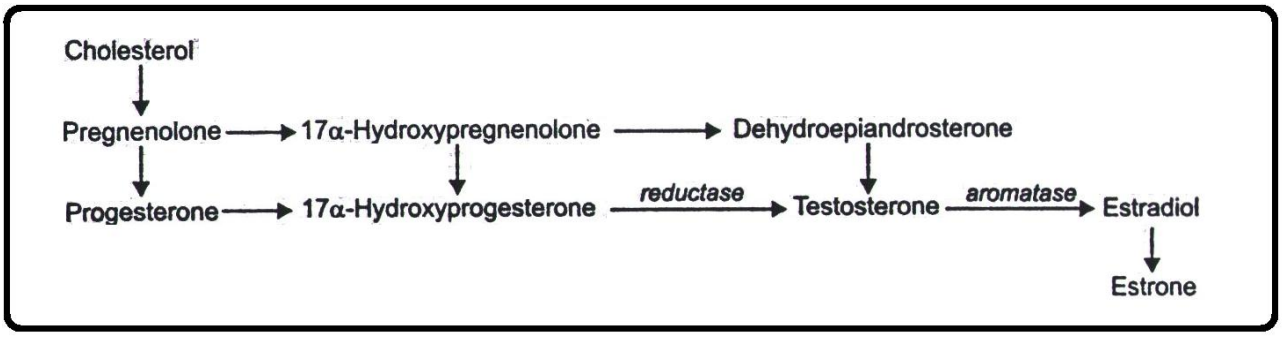
1. **Explain the steps in the biosynthesis of androgens and their regulations.**
   - *Sites of synthesis:* Testes, ovaries, adrenal cortex, and placenta.
     Testicular androgens are synthesized from cholesterol derived from:
     1) Leydig cells in the testes, which synthesize cholesterol and store it as cholesterol ester.
     2) Plasma by receptor-mediated uptake.
   - ***Rate-limiting step:*** Cholesterol delivery to the inner mitochondrial membrane by the transport protein, steroidogenic acute regulatory protein (STAR).
   - *Steps:*
     1) Conversion of cholesterol to *pregnenolone* by mitochondrial enzyme complex cytochrome P450 side chain cleavage (P450 see), which catalyzes oxidative side chain cleavage of cholesterol. This step is regulated by LH and ACTH.
     2) Translocation of pregnenolone from Leydig cells to the endoplasmic reticulum where the remaining biosynthetic reactions occur.
     3) Formation of testosterone from pregnenolone by the concerted action of the enzymes such as 3β-hydroxysteroid dehydrogenase (3β-OHSD), $\Delta^{5,4}$ isomerase, 17α-hydroxylase. C_{17-20} lyase, and 17β-hydroxvsteroid dehydrogenase (17β-0HSD).
     4) In addition in testosterone, small quantities of dehydroepiandrosterone, androstanediol, dihydrotestosterone (DHT), and *17β-estradiol* are also synthesized by the testes.
     5) *Estradiol* is derived mostly from aromatization of testosterone in peripheral tissues in males
     6) In females, testosterone is mostly derived from the adrenals, and only one-third is synthesized in the ovaries.



   - Regulation:
     - The anterior pituitary hormone LH regulates the synthesis and secretion of testosterone by the following sequence of events:
       - Binding to receptors on the plasma membrane of Leydig cells.

- Activation of adenylate cyclase
- Increase in cAMP levels.
- Increase in the rate of cholesterol side chain cleavage.

➢ ***Regulation of LH secretion:***

- By GnRH from the hypothalamus.
- Feedback by testosterone that may be exerted at the level of the hypothalamus or the anterior pituitary.

➢ *The regulatory effect of ACTH on the adrenals is similar to that of LH.*

➢ ***FSH***

- Sensitizes Leydig cells to the actions of LH by increasing the number of LH receptors.
- Promotes the synthesis of androgen-binding protein (ABP) by the Senoli cells.
- ABP binds testosterone and transports it from the Leydig cells to the site of spermatogenesis.
- Secretion is inhibited by inhibin produced by the Sertoli cells.

2. **How is testosterone transported?**

About 97-99% of testosterone is transported in circulation bound to the following proteins:

- *Sex hormone-binding globulin (SHBG),* or *testosterone-estrogen-binding globulin (TEBG),* a β-globulin synthesized in the liver, accounts for most of the testosterone binding. SHBG synthesis is increased by estrogens and decreased by androgens.
- Plasma albumin.

The free fraction, which constitutes only 1-3% of total testosterone, is the biologically active hormone.

3. **How is testosterone metabolically activated and inactivated?**

Metabolic activation

- Less than 10% of testosterone is activated to *dihydrotestosterone (DHT)* or *estradiol*, and 90% is: converted to excretory metabolites.
- Testosterone is converted to its metabolically active form dihydrotestosterone by the enzyme *5α- reductase* in male accessory organs, brain, skin, and hair follicles. DHT is a potent androgen.
- Aromatization of testosterone forms estradiol in peripheral tissues such as adipose tissue.

Metabolic inactivation

- Metabolic inactivation of testosterone takes place in the liver.
- Testosterone is converted to the 17-ketosteroids, androsterone and etiocholanolone, which undergoes conjugation with glucuronate or sulphate to form water-soluble compounds that are excreted in the urine.

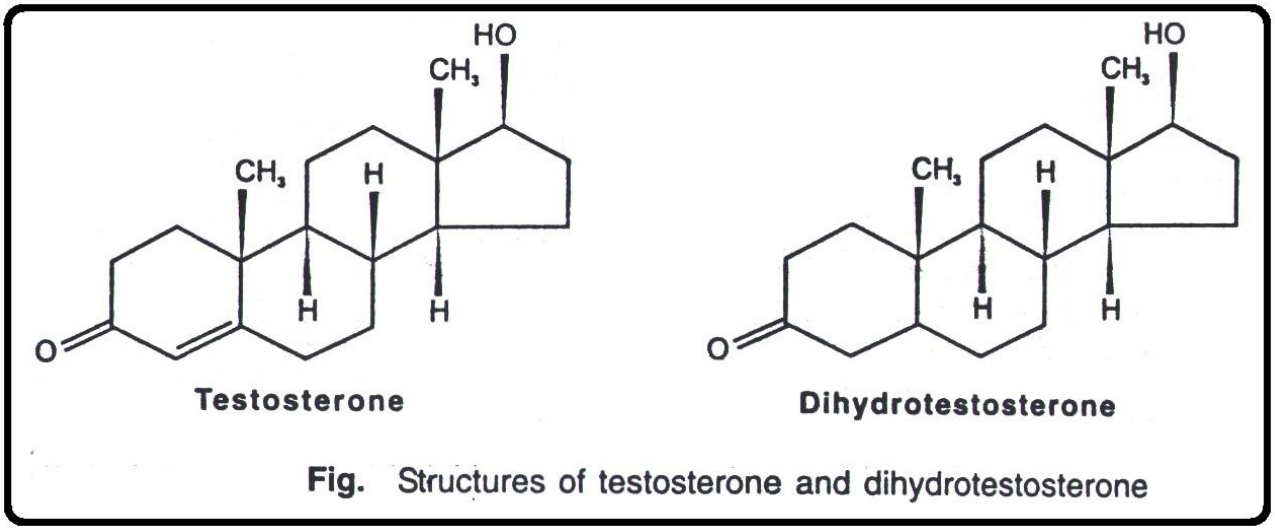


Fig. Structures of testosterone and dihydrotestosterone

4. **Elucidate the biological actions of testosterone?**

Testosterone and DHT exert the following biological effects:

- *Sexual differentiation during embryonic development, and development of male secondary sexual characteristics*. Testosterone is necessary for sexual differentiation and spermatogenesis, whereas DHT is essential for development of the male external genitalia. Both testosterone and DHT are required for the male phenotype.
- *Anabolic and growth promoting effects* such as growth of bone, increase in skeletal muscle mass, and redistribution of fat.

Mechanism of action:

- Free testosterone enters the cytoplasm of target cells through the plasma membrane where it is converted to DHT.
- Both testosterone and DHT bind to a specific intracellular receptor and the hormone-receptor complex binds to nuclear chromatin selectively activating transcription of specific genes.
- Testosterone increases the activity of DNA polymerase and thymidine kinase, and the synthesis of androgen-binding protein.

5. **Outline the causes and diagnostic tests for hypogonadisim**.

Hypogonadism refers to a clinical condition characterized by defective spermatogenesis and/or testosterone production.

- ***Causes :*** Hypogonadisim may be calssified into two types:
  - ***Primary hypogonadism*** due to testicular disease with defective seminiferous tubular function, decreased spermatogenesis, and infertility, or defective Leydig cell function with failure of spermatogenesis.
    - ✓ *Klinefelter's syndrome* with 47 XXY genotype and enzyme defects such as 5α-reductase.
    - ✓ Bilateral testicular torsion.
    - ✓ Radiation and cytotoxic drugs.
  - ***Secondary hypogonadism*** may be due to:
    - ✓ Pituitary disorders panhypopituitarism, prolactinomas.
    - ✓ Hypothalamic disorders such as Kallman syndrome.
- ***Diagnostic Tests***
  - *Measurement of plasma testosterone:* Levels are low in both primary and secondary hypogonadism.
  - *Measurement of LH and FSH*: Plasma LH is increased in Leydig cell failure, and plasma FSH in defective seminiferous tubular function. Decrease in both LH and FSH with oligospermia suggestive of pituitary or hypothalamic disease.
  - *hCG stimulus test:* Administration of human chorionic gonadotropin (hCG) to test Leydig cell function.
    - ✓ Testosterone is estimated in blood drawn at 9.00 hours.
    - ✓ hCG 2000 IU is administered by intramuscular injection on day 1 and 3, and blood withdrawn on the fifth day for testosterone estimation.
    - ✓ An increase in plasma testosterone is seen in normal subjects, whereas in primary testicular failure, the response is decreased or absent
    - ✓ The response may, however, be normal in secondary testicular failure.
  - GnRH test is used in the differential diagnosis of hypogonadism.
    - ✓ The test involves injection of GnRH (100 ug i.v.) and measurement of plasma LH and FSH before, and at 30 and 60 minutes after GnRH injection.
    - ✓ In pituitary disease, the response of LH and FSH to GnRH may be normal, decreased, or absent; whereas in hypothalamic disease, the response may be delayed, normal, or decreased.

6. **Give a brief account of the biosynthesis of estrogen and its regulation.**
   - *Site of synthesis:* Ovary in non-pregnant females, the testes in males. Estrogen biosynthesis -also occurs in the liver, muscle, adipose tissue, hypothalamus, and hair follicles.
   - *Precursor molecule:* Testosterone.
   - *Conversion of testosterone to estradiol* is accomplished by a microsomal enzyme system called the aromatase complex in a process involving three successive hydroxylations and requiring three molecules each of NADPH and O_2.
   - In males, peripheral aromatization of testosterone accounts for significant production of estrogens.
   - *Adrenal androgens* are responsible for estrogen synthesis in postmenopausal women and for about 50% of estrogen synthesis during pregnancy.

Regulation

- LH stimulates theca cells to synthesize androgen precursors of estrogens.
- **FSH**
  - ✓ Stimulates growth of the ovarian follicle.
  - ✓ stimulates estrogen synthesis by granulosa cells.
  - ✓ Stimulates development of LH receptors on both theca and granulsoa cells.
  - ✓ In inhibited by **inhibin**.
- Secretion of LH and FSH
  - ✓ Is regulated by GnRH.
  - ✓ Is inhibited by estrogen.

7. **Discuss the causes, diagnosis, and management of amenorrhea.**

Amenorrhea refers to the absence of menstruation.

❖ Causes:
- Non-endocrinal causes such as pregnancy and menopause.
- Endocrinal causes such as:
  1) *Primary ovarian failure* as in *Turner's syndrome* with 45X0 karyotype, characterized by developmental abnormalities, delayed puberty, low plasma estrogens, and increase in FSH and LH.
  2) *Polycystic ovary syndrome* (Stein-Leventhal syndrome), characterized by large polycystic ovaries, high plasma LH levels, chronic anovulation, obesity, androgen excess, hirsutism, and infertility.
  3) *Hypogonadotrophic hypogonadism* resulting from pituitary or hypothalamic disease as in:

- ✓ Panhypopituitarism following treatment of pituitary adenomas by surgery or radiation.
- ✓ Prolactinomas.
- ✓ Kallman syndrome, due to defective GnRH synthesis or secretion, characterized by sexual1 immaturity and eunuchoid habitus.
- ✓ Deficiencies of GH, ACTH, thyroid hormone, and vasopressin.
- ✓ Excessive weight loss with anorexia nervosa.
- ✓ Malignancies.
- ✓ End-stage kidney disease.

❖ ***Diagnosis***

- Increase in plasma FSH with decrease in estradiol suggests ovarian failure.
- Increase in plasma LH in the absence of pregnancy indicates polycystic ovary syndrome.
- Normal or low plasma FSH and LH levels with low plasma estradiol levels suggest pituitary or hypothalamic disorder.

❖ ***Management***

- Bromocriptine administration in amenorrhea due to a pituitary tumor.
- Cyclical estrogen and progesterone replacement in patients with ovarian, pituitary, or hypothalamic disease, when fertility is not required.
- Administration of FSH and LH in pituitary failure if fertility is required.
- Administration of clomiphene in hypothalamic disease to block estrogen receptors in the hypothalamus, and to stimulate GnRH with secretion of LH and FSH.

8. **Outline the laboratory tests for the investigation of infertility.**

The laboratory tests for investigation of infertility include the following:

❖ ***In females:***

- Plasma progesterone level is a reliable indicator of ovulation. A value of < 10 nmol/L indicates failure in ovulation or luteal function.
- Ovarian ultrasound examination to monitor follicular development and ovulation.
- Evaluation of luteal function by histological examination of an endometrial biopsy specimen.

❖ ***In males:***

- Semen analysis.
- Evaluation of plasma FSH.
- Low plasma testosterone with increase in LH indicates Leydig cell failure.
- A decrease in both plasma LH and testosterone requires evaluation of pituitary and hypothalamic function.

- High plasma FSH indicates failure of seminiferous tubules, while low FSH woth oligospermia pinpoints pituitary or hypothalamic disorder.
- High plasma prolactin indicates pituitary tumor.

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Good Luck

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Mohammed Al-Haddad

ممثل المرحلة : حسين شاكر محمد

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"Good advice is always certain to be ignored, but that's no reason not to give it."

Agatha Christie, British Novelist