism occurs mainly in Liver as conjugated metabolites (e.g., as Glucuronides) for excretion in urine
- Small amount of Cortisol is excreted unchanged in the urine
    - Healthy individuals, urinary Cortisol excretion is less than 250 nmol/24hour
    - Cortisol to Creatinine ratio in early morning urine is less than 25umol Cortisol to 1.0 mol Creatinine
- Products of Cortisol metabolism is excreted in urine as **17-Hydroxy-Cortico-Steroids** (**17-OHCS**)

What are the functions of Cortisol and other Glucocorticoids?

- Glucocorticoids affect Carbohydrate, Fat and Protein metabolism
- Cortisol stimulates:
    - Gluconeogenesis, Uptake and Degradation of Amino Acids and Ketogenesis in Liver
    - Lipolysis in Adipose tissue
    - Protein degradation in Muscle
- Cortisol helps to regulate stress response
- Glucocorticoids are involved in regulation of Sodium and Water homeostasis
- Glucocorticoids act as Anti-inflammatory or Immunosuppressive Agents
- Glucocorticoids are Insulin Counter Regulatory Hormones
    - Increase in blood glucose due to excess Glucocorticoid activity is known as **Adrenal Diabetes**
- Prolonged excess Glucocorticoids release may lead to damage of beta cells in Pancreas causing Diabetes Mellitus
- Glucocorticoids decrease protein matrix of bone through their protein catabolic effect, causing increased loss of Ca^{2+} from bone, resulting in Osteoporosis

ALDOSTERONE (MAIN MINERALOCORTICOID)

How is Aldosterone (Mineralocorticoid) produced? (**Fig. 1**)

- Natural or Synthetic steroids with Aldosterone-like effects are called Mineralocorticoids
- Aldosterone is a 21-Hydroxyl steroid hormone synthesized from Cholesterol
- Steroidogenesis flow chart shows the pathway for biosynthesis of Cortisol
- Regulation of Aldosterone synthesis and release is different from Cortisol

What are the factors that can affect the release of Renin?

- Renin a proteolytic enzyme produced by **Juxtaglomerular Apparatus** in Kidneys is released into the circulation in response to certain factors
- Factors that can influence release of Renin include:

- **Stimulate of Renin release**:
  - Dehydration, Decreased blood pressure, Fluid or blood loss, Salt depletion, Change from supine to erect posture, Beta-Adrenergic agents and Prostaglandin

- **Inhibits of Renin release**:
  - Increased blood pressure, Change from erect to supine posture, Salt loading. Prostaglandin inhibitors, Beta-Adrenergic antagonists, Potassium, Vasopressin, and Angiotensin-II

Outline the Renin-Angiotensin-Aldosterone (RAA) system that regulates secretion of Aldosterone (Fig. 4):

- Renin is secreted in response to either fall in Renal Blood Pressure, decrease Osmolality in blood (low [Na^+])
- Renin converts Angiotensinogen in plasma to Angiotensin-I (AI),
- Angiotensin Converting Enzyme (ACE) produced in Lungs converts Angiotensin-I to Angiotensin-II (AII)
- Angiotensin-II acts on appropriate cells in Adrenal Cortex to increase biosynthesis and secretion of Aldosterone
- Enzyme Angiotensinases terminates action of Angiotensin-II
- Aldosterone acts on Tubule to promote Na^+ reabsorption in exchange for urinary loss of K^+ and H^+

How does ACTH affect secretion of Aldosterone?

- High plasma concentration of ACTH may increase biosynthesis of Aldosterone by increasing availability of steroid substrates (e.g., cholesterol) in Adrenal cortex
- ACTH Control mechanism is relatively unimportant, except possibly in stress conditions and in Congenital Adrenal Hyperplasia due to deficiency of enzyme 21-Hydroxylase

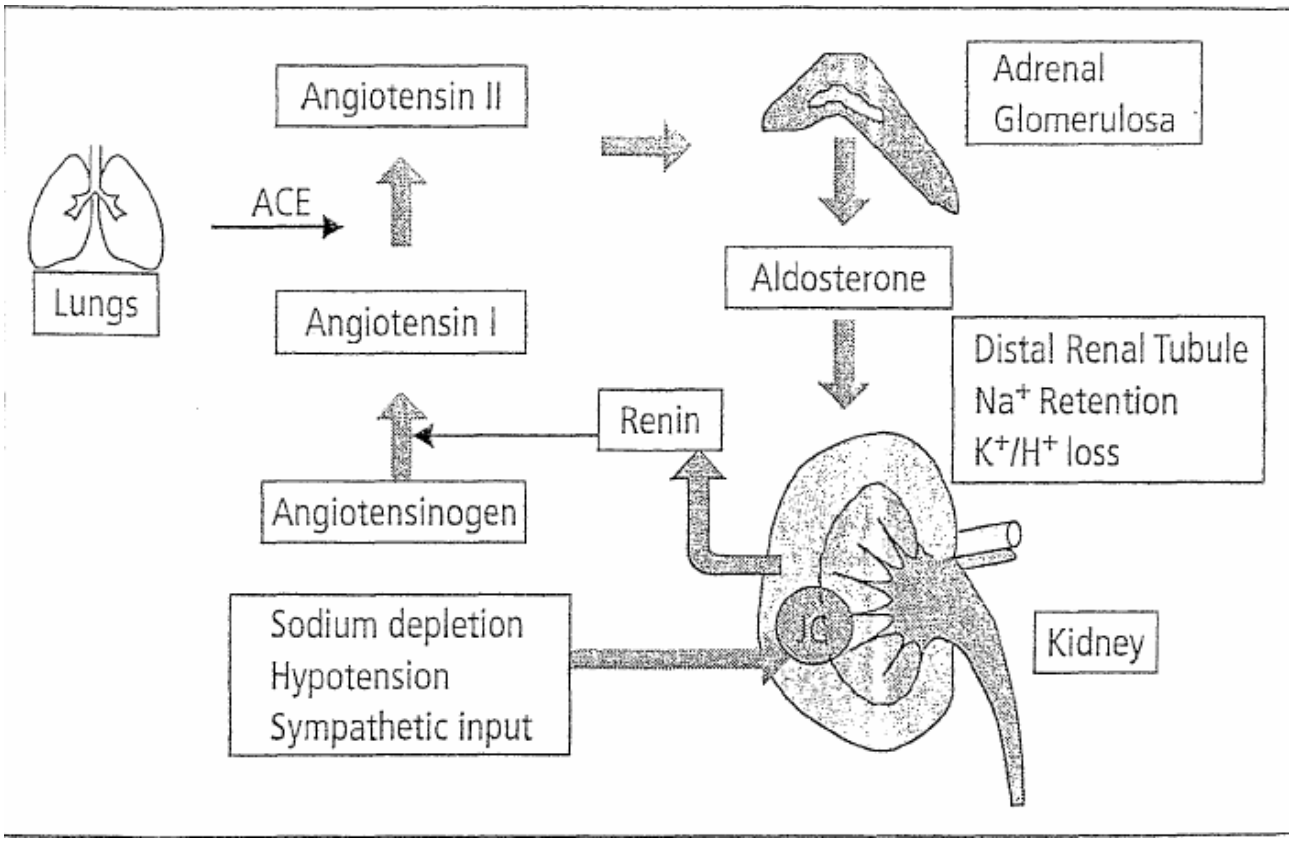
How are Aldosterone and other Mineralocorticoids transported in Plasma?

- Aldosterone and other Mineralocorticoids do not have any specific plasma transport protein, they form very weak bonds with albumin
- Aldosterone is very rapidly cleared from plasma by the Liver
- Liver forms Tetra-hydro-Aldosterone–3–Glucuronide, which is excreted in the urine.

What are the functions of Aldosterone?

- Aldosterone is a major regulator of Water and Electrolyte Balance
- Primary function of Aldosterone is regulation of Na^+ ions by Distal Renal Tubules
- Aldosterone stimulates re-absorption of Na^+ ions and secretion of K^+ and H^+
- **Excess Aldosterone** results in Sodium Retention, Hypokalemia, and Alkalosis
- **Aldosterone deficiency** results in Sodium Loss, Hyperkalemia, and Acidosis
- Hyperkalemia stimulates Aldosterone release to improve Potassium excretion
- Aldosterone is the first-line defense against Hyperkalemia

Fig. 4: Renin-Angiotensin-Aldosterone (RAA) system that regulates secretion of Aldosterone



Major References for further reading:

1. G. Beckett, S. Walker, P. Rae and P. Ashby; 7th Edition, 2005; Lecture Notes: Clinical Biochemistry, Blackwell Publishing
2. Textbook of Biochemistry with Clinical Correlations; Edited by TM Delvin, 3rd Ed.
3. V. L. Davidson and D. B. Sittman, Biochemistry Clinical Cases and Correlations 3rd Edition; Harwal Publishing

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ADRENAL FAILURE (INSUFFICIENCY) – DIAGNOSTIC TESTS

What is the basic classification of Adrenal Failure?

- Basic classification of Adrenal failure or Adrenal Insufficiency (Adreno-Cortical Hypo-function) depends on location of lesion:

- **Primary**:
  - Primary Adrenal Failure or Primary Adrenal Insufficiency (Addison's Disease) is due to:
    - Failure of Adrenal Gland caused by destruction of Adrenal Gland itself
  - Cortisol and Aldosterone production may be affected

- **Secondary**:
  - Secondary Adrenal Failure or Secondary Adrenal Insufficiency:
    - Hypothalamic or Pituitary disease leading to deficiency of ACTH (Corticotrophin) or After long-term use of Steroid therapy

What are the causes of Primary Adrenal Failure?

- Several causes including:
- Infective (Tuberculosis, Meningococcal, HIV, etc)
- Autoimmune Adrenalitis
- Metastasis (most commonly from Lung and Breast Carcinomas),
- Hemorrhage,
- Metabolic failure, such as, insufficient hormone production, caused by
  - Congenital Adrenal Hyperplasia (CAH),
  - Enzyme inhibitors, such as Metyrapone
  - Cytotoxic agents (e.g. Etomidate)

List some signs and symptoms of Primary Adrenal Failure

- **Some non-specific signs and symptoms include**: Weakness, Abdominal Pain, Nausea, Weight Loss, Hypotension, Shock, Acid-Base Disturbance, Lack of Libido, Loss of body hair in women, and Psychiatric changes

- **Some specific signs and symptoms include:** Hypoglycemia with Hyponatremia, Hyperkalemia, Raised Serum Urea levels, **Hyper-pigmentation** (affecting Buccal mucosa), Scars and Skin creases

These conditions are life threatening and requires urgent investigation if suspected

List some signs and symptoms of Secondary Adrenal Insufficiency

- Some signs and symptoms of Secondary Adrenal Insufficiency may be identical to those of Primary Adrenal Insufficiency,
- Hyper-pigmentation does not occur in Secondary Adrenal Insufficiency {**WHY???}**

- Because of Insufficient production of ACTH and other products of Proopiomelanocortin (POMC) metabolism {i.e., Melanocyte Stimulating Hormones (MSH)}

What Biochemical results are indicative of Primary Adrenal Insufficiency?

- Biochemical results indicative of Primary Adrenal Insufficiency include:
  - Hyponatraemia, Hyperkalaemia, Elevated Serum Urea and Hyper-pigmentation

What is the Biochemical basis for Hyponatraemia, Hyperkalaemia, Elevated Serum Urea and Hyper-pigmentation in patients with Primary Adrenal Insufficiency?

- Lack of Aldosterone leads to pathological Sodium loss in Kidneys, resulting in contraction of Extracellular Fluid Volume (Hypovolaemia), causing Hypotension and Pre-renal Uremia
- Patients may develop life-threatening Sodium depletion and Potassium Retention due to Aldosterone Deficiency
- Hypovolaemia and Hypotension stimulate AVP Secretion, thus causing Water Retention
- Absence of Cortisol impaired ability of Kidneys to excrete water, which leads to Hyponatraemia
- Overall effect causes reduction in Total Body Water, which is reflected by increase in Serum Urea
- Absent of Cortisol causes failure in Negative Feedback control, resulting in excessive secretion of ACTH from Anterior Pituitary
- Structure of ACTH contains part of Amino Acid sequence of Melanocyte-stimulating hormone (MSH)
- Excessive secretion ACTH causes darkening of skin and mucus membranes, resulting from the action of MSH

SCREENING AND DIAGNOSTIC TESTS FOR ADRENOCORTICAL INSUFFICIENCY (ADRENAL FAILURE):

What basal laboratory tests are done on a patient suspected of having Adrenal Insufficiency?

- Suspected case of Adrenal Insufficiency should be referred to hospital for Screening and Diagnosis
- Before patient is given Cortisol, blood should be collected for Basal measurements of:
  - Plasma Urea, Electrolytes, Glucose, Serum Cortisol and Plasma ACTH concentration
- Patients intake of Sodium must be adequate whilst investigations proceed

Note the following with respect to Cortisol and ACTH measurements:

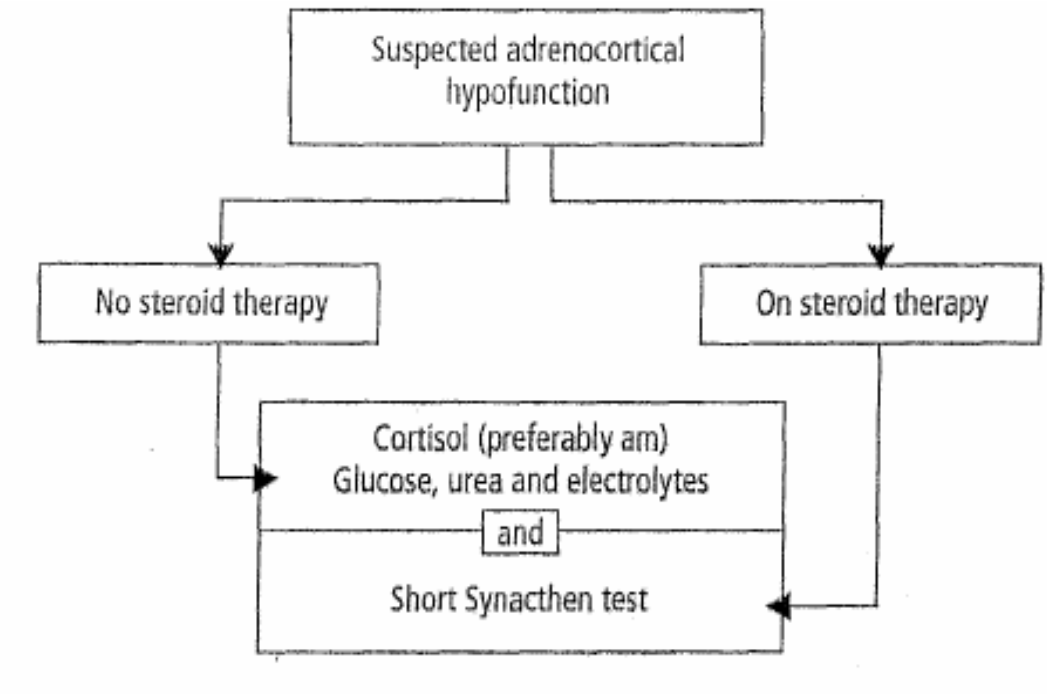
- Normal Serum [Cortisol] at 8 a.m., or Normal 24-hour Urinary Free Cortisol, does not exclude Primary Adrenocortical Insufficiency **Why?**
  - Because the patient may be able to maintain Normal Basal output of Cortisol but **is unable** to secrete adequate amounts of Cortisol in response to Stress
- Serum [Cortisol] below **50**nmol/L at 8 a.m. is strong presumptive evidence for Primary Adrenal Failure

- Diagnosis of Primary Adrenal Failure is unlikely, if Serum [Cortisol] is **550**nmol/L or more at 8 a.m. (in absence of Steroid Therapy)
- Diagnostic accuracy for Primary Adrenal Insufficiency is greatly improved when serum [Cortisol] and [ACTH] are measured at the same time; **Why?**
    - Because Low Serum [Cortisol] < 200nmol/L, and raised [ACTH] > 200nmol/L may be diagnostic of Primary Adrenal Failure

List some Biochemical tests that can be used for Screening and Diagnosis of the Adrenal Failure

- Biochemical tests used for diagnosis of Adrenal failure are:
    - Short Cosyntropin (Synacthen, Cortrosyn or Tetracosactrin) Test
    - Depot (Long) Synacthen test
    - Prolonged Cosyntropin Stimulation (Rose) test
    - Corticotrophin Releasing Hormone (CRH) stimulation test
    - Rapid Synacthen stimulation test

Fig. 1: Suggested plan for Investigation of Adrenocortical Failure



What is the procedure for the Short Cosyntropin test?

- Short Cosyntropin (Synacthen, Cortrosyn or Tetracosactrin) test indicates ability of Adrenal Cortex to respond to ACTH
    - {***Cosyntropin is the Synthetic 1 – 24 analogue of ACTH; Trade name for Cosyntropin is Synacthen or Cortrosyn***}

Procedure for Short Cosyntropin test includes the sequence:

- Measure Baseline Plasma [Cortisol] and [Aldosterone]
- Patient is given **0.25**mg of Synacthen as an Intravenous bolus or as Intra-muscular Injection
- Measure Plasma [Cortisol] and [Aldosterone] again after 30 minutes
- Blood samples for Aldosterone can be held until results of Cortisol response are known

TAKE NOTE:

- Normally, Baseline Plasma [Cortisol] should be within the Reference Range (280 – 720 nmol/L at 08.00 am to 10.00 am)
- Acceptable Baseline Plasma [Cortisol] > 225nmol/L
- There should be an increment of more than 200nmol/L Plasma [Cortisol] after Synacthen, and
- Final Plasma [Cortisol] should be greater than 500nmol/L
- Aldosterone response in a Synacthen test is blunted or absent in patients with Primary Adrenal Insufficiency
- In Secondary Adrenal Insufficiency, Aldosterone response is normal (an increase of two times the baseline value) in the Synacthen test, because the Renin-Angiotensin Axis is not affected by decreased Corticotrophin (ACTH)

How are the results of the Short Cosyntropin test interpreted?

- Interpretation Short Cosyntropin test results involves assessment of data obtained with respect to set criteria
- Three criteria should be met for normal response, these criteria are:
    - **Baseline Plasma [Cortisol] should be > 225nmol/L**
    - **Final Plasma [Cortisol] should be > 500nmol/L**
    - **Increment in [Cortisol] should be at least 200nmol/L**

- For a patient to be declared as normal all Three Criteria must be satisfactory
- Normal response to Short Synacthen test excludes Primary Adrenocortical Insufficiency
- Failure to meet any of the criteria indicates Adrenocortical Inadequacy
- Elevated Plasma [ACTH] will confirm diagnosis of Primary Adrenal Insufficiency in a patient with abnormal response to Short Synacthen test

What addition test should be carried out if the results from the Short Synacthen test are equivocal (unclear)?

- Patients with equivocal responses (i.e., unclear responses) to Short Synacthen test may be re-tested after Stimulation of Adrenal Cortex with ***depot*-Synacthen**
- This longer acting material (1.0 mg) should be given intramuscularly daily for three days
- On the fourth day the Short Synacthen test should repeated
    - If the normal criteria for Short Synacthen test are fulfilled on the second testing, then Adrenal Insufficiency is not of Primary origin
    - Such a result points towards Secondary Adrenocortical Insufficiency

What are some of the factors that can affect the Short Synacthen test?

- Some factors that can affect the Short Synacthen test to the point of invalidating the tests include:
    - Severe Emotional Stress,
    - Treatment with Glucocorticoids within 12-hours prior to Synacthen test,
    - Taking of Estrogen-containing Oral Contraceptives

What is the use and procedures for Prolonged Cosyntropin Stimulation (Rose) test?

- Prolonged Cosyntropin-Stimulation (Rose) test can be used to differentiate Primary from Secondary Adrenal Insufficiency
- Procedure for Prolonged Cosyntropin Stimulation (Rose) test:
- Measure Baseline Plasma [Cortisol
- Measure 24-hour Urinary [17-Hydroxycorticosteroids] (17-OHCS)
- Over the next 48-hours, patient is continuously given infusion containing 0.25mg Cosyntropin
- On Second day of infusion, measure Plasma [Cortisol] and the 24-hour Urinary [17-OHCS]
- At the end of the infusion measure the final Plasma [Cortisol]

How are the results of the Prolonged Cosyntropin Stimulation (Rose) test interpreted?

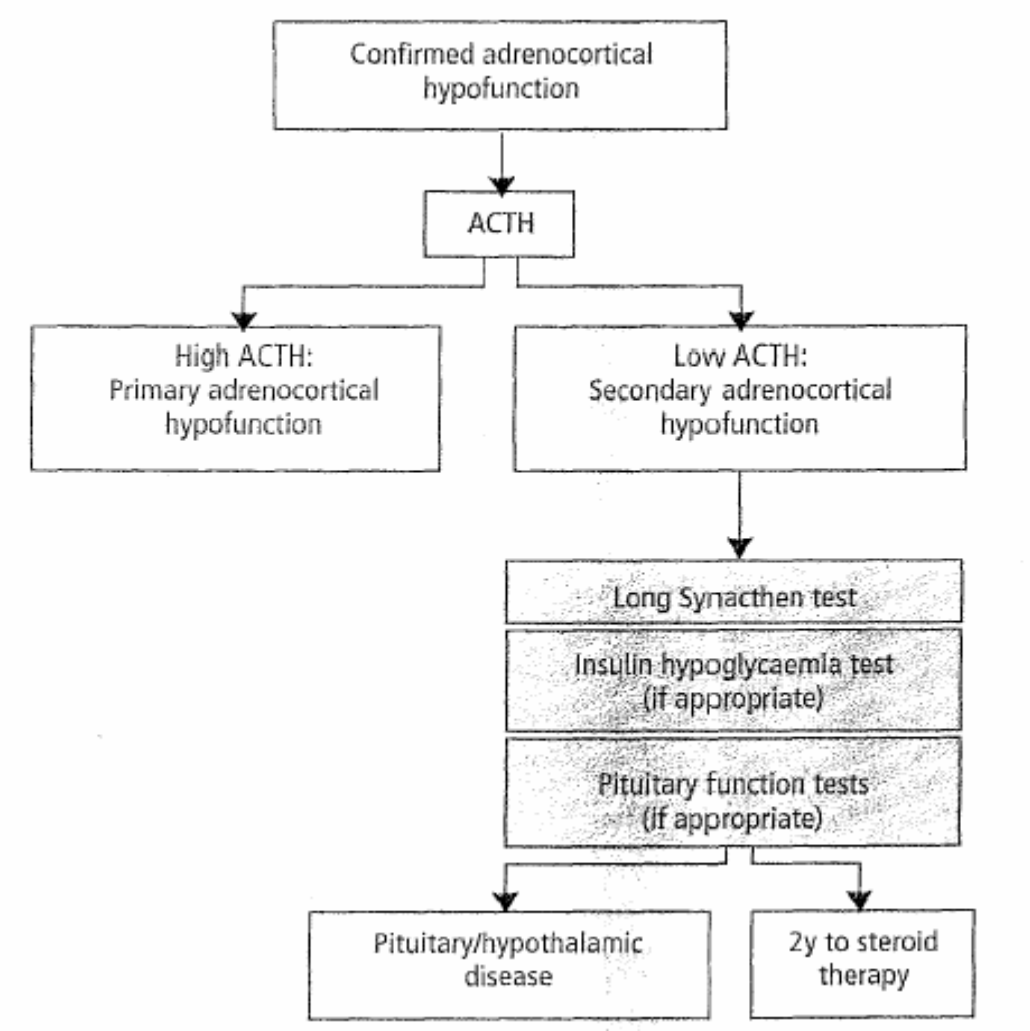
- In Primary Adrenal Insufficiency, no change is seen in Plasma [Cortisol] or 24-hour Urinary [17-OHCS] **WHY??**
    - Because in Primary Adrenal Insufficiency Cortisol is not produced
- In Secondary Adrenal Insufficiency, Incremental Increase occurs over the course of the Infusion **WHY??**
    - Because the problem is **not Primary,** Cosyntropin action stimulates Adrenal Cortex, which then produces Cortisol
- Results indicate that Adrenal Cortex has undergone Atrophy because of Insufficient ACTH stimulation; however, with longer stimulation, the Adrenal Cortex is capable of functioning

Diagnosis of Secondary Adrenocortical Insufficiency:

How can Secondary Adrenocortical Insufficiency be diagnosed?

- Low Serum [Cortisol] accompanied by Low Plasma [ACTH] would support a diagnosis of Adrenocortical Insufficiency Secondary to Hypothalamic or Pituitary disease

- In such cases, whilst the Atrophied Adrenocortical cells may fail to respond in the Short Synacthen test, the Adrenal Cortex can become responsive over a longer period of stimulation using the **Depot (long) Synacthen test**



Procedure for the Depot (Long) Synacthen Test:

- Measure Baseline Serum [Cortisol]
- Patient is then administered Depot Synacthen (1.0 mg) Intramuscularly on three successive days
- On each of the Three days Serum [Cortisol] must be measured between Five to Eight hours after administration of the Depot Synacthen

How are the results interpreted?

- In Primary Adrenocortical Insufficiency, Serum [Cortisol] will NOT INCREASE above 600nmol/L at 5 to 8 hours after the Third injection
- In Secondary Adrenocortical Insufficiency, Stepwise increase in Serum [Cortisol] will be apparent after successive administration of the Depot Synacthen

TAKE NOTE:

- Poor responses to Prolonged Synacthen tests may occur in patients with Hypothyroidism (both Primary and Secondary)
- In patients with Hypothyroidism, Adrenal Function cannot be satisfactorily assessed until the Thyroid Deficiency has been corrected
- Once a decision has been made as to whether Adrenal Insufficiency is Primary, or Secondary, the appropriate Imaging Technique should be used to rule out other treatable causes

Major References for further reading:

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