STEROIDOGENESIS: An Overview

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What are the major Steroid hormones?

- **Hormones synthesized from Cholesterol**; Examples:
- **Progesterone**: Corpus Luteum, involves in changes in Luteral phase of menstrual cycle, differentiation factor for mammary glands;
- **Estradiol (Oestradiol)**: Ovary, responsible for secondary female sex characteristics;
- **Testosterone**: Testes, responsible for secondary male sex characteristics;
- **Aldosterone**: Mineralocorticoid from Adrenal Cortex;
- **Cortisol**: Glucocorticoid from Adrenal Cortex;

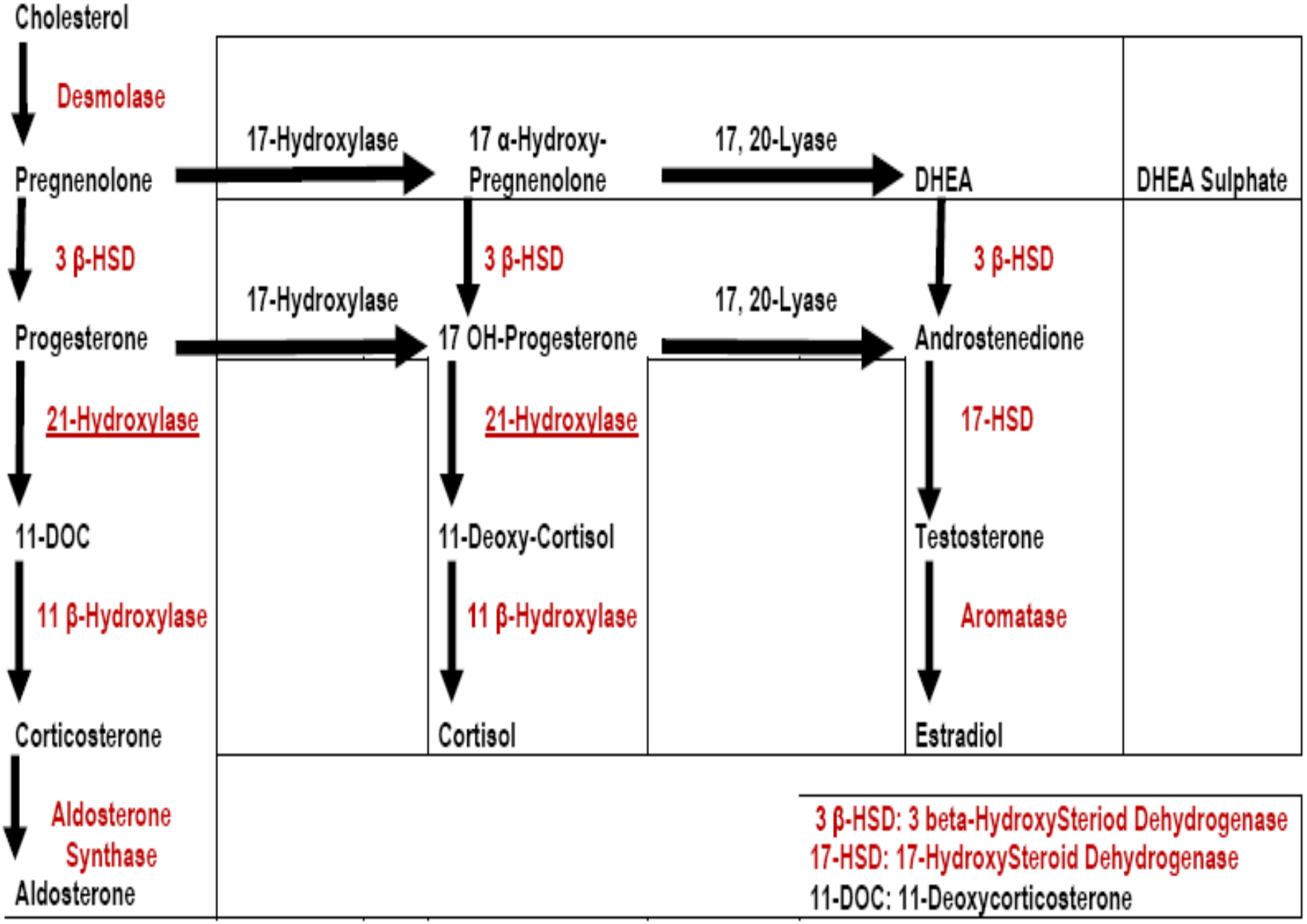
Outline the pathways for biosynthesis of steroid hormones

- Pathways are presented as a flow chart;
- Steroid hormone synthesized in tissue depends on:
  - Complement of Peptide Hormone Receptors,
  - Response to Peptide Hormone Stimulation,
  - Genetically Expressed Complement of Enzymes;

- **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?

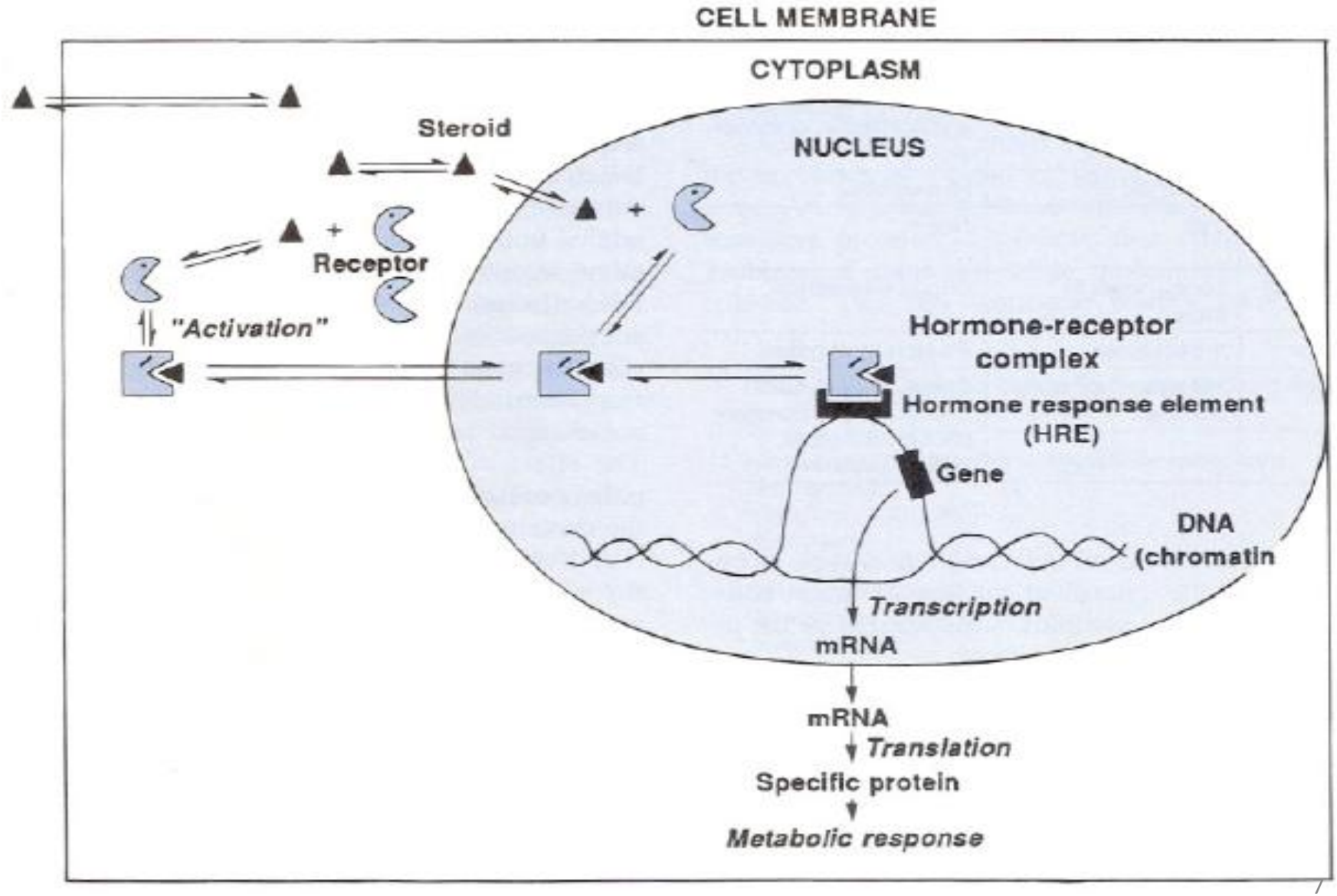
- They are hydrophobic,
- Bound to Specific Hormone Binding Glycoproteins in plasma (bound fractions of steroid hormones);
- **Small amount remains Free in plasma** (unbound fraction);
- **Unbound or "Free" fraction of steroid hormone in blood plasma is Biologically Active Fraction,**
- Measurement of "**Free fraction**" or **binding protein** level is important in diagnosis of patients with certain steroid hormone disorders;

What is the general mode of action of steroid hormones?

- Free fraction goes through membrane in target tissues, binds to Intracellular receptors to form **Steroid Hormone-Receptor (SHR) Complex**,
- Complex exerts action on Nucleus in Target cells,
- **SHR Complex** binds to Specific Nucleotide Sequences on DNA of Responsive Genes,
  - Specific Nucleotide Sequences in DNA are called **Hormone Response Elements (HRE)**,
- Interaction of **SHR 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 mostly bound to **Sex Hormone Binding Globulin (SHBG)**;
- **SHBG** has higher affinity for Testosterone than Oestradiol;
- **Testosterone decreases SHBG** synthesis in liver,
- **Estradiol stimulates SHBG synthesis in** liver,
  - SHBG levels in female is about twice that in male,
- **Factors that alter SHBG levels in blood alter Ratio of Free Testosterone to Free 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 increases SHBG level in blood and Testosterone decreases it, this system functions as a Biological Servomechanism;

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

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

What axis regulates secretion of sex steroid hormones?

- Regulation is by Negative Feedback mechanism on **HPG –Axis (Figs 3 & 4)**
  - **HPG-Axis: Hypothalamic-Pituitary-Gonadal Axis**;
- Hypothalamus releases **Gonadotrophin-Releasing Hormone (GnRH)**,
- **GnRH** acts on Anterior Pituitary to produce **Gonadotrophins**:
  - **Luteinizing Hormone (LH),**
  - **Follicle-Stimulating Hormone (FSH)**

- **Gonadotrophins** act cooperatively on Ovaries in females and Testes in males to stimulate Sex Hormone secretion and reproductive processes;
- **Inhibin** produced by Gonads feed back inhibits production of FSH;

Fig. 3: Hypothalamus –Pituitary-Testicular (HPT) Axis, for feedback regulation of Testosterone secretion (Clinical Biochem 9th Ed, 2013)

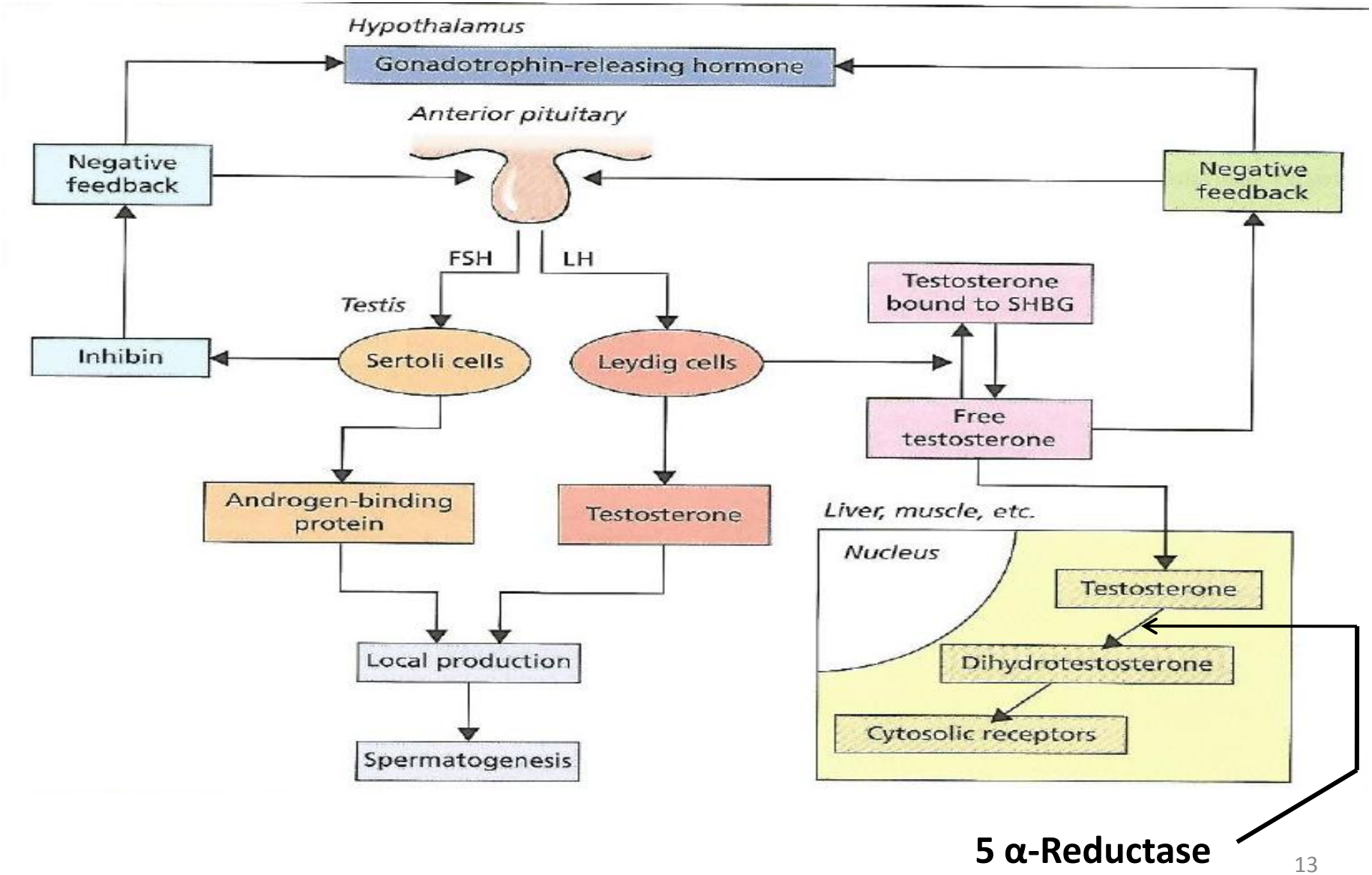
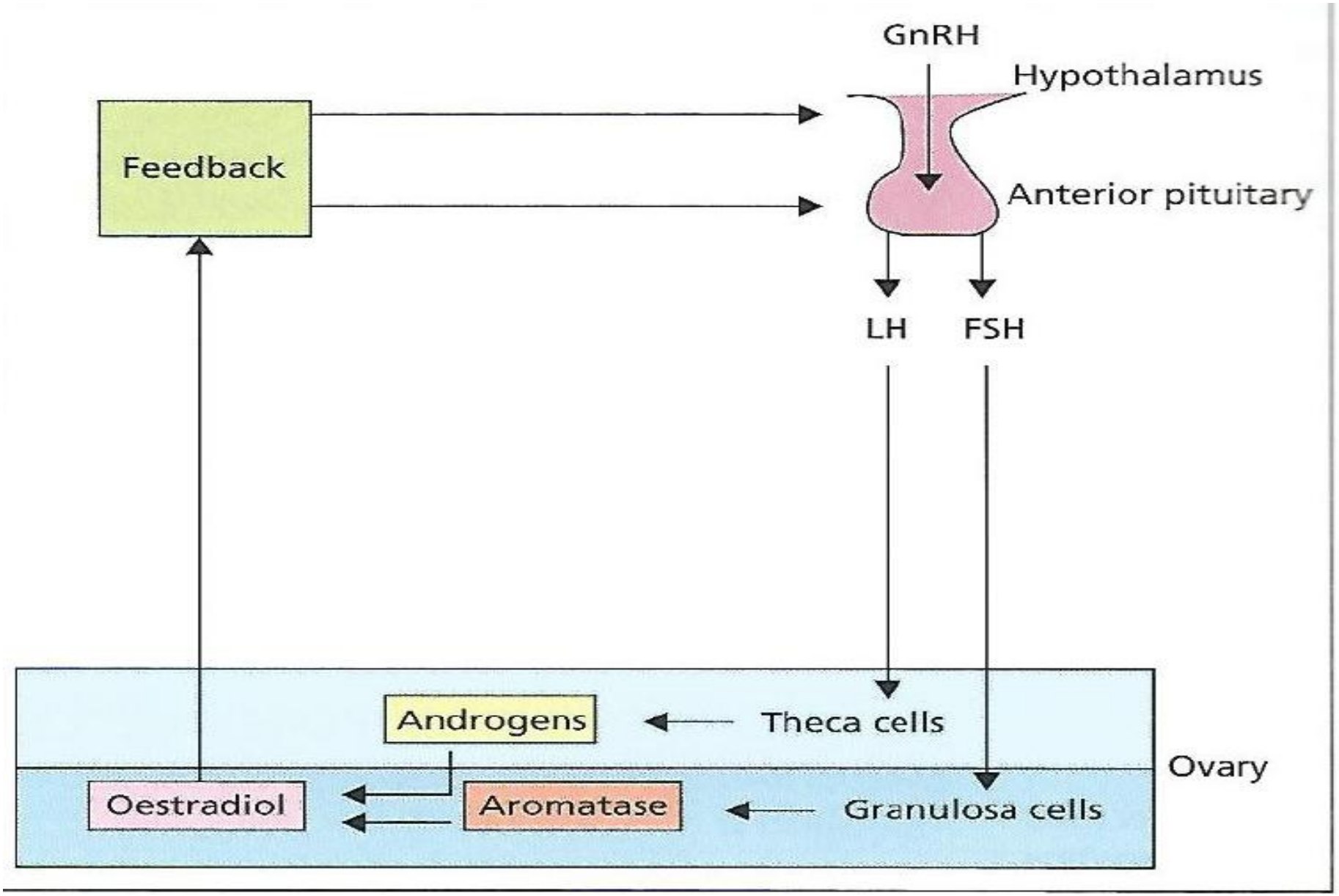


Fig. 4: Hypothalamus –Pituitary-Ovarian (HPO) Axis, for feedback regulation of Oestradiol secretion (Clinical Biochem 9th Ed, 2013)



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
  - Clitoral enlargement,
  - Hair growth in a male pattern,
  - Deepening of the voice,
  - Breast atrophy;
- Tumors of ovary or adrenal are the likely cause;

Why carry out Androgen Screen in female?

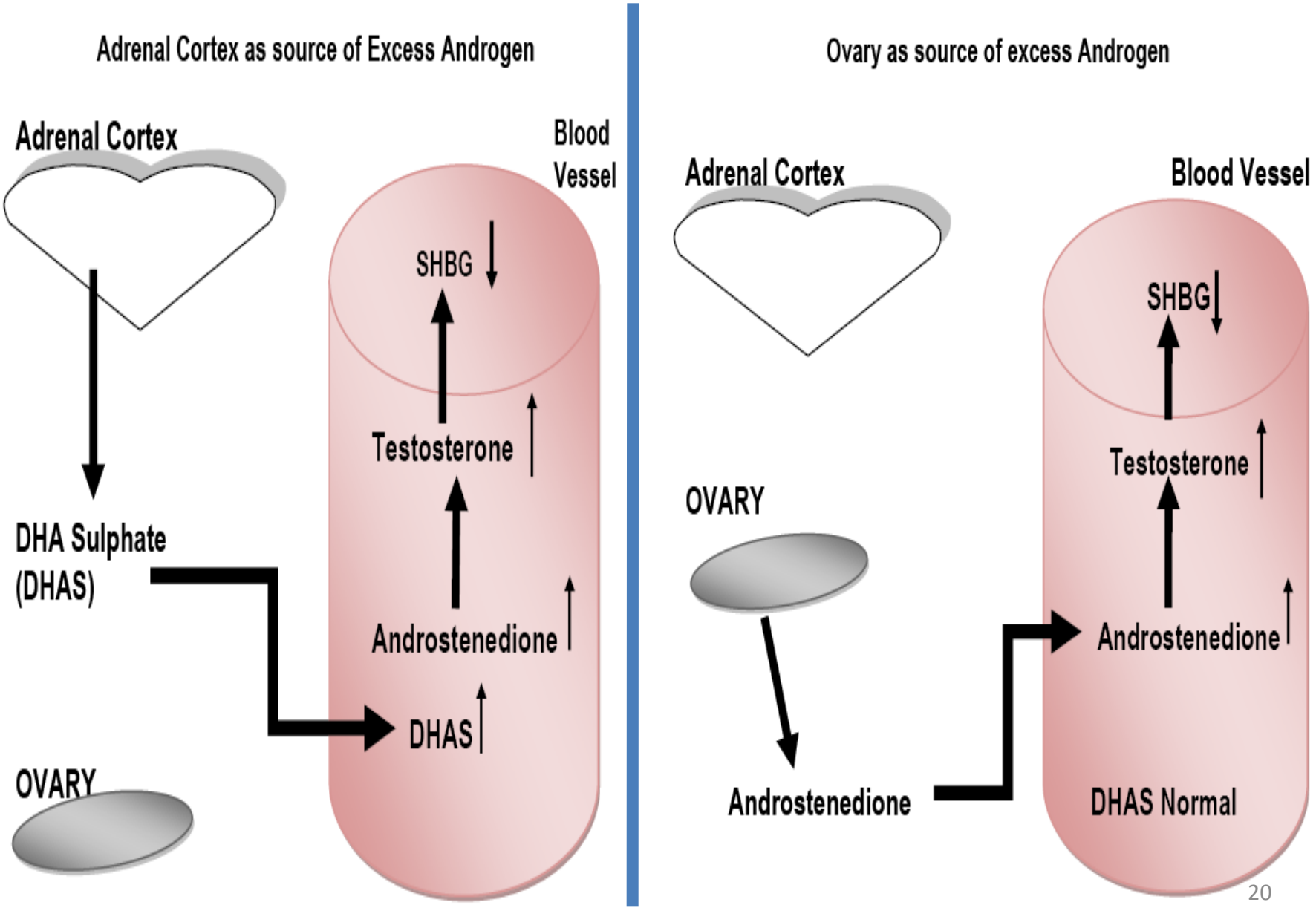
- Observation of elevated Testosterone in a female should always be investigated further;
- A decrease in plasma level of SHBG is evidence of elevated Androgen, because Testosterone inhibits synthesis of SHBG;
  - It may not be clear whether the source of the Testosterone is from Ovary or Adrenal Cortex;

- "**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),**
  - **Androstenedione;**

- **Figs. 5a, 5b:** Schematic diagrams of Androgen Screen
- If **DHA Sulfate** and **Androstenedione** are elevated
  - It suggests **Adrenal gland** is overproducing Androgens;

- If **DHS Sulfate** is 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 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 (> 30nmol/L):
  - Patient has ovulated; there is no need for further Endocrine Investigation;
- Other causes of subfertility should be sought;
- If Progesterone is low (< 10nmol/L), ovulation has not occurred;

- In cases of Oligomenorrhoea or Amenorrhoea, hormone measurements may be diagnostic;
- Established protocols for Investigation are 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

Polycystic Ovarian Syndrome (PCOS)

- Indicated by:
  - **Elevated plasma LH,**
  - **Normal FSH**;
- Oestradiol measurements are often unhelpful,
- Hirsutism, a feature of PCOS, is associated with raised plasma [Testosterone] and low [SHBG];

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**:
  - Tissues fail to respond to Insulin,
  - Ability of Insulin to dispose of glucose in Skeletal Muscle, Adipose tissue, Liver and other tissues is compromised;
- When Insulin Resistance or low Insulin Sensitivity exists, the body attempts to overcome this resistance by secreting more Insulin from Pancreas;
- This compensatory state of **Hyperinsulinemia is used as marker for Insulin Resistance Syndrome**;

State some causes for Insulin Resistance

- Reduced number or affinity of Insulin Receptors,
- Normal Insulin binding, but abnormal Post-Receptor responses, such as, problems with activation of Glucose Transporter,
- High expression of Tumor Necrosis Factor-α (TNF-α) in fat cells of Obese Individuals,
  - The greater the quantity of body fat in susceptible individual, the greater the resistance of Insulin-Sensitive cells to action of Insulin;

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;**

How does Hyperinsulinemia relates to infertility in female?

- Despite Insulin Resistance in Adipocytes and Muscle:
  - **Ovary remains relatively sensitive to Insulin,**
  - **Both Insulin and Insulin-like Growth Factor-1 have stimulatory effects on production of Androgen by the Ovary;**

- **Hyperinsulinemia** is the central, probably heritable, biochemical abnormality in PCOS;
- **Hyperinsulinemia** leads to **Hyper-Androgenism**:
  - **Ovarian over production 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 plasma [LH] & [FSH]:
  - **Ovarian underproduction of Estrogen,**
  - **Abnormal production of Progesterone,**
  - **Overproduction of Testosterone, which may results in Amenorrhea and Infertility;**

What is the biochemical basis for PCOS?

- Several theories have been suggested, including:
- **Evidence of Autosomal Transmission related to strong Genetic Clustering**,
  - **A Gene or Series of Genes causes ovaries to become Sensitive to Insulin stimulation, causing the ovary to overproduce Androgen, while blocking Maturation of Follicles**;

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

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

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

- **Reduction in plasma levels of IGF-1 Binding Protein causes increase in plasma level of circulating Free IGF-1, which enhances Ovarian Androgen production**;

- In most cases of PCOS the Ovary is the major site of excess Androgen production,
  - Some women with PCOS may have Adrenal contribution to the increased Androgen production;

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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?

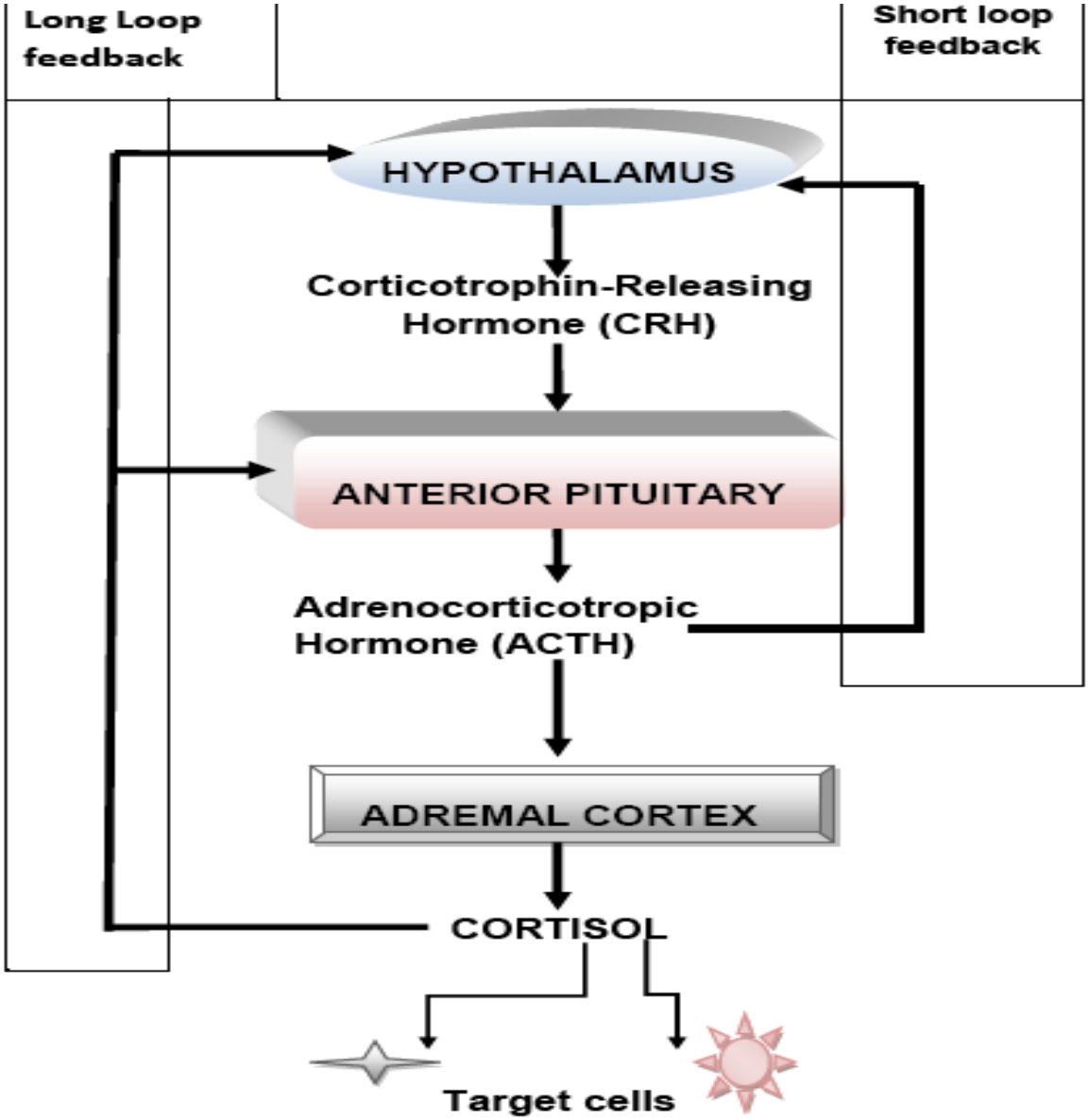
- Pathway for biosynthesis of Cortisol is presented in Flow diagram in **Fig. 1,**
- Cortisol is synthesized from Cholesterol delivered to Adrenal Gland mainly by LDL-Cholesterol;
- Number of LDL receptors is increased when the Adrenal Glands are stimulated by **Adreno-Cortico-Trophic Hormone (ACTH, 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)



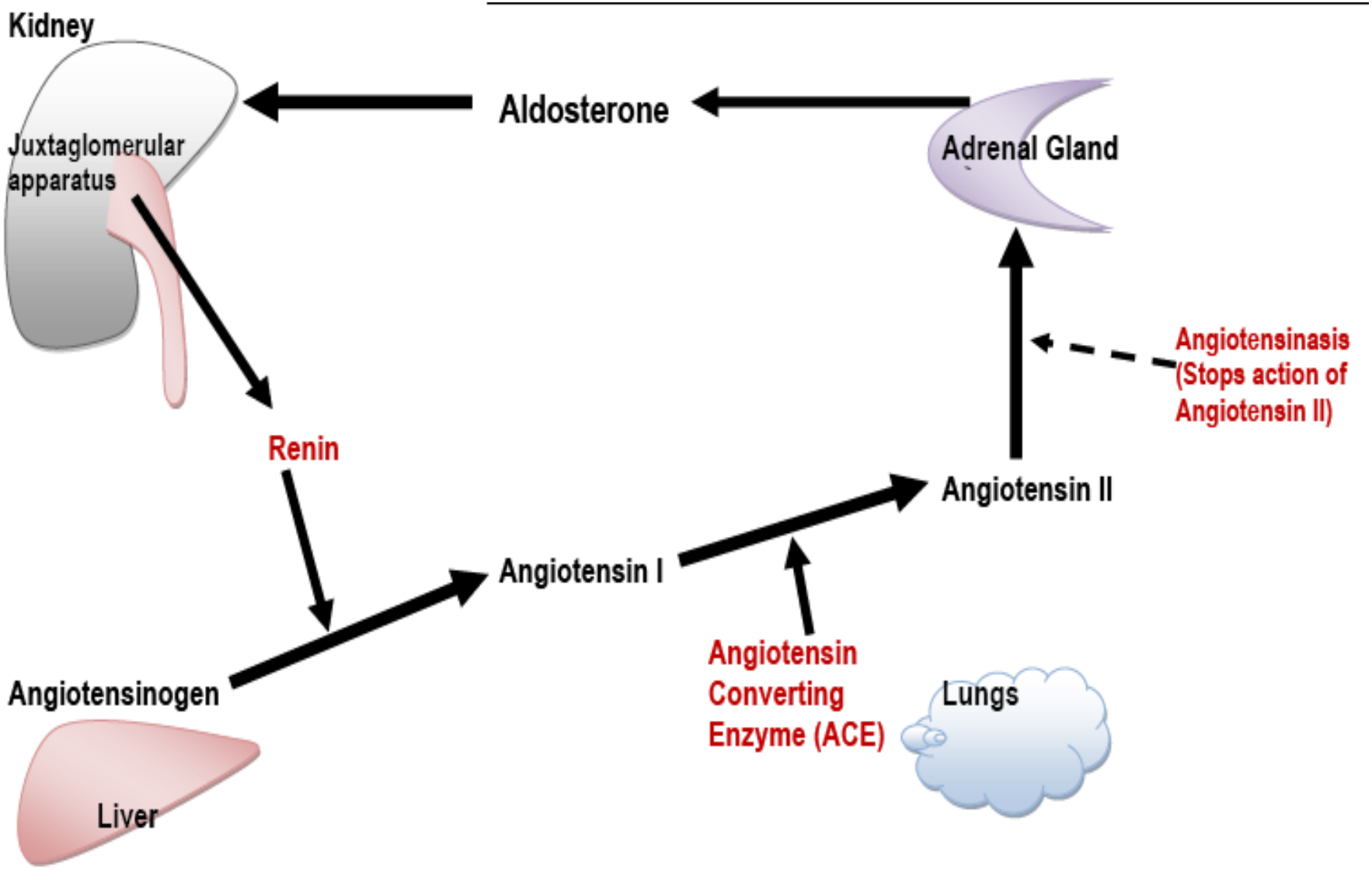
Cortisol Binding in Plasma

- 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