STEROIDOGENESIS: An Overview

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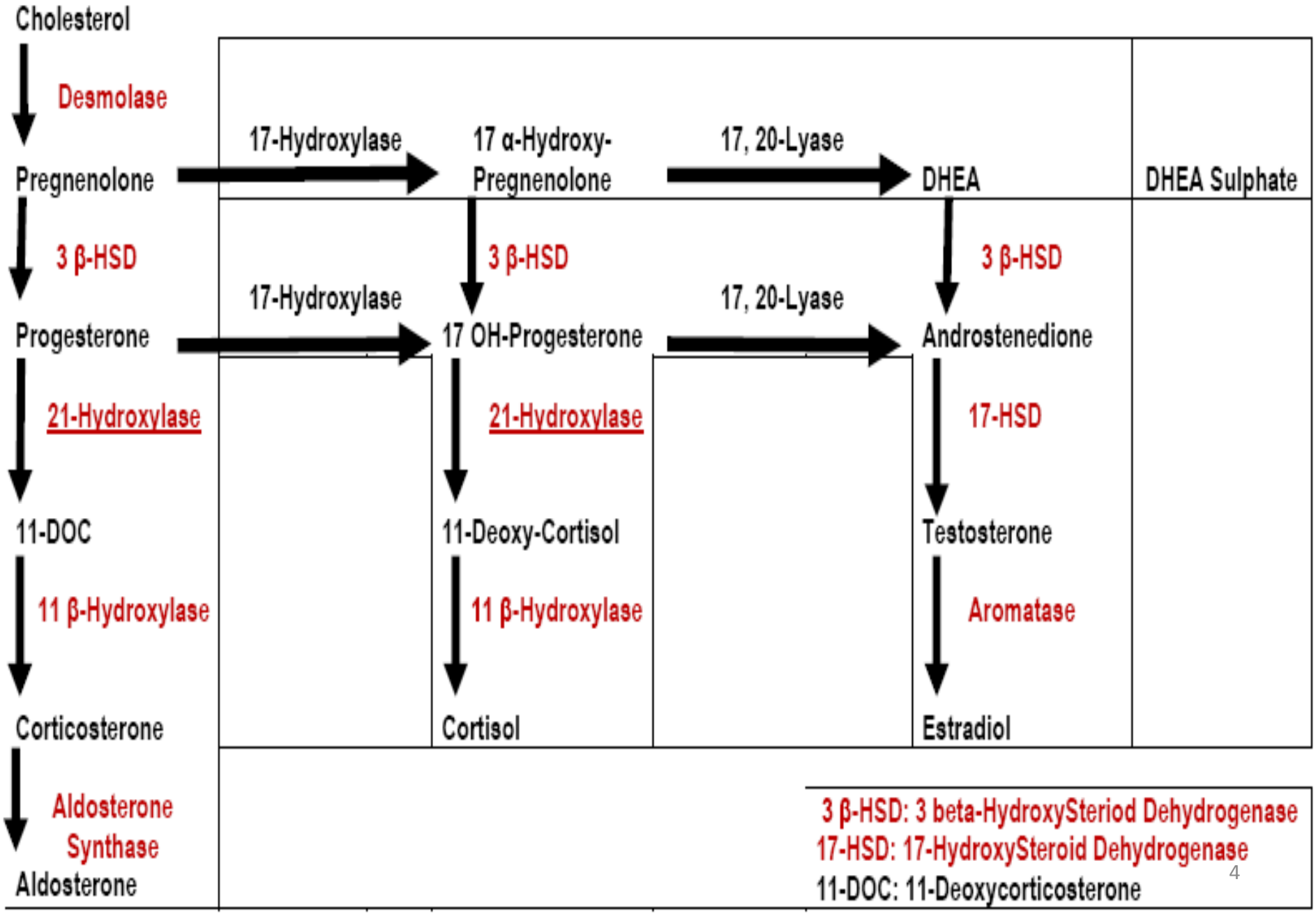
What are the Steroid hormones?

- **Hormones that are synthesized from Cholesterol**;
- Some important steroid hormones in humans:
- **Progesterone**: secreted in Corpus Luteum, responsible for changes in luteral phase of menstrual cycle, differentiation factor for mammary glands;
- **Estradiol (Oestradiol)**: An Oestrogen, produced in ovary, responsible for secondary female sex characteristics;
- **Testosterone**: An Androgen, produced in testes, responsible for secondary male sex characteristics;
- **Aldosterone**: Principal Mineralocorticoid from Adrenal Cortex;
- **Cortisol**: Principal Glucocorticoid from Adrenal Cortex;

Outline the pathways for biosynthesis of steroid hormones

- Pathways for biosynthesis of steroid hormones are usually presented as a flow chart;
- Specific steroid hormone synthesized by a given tissue depends upon:
  - Complement of Peptide Hormone Receptors on the tissue,
  - The tissue response to Peptide Hormone Stimulation,
  - The Genetically Expressed Complement of Enzymes in the tissue;
- Thus, flow chart does not go to completion in all tissues;
- **Fig. 1**: Schematic diagram of pathways for biosynthesis of different steroid hormones;

Fig. 1: Flow diagram of pathways for biosynthesis of steroid hormones



How do steroid hormones exist in blood plasma?

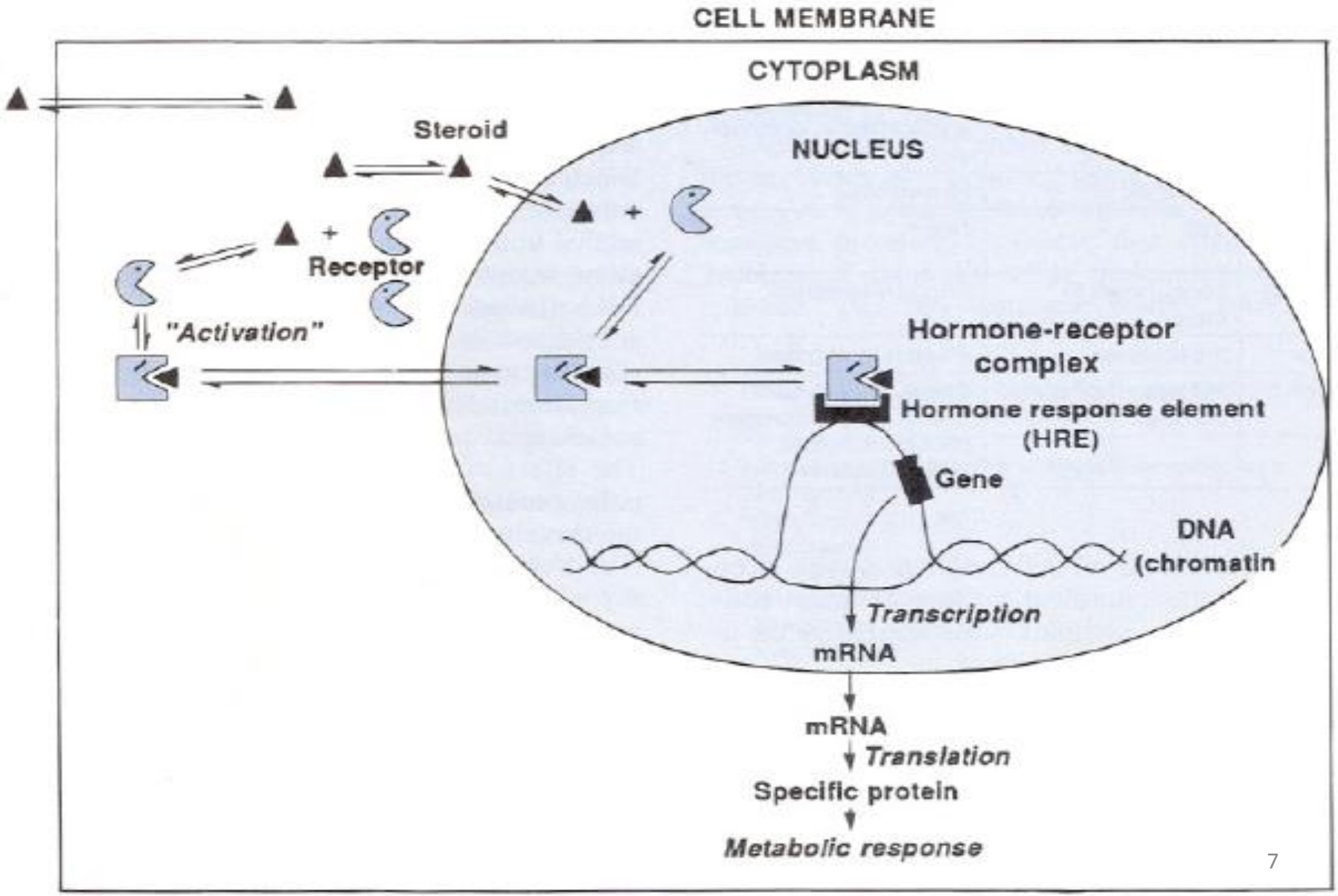
- Steroid hormones are hydrophobic, thus they bind to Specific Hormone Binding Glycoproteins in blood plasma (bound fractions of steroid hormones);
- Small amount of Steroid hormone usually remains Free in plasma (unbound fraction);
- **Unbound or "Free" fraction of steroid hormone in blood plasma is the biologically active fraction,**
- Measurement of the "Free fraction" of steroid hormone or the binding protein level is important in the diagnosis of patients with certain steroid hormone disorders;

What is the general mode of action of steroid hormones?

- Free Fraction of Steroid hormone pass through cell membrane in target tissues and bind to Intracellular receptors, forming Steroid Hormone-Receptor Complex,
- The Complex exerts its action on Nucleus of Target cells,
- Steroid Hormone-Receptor Complex binds to Specific Nucleotide Sequences on DNA of Responsive Genes,
  - Specific Nucleotide Sequences in DNA are called **Hormone Response Elements (HRE)**,
- Interaction of steroid hormone-receptor complexes with DNA leads to altered rates of Transcription of associated Genes in Target cells; (**Fig. 2**)

Fig 2: Schematic diagram, mode of action of steroid hormones

(Harper's Biochem, 24th Ed , 1996)



How does SHBG affect plasma levels of Sex Steroid Hormones?

- Testosterone and Oestradiol circulate in blood plasma mostly bound to Sex Hormone Binding Globulin (SHBG);
- SHBG has higher affinity for Testosterone than Oestradiol,
- **Testosterone decreases SHBG** synthesis in the liver,
- **Estradiol stimulates SHBG synthesis in** the liver,
  - SHBG levels in females is about twice that in males,
- Factors that alter the concentrations of SHBG in blood plasma alter the Ratio of Unbound Testosterone to Unbound Oestradiol,

- In both sexes the effect of:
  - An increase in SHBG level in blood plasma is to increase Oestradiol-like effects, (**Why?**)
  - A decrease in SHBG level in blood plasma is to increase Androgen effects (**Why?)**

- As Oestradiol itself increases SHBG level in blood plasma and Testosterone decreases it, this system functions as a Biological Servomechanism;

- Testosterone and SHGB concentrations in plasma are sometimes reported by laboratory as a Ratio **Free Androgen Index (FAI)**, which gives a clearer indication of Androgen status than does plasma Testosterone alone;

$$\text{FAI} = \frac{[\text{Total Testosterone}]}{[\text{SHBG}]}$$

What axis regulates secretion of sex steroid hormones?

- Regulation of secretion of sex steroids is by Negative Feedback mechanism on **HPG –Axis (Figs 3a, 3b & 4)**
  - **HPG-Axis:** Hypothalamic-Pituitary-Gonadal Axis;
- Hypothalamus releases Gonadotrophin-Releasing Hormone (GnRH), which acts on Anterior Pituitary to produce Gonadotrophins:
  - Luteinizing Hormone (LH),
  - Follicle-Stimulating Hormone (FSH)
- Gonadotrophins act cooperatively on the Ovaries in females and Testes in males to stimulate Sex Hormone secretion and reproductive processes;
- Inhibin produced by the Gonads feed back inhibits production of FSH;

Fig. 3a: Hypothalamus –Pituitary-Testicular Axis, for Feedback regulation of secretion of Testosterone

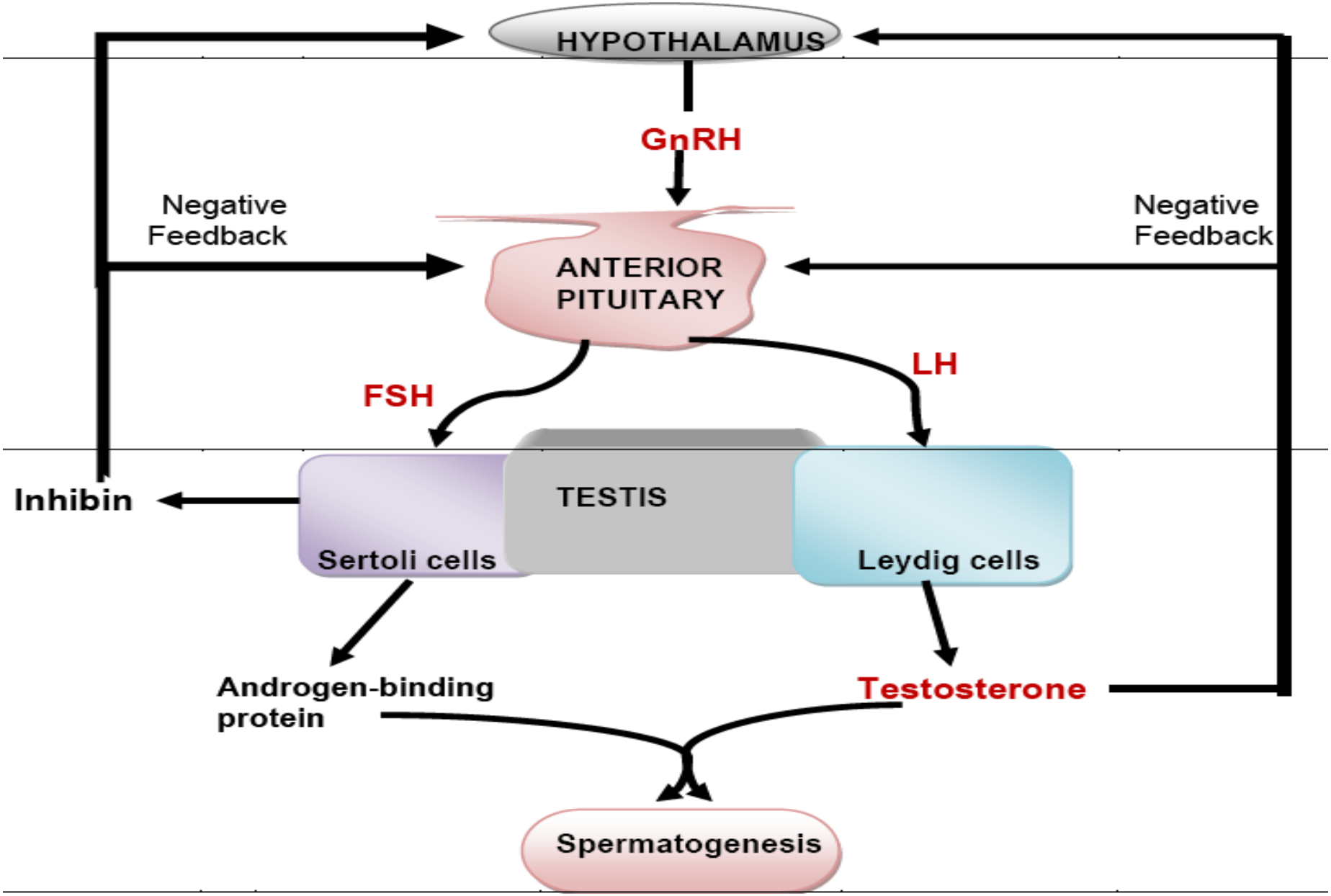


Fig 3b: Conversion of Free Testosterone to Dihydrotestosterone

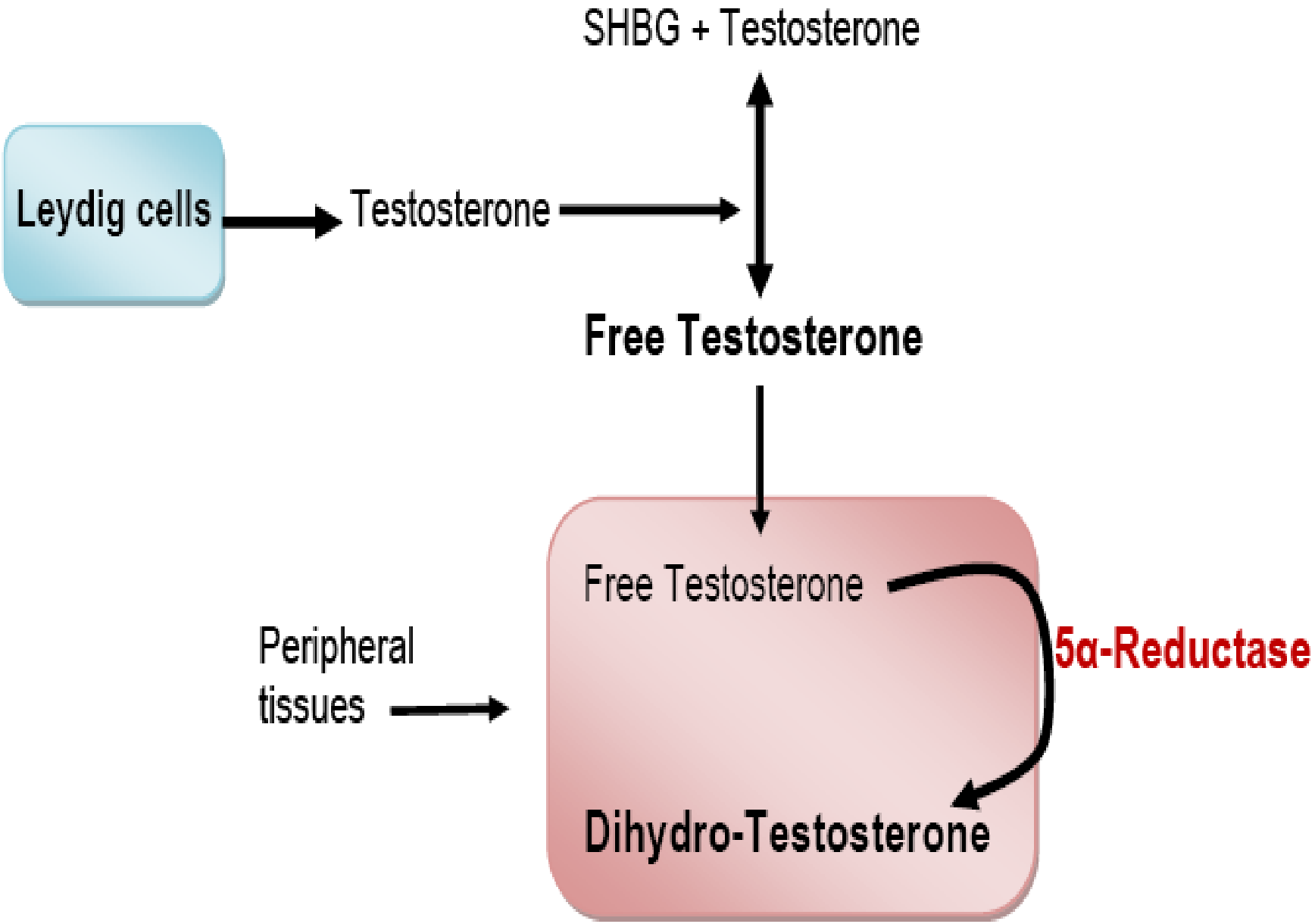
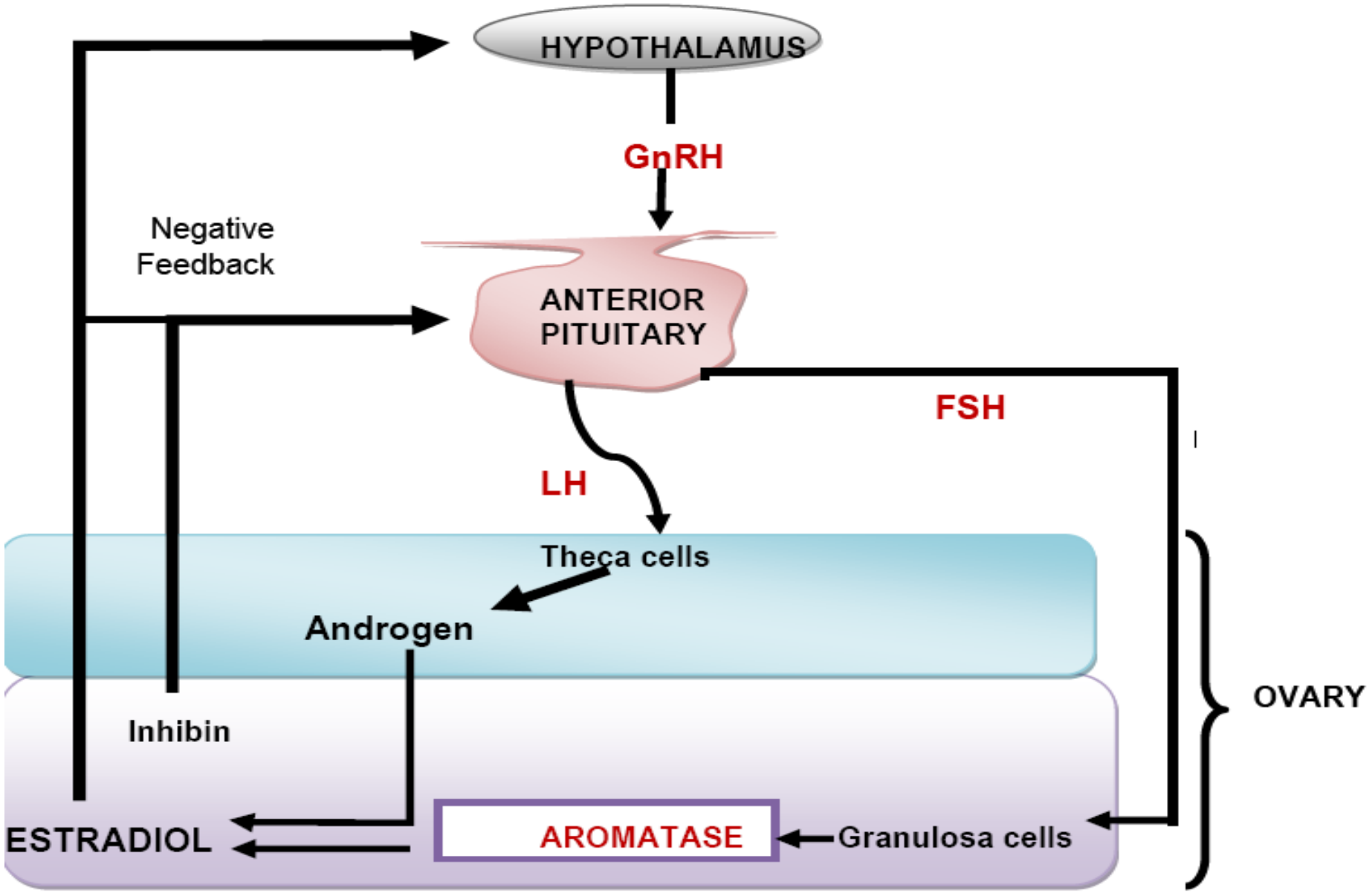


Fig. 4: Hypothalamus –Pituitary-Ovarian- Axis, for Feedback regulation of secretion of Estradiol



TAKE NOTE:

- Plasma levels of Oestradiol are low before puberty, but rise rapidly and fluctuate cyclically during reproductive life;
- After Menopause, plasma Oestradiol levels fall despite high circulating levels of Gonadotrophins,
- Normal hormonal control of Menstrual Cycle depends on the interaction of hormones secreted from Hypothalamus, Anterior Pituitary and Ovary;

What are some disorders of Female Sex Hormones?

- Some disorders include: **Sub-fertility, Amenorrhoea, Oligomenorrhoea**;
- **Hirsutism**: Increase body hair, male pattern distribution;
  - In most cases it is genetic in origin and benign,
  - May be due to Polycystic Ovarian Syndrome (PCOS),
- **Virilism**: Although uncommon it is a sign of serious disease; Testosterone levels are usually elevated;
- Evidence of excessive Androgen action may occur, e.g.,:
  - Clitoral enlargement,
  - Hair growth in a male pattern,
  - Deepening of the voice and
  - Breast atrophy;
- Tumors of the ovary or of the adrenal are the likely cause;

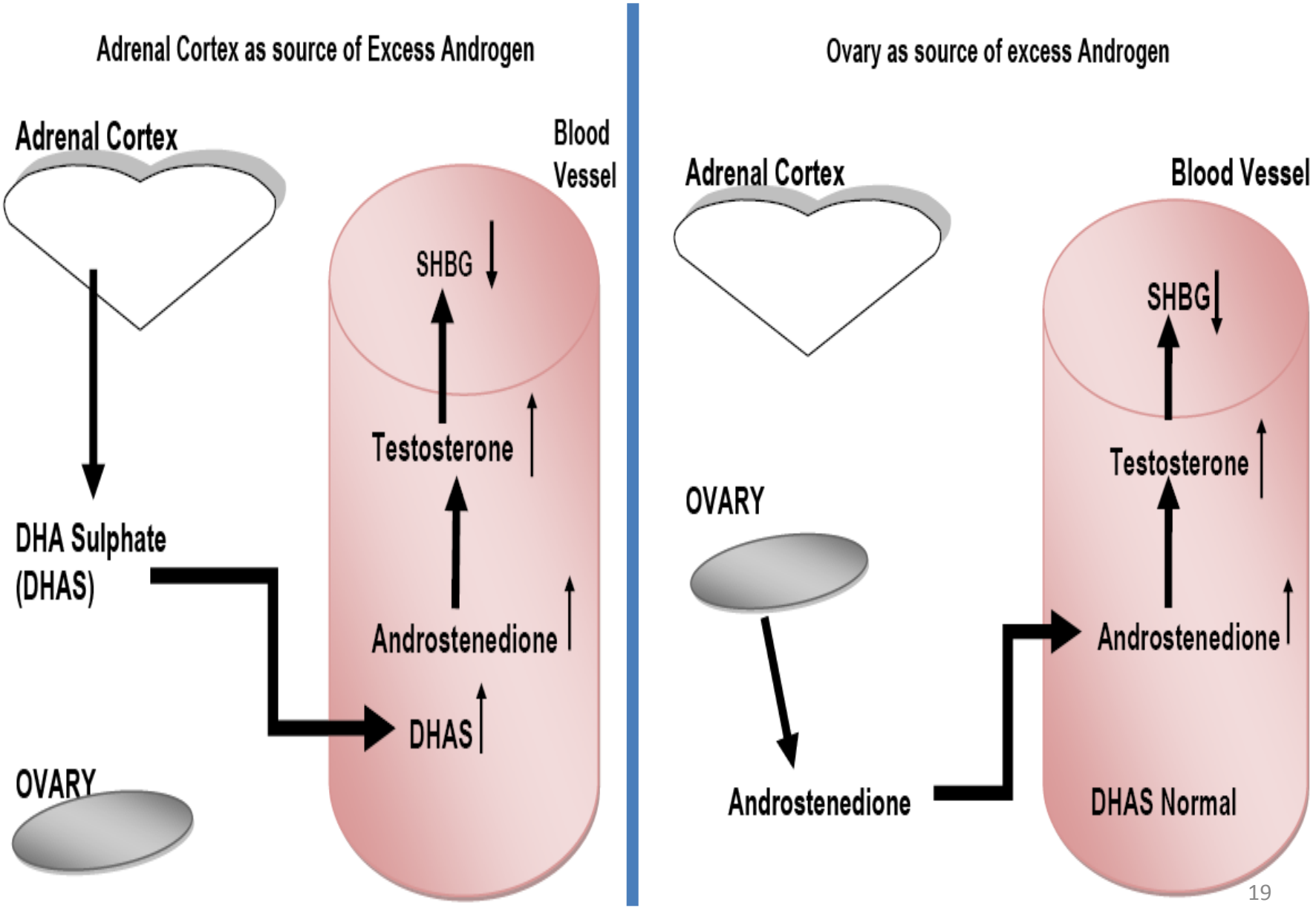
Why carry out the Androgen Screen in female?

- Observation of an elevated Testosterone in a female should always be investigated further;
- A decrease in plasma level of SHBG is evidence of elevated Androgen, because Testosterone inhibits synthesis of SHBG;
- It may not be immediately apparent whether the source of the Testosterone is the Ovary or the Adrenal Cortex;
- "**Androgen Screen**" may be used to establish the source of the Testosterone;
- Androgen Screen is carried out by measuring the levels of other Androgens in plasma, such as:
  - **Dehydroepiandrosterone Sulfate (DHAS), and**
  - **Androstenedione;**

- Schematic representation of Androgen Screen shown in **(Figs. 5a & 5b)**:
- When **DHA Sulfate** and **Androstenedione** are elevated, it suggests **Adrenal gland** is overproducing Androgens;

- When **DHS Sulfate** in normal but **Androstenedione** is elevated, it suggests **Ovary** is overproducing Androgen;

Figs 5a & 5b: Adrenal Screen in female



Endocrine Investigation in the Sub-fertile Female

- Investigation of Infertile Female depends on Phase of Menstrual Cycle;

If there is a Regular Menstrual Cycle:

  - Progesterone should be measured in the middle of the Luteal Phase (day 21);
- If Progesterone is high (> 30 nmol/L):
  - Patient has ovulated and there is no need for further Endocrine Investigation;
- Other causes of subfertility should be sought;
- If Progesterone is low (< 10 nmol/L), ovulation has not occurred;

- For females with Irregular or Absent Menstruation (Oligomenorrhoea or Amenorrhoea) or who are not ovulating, hormone measurements may be diagnostic;
- The established protocol for Investigation can be used;
- Measurement of Oestradiol and Gonadotrophin levels in plasma may detect:
  - Primary Ovarian Failure, or
  - Polycystic Ovarian Syndrome;
- Measurement of Prolactin, and Androgens may assist

What are some of the Endocrine causes of Subfertility in females?

- **Primary Ovarian Failure**:
  - Indicated by elevated Gonadotrophins and Low Oestradiol concentration (Post-Menopausal Pattern);
  - Hormone replacement therapy assists libido and prevents Osteoporosis, but may not restore fertility,
- **Hypogonadotrophic – Hypogonadism**:
  - Subnormal Gonadotrophins and Oestradiol concentrations suggests the presence of Hypothalamic-Pituitary lesion;
  - Mechanisms responsible for Amenorrhoea or Oligomenorrhoea in female with normal Gonadotrophin and Oestradiol concentrations remain to be elucidated;

- **Hyperprolactinaemia**:
  - Prolactin acts on Mammary Glands to control lactation,
  - Gonadal function is impaired by elevated levels of plasma Prolactin;
  - Hyperprolactinaemia can cause Infertility in both sexes;
  - An early indication in women is Amenorrhoea and Galactorrhoea;
- **Some causes of Hyperprolactinaemia** include:
  - Stress;
  - Drugs (e.g. Estrogens, Phenothiazines, α-Methyl Dopa)
  - Primary Hypothyroidism (TRH can stimulate Prolactin);
  - Pituitary diseases;

- **Polycystic Ovarian Syndrome (PCOS)**:
  - Indicated by elevated plasma LH and normal FSH;
  - Oestradiol measurements are often unhelpful,
  - Hirsutism, a feature of this condition, is associated with raised Testosterone and subnormal sex hormone binding protein concentrations;

LET US TAKE A BRIEF LOOK AT PCOS

- Before considering PCOS, one needs to relate **Insulin Resistance** to **Hyperinsulinemia** in females;

What is Insulin Resistance?

- Insulin Resistance is a poorly understood phenomenon in which tissues fail to respond to Insulin,
- Some of the reasons may include:
  - Reduced number or affinity of Insulin Receptors,
  - Others may have normal Insulin binding, but abnormal Post-Receptor responses, such as, problems with activation of Glucose Transport,
  - High expression of Tumor Necrosis Factor-α (TNF-α) in fat cells of Obese Individuals,
    - The greater the quantity of body fat in a susceptible individual, the greater the resistance of normally Insulin-Sensitive cells to the action of Insulin;

- **Insulin Resistance simply means**:
  - Ability of Insulin to dispose of glucose in the Liver, Skeletal Muscle, Adipose tissue and other peripheral tissues is compromised;
- When Insulin Resistance, or Reduced Insulin Sensitivity exists, the body attempts to overcome this resistance by secreting more Insulin from the Pancreas;
- This compensatory state of **Hyperinsulinemia** (high insulin levels in blood) **is used as a marker for the Insulin Resistance Syndrome**;

What causes Insulin Resistance?

- Exact mechanism is not fully known, but Hypothesis have been proposed, including:
  - **Post-Receptor Defect in Adipose Tissue;**
  - **Abnormalities in regulation of Expression of Insulin Gene;**
- Despite Insulin Resistance in Adipose Tissue and Skeletal Muscle:
  - **Ovary remains relatively sensitive to Insulin, and also**
  - **Both Insulin and Insulin-like Growth Factor-1 have stimulatory effects on the production of Androgen by the Ovary;**

How does Hyperinsulinemia relate to infertility in female?

- The central, probably heritable, biochemical abnormality of PCOS is Hyperinsulinemia;
- Hyperinsulinemia leads to **Hyper-Androgenism**:
  - Ovarian overproduction of Testosterone,
  - Adrenal overproduction of Androgens (DHA Sulfate and Androstenedione),
- Increased Testosterone (or Androgens) affects HPO axis in the female, leading to abnormal production of LH and FSH;
- Consequences of abnormal LH and FSH production include:
  - Ovarian underproduction of Estrogen, along with
  - Abnormal production of Progesterone,
  - Overproduction of Testosterone, which consequently results in Amenorrhea and Infertility;

What is the biochemical basis for PCOS?

- Biochemical basis of PCOS is not clearly understood;
- A number of theories have been suggested, including:
  - Evidence of Autosomal Transmission related to strong Genetic Clustering,
    - **A Gene or Series of Genes causes the ovaries to become Sensitive to Insulin stimulation, causing the ovary to overproduce Androgen, while blocking the Maturation of Follicles**;

- **Major underlying disorder in PCOS is Insulin Resistance, with resultant Hyperinsulinemia stimulating excess production Androgens by the Ovaries**;

How does defect in Insulin metabolism promote Hyper-Androgenism in PCOS?

- Exact mechanism whereby defects in Insulin metabolism promote increased Androgen activity in PCOS is not fully understood;
- A number of Hypotheses have been suggested:
  - **Insulin inhibits the biosynthesis of SHBG in the liver, which leads to an increase in plasma level of Free Testosterone**,
  - **Insulin also inhibits the biosynthesis of Insulin-like Growth Factor-1 (IGF-1) Binding Protein in the liver,**

- Reduction in plasma levels of IGF-1 Binding Protein causes an increase in plasma level of circulating Free Insulin-like Growth Factor –1 (IGF-1), which further enhances Ovarian Androgen production;
- In most cases of PCOS the Ovary is the major site of excess Androgen production, but some women with PCOS may have an Adrenal contribution to the increased Androgen production;

OTHER STEROID HORMONES IN BRIEF

- Adrenal Cortex is the site of 2 important steroid hormones:
- **Cortisol:** Main **Glucocorticoid** in humans:
  - Glucocorticoids are 21-Carbon steroids,
  - They promotes Gluconeogenesis,
- **Aldosterone:** Main **Mineralocorticoid** in humans:
  - Mineralocorticoids are 21-Carbon steroids,
  - They promotes retention of Na^+ ions and excretion of K^+ and H^+ ions, particularly in the kidneys;

What is the pathway for biosynthesis of Cortisol?

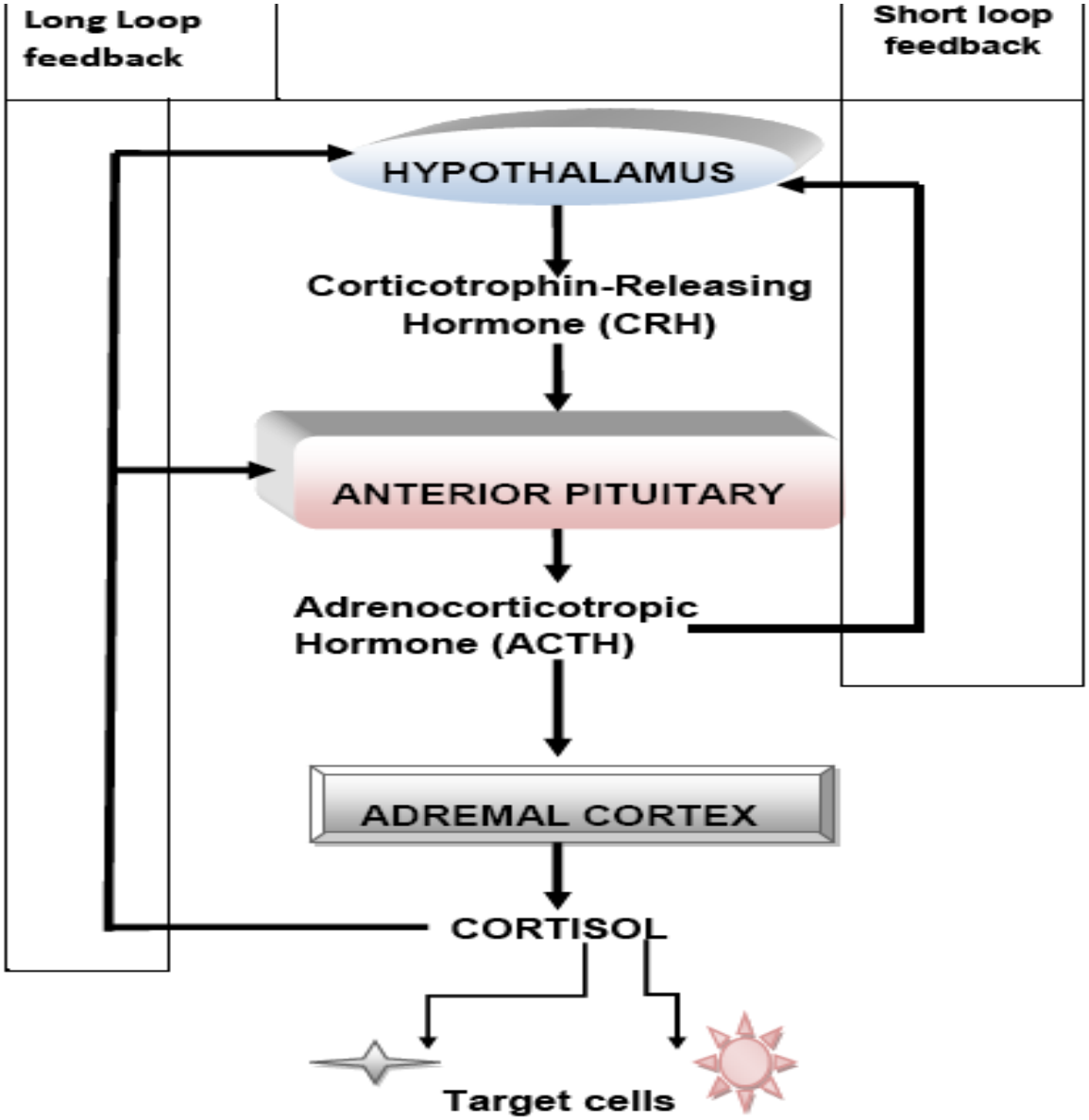
- Pathway for biosynthesis of Cortisol is presented in Flow diagram in **Fig. 1,**
- Cortisol is synthesized from Cholesterol delivered to Adrenal Gland mainly by LDL-Cholesterol;
- Number of LDL receptors is increased when the Adrenal Glands are stimulated by **Adreno-Cortico-Trophic Hormone (ACTH, or Corticotrophin**),
- Adrenal glands are also capable of synthesizing Cortisol via a minor pathway using Acetate,

How is the secretion of Cortisol regulated?

- Cortisol secretion is regulated via Hypothalamic-Pituitary-Adrenocortical axis (HPA- axis) with classic Negative Feedback Control (**Fig. 6**);
- Corticotrophin-Releasing Hormone (CRH) is secreted by Hypothalamus under influence of Cerebral Factors;
- Binding of CRH to Anterior Pituitary induces production of a large compound **Pro-opiomelanocortin (POMC)**,
- POMC is cleaved into various fragments, including **ACTH, Melanocyte-Stimulating Hormones (MSH), Beta-Lipotrophins, and Beta-Endorphins**;

- ACTH acts on Adrenal Cortex stimulating synthesis and secretion of Cortisol;
- Hypothalamic secretion of CRH and Pituitary secretion of ACTH are regulated by Cortisol in Negative Feedback Loops;
- In humans, only Cortisol exerts Negative Feedback on ACTH release;
- In cases of enzyme deficiencies (e.g., 21-Hydroxylase):
  - Cortisol is not produced, thus ACTH secretion cannot be regulated by Negative Feedback;
  - Continuous action of ACTH on Adrenal gland causes Adrenal Hyperplasia; Clinical condition called Congenital Adrenal Hyperplasia (CAH);

Fig. 6: Negative Feedback Control of Cortisol Hypothalamic-Pituitary-Adrenocortical Axis (HPA-Axis)



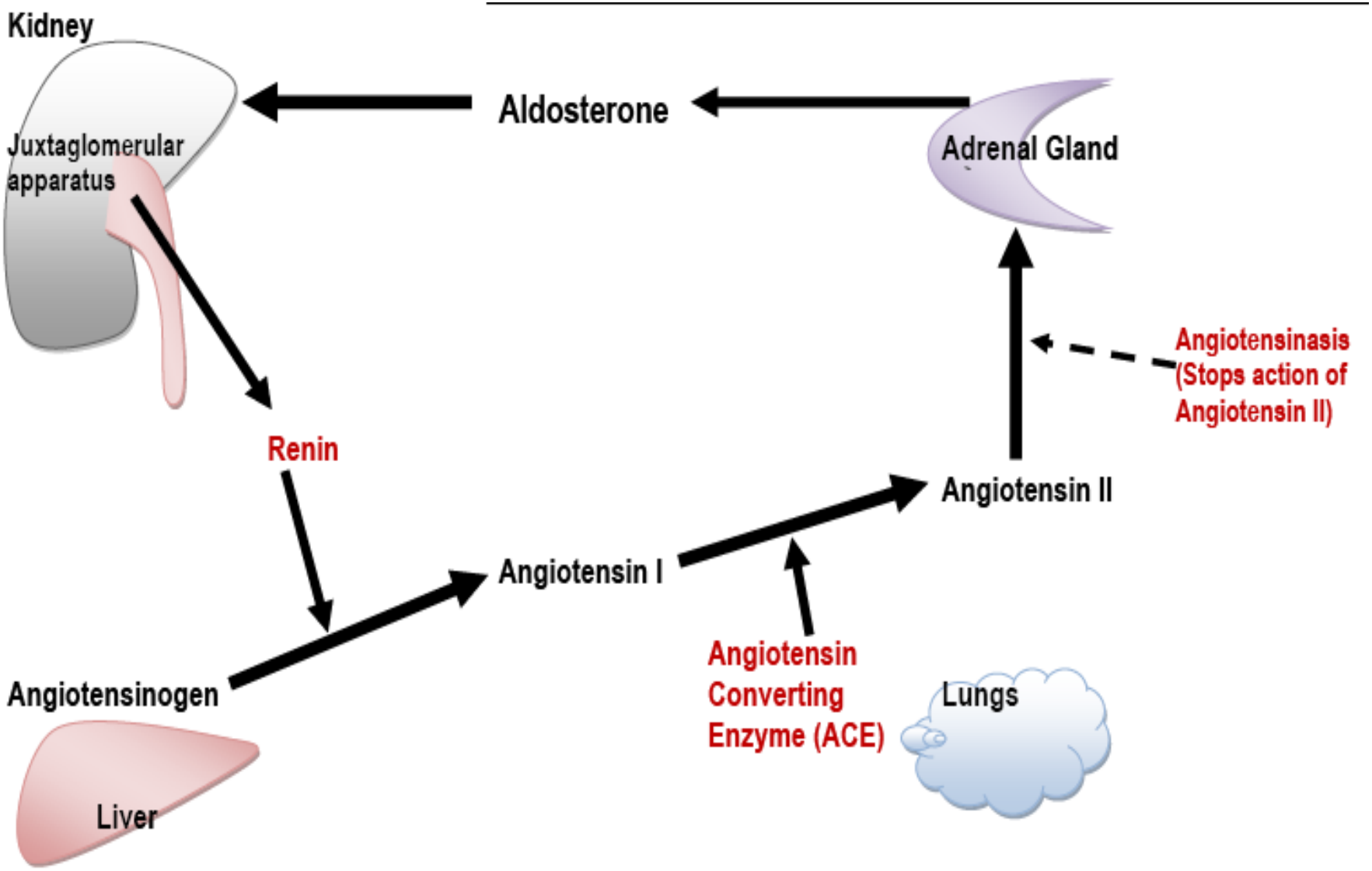
Cortisol Binding in Plasma

- Cortisol secreted by Adrenal Cortex is transported in plasma mainly bound to **Corticosteroid-Binding Globulin (CBG, also called Transcortin);**
- Free Fraction of Cortisol in plasma is biologically active,
- Plasma level of CBG is affected by several factors:
  - Pregnancy and Oestrogen treatment (Oral contraceptives) increases Plasma CBG level;
  - Hypo-proteinaemic state (e.g., Nephrotic Syndrome) causes decrease in plasma CBG level,
  - Parallel changes occur in plasma levels of total Cortisol,
- Cortisol metabolism occurs mainly in the Liver, and products of Cortisol metabolism can be detected in urine as **17-Hydroxycorticosteroids** (**17-OHCS**),

ALDOSTERONE (Mineralocorticoid)

- Primary Regulators of Aldosterone secretion:
  - Renin-Angiotensin system: Stimulates Aldosterone production (**Fig. 7**)
  - Increased plasma K^+ ions stimulates Aldosterone production,
  - Decreased plasma K^+ ions inhibit Aldosterone production

Fig. 7: Renin-Angiotensin-Aldosterone Axis for regulation of Aldosterone secretion



- **Some actions of Aldosterone**:
- Primary role is Na^+ metabolism,
- Primary target is Distal Tubules,
- Actions of Aldosterone cause Kidneys, Gut, Salivary and Sweat Glands to maintain Electrolyte Balance,
- Aldosterone stimulates re-absorption of Na^+ ions and secretion of K^+ and H^+ ions,
- Aldosterone deficiency causes Hyponatraemia, Hyperkalemia, Acidosis;
- Effect on Na^+ and K^+ ions depends on plasma levels:
  - Increased Na^+ uptake = Increased K^+ secretion

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