Unit-5

÷ Sex Hormone ÷

→ The sex hormone are the basically steroidal in nature they are steroidal hormone. synthesies in our body.

⇒ The sex steroidal hormone are release from the adrenyl cortex, basically they are release from the Zonareticuleris part of the adrenyl cortex they are called sex steroid hormone.

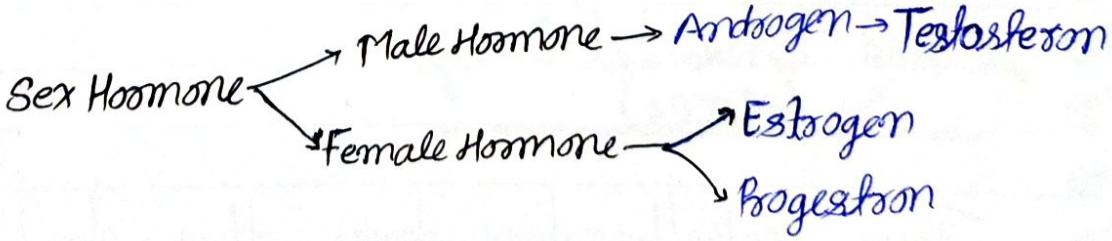
⇒ The sex hormone can be classified on the male or female it is of two type.

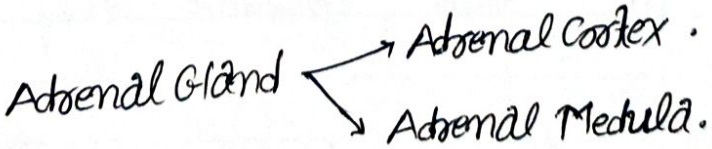
(i) Male hormone

(ii) Female hormone

→ The male hormone basically called Androgen Example is testosteron which is release from the testis of the male.

→ And the female hormone is of two type (i) Estrogen (ii) Progestron.





÷ Estrogen ÷

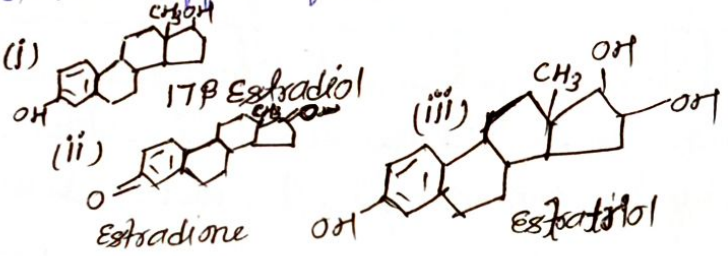
→ Estrogen is a female hormone the chemical name is 17 β Estradiol.

→ They contain the steroidal ring of 18 carbon and they have two hydroxy group.

→ The estrogen hormone was first discovered and isolated by Loewe and Longe in 1926. this is from urine of pregnant Lady.

→ He said that in the urine of Pregnant lady Estrogen is available into three diff-2 form.

(i) Estradiol
(ii) Estradione
(iii) Estratriol



→ The natural Estrogen do not given orally have the good oral absorption but they are not given orally because they have high first Pass metabolism. so to avoid the first pass metabolism natural estrogen never given in the form of oral.

→ There are two types of synthetic estrogen molecule which are given orally because they do not undergoes first pass metabolism.

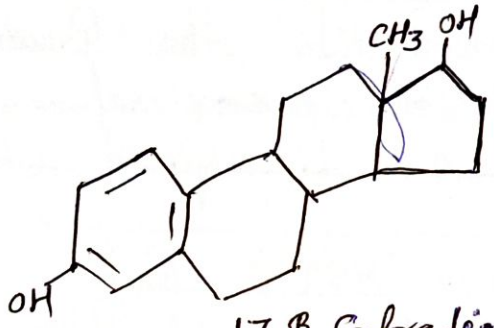
Ex- Ethynil Estradiol, Diethylstibesteral.

→ After the releasing of steroidal hormone they goes into the blood and bind with the globulin protein. and they form the SSBG complex (Specific steroid Binding Globulin) and goes into the diff-2 part of the body.

→ After binding with the receptar they goes into the liver and from liver they are metabolise by the oxidation & Hydroxylation process. into Estradione and Estradiol. and they are metabolic product.

⇒ When we take the sample of urine of pregnant lady we can find these molecule very well.

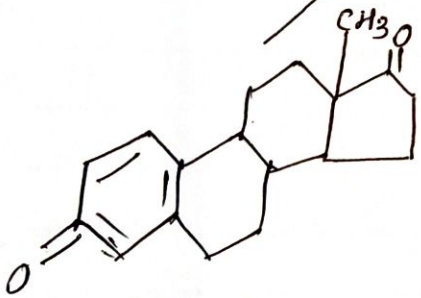
→



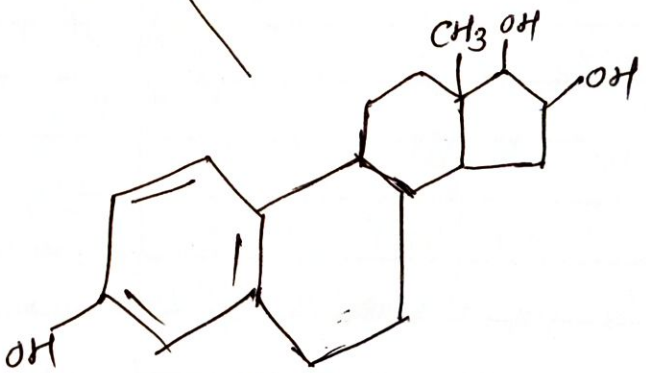
17 β Estradiol

Oxidation

Hydroxylation



Estradione



Estradiol

Pharmacology of Estrogen:-

→ It works as female sex hormone.

→ Basically the estrogen helps to the development of the different sexual part of the female body for example they developed the ovary for the ovulation, and they developed the endometrium wall after the menstrution cycle. and they developed the deff ~ menstrution cycle phase.

→ Estrogen developed the secondry character of the female like, change in the voice, change in the nature, development of gonades, menstrutation cycle, development of breast.

→ During the pregnancy when the estrogen level is high in the female then the level of two hormone FSH (Follicular stimulating hormone) LH (Leutinising Hormone) is decrease by the negative feedback mechanism.

→ Eatrogen can also produce some week anabolic effect like lipolysis decrease of the cholesterol level Increase In the HDL Level.

→ It is also carcinogenic in nature. it may develope the cancer of ovary & breast for prolong release of the drug.

Adverse Effect:- (i) Nausea Vomiting.
(ii) Anorexia.
(iii) Mild diarrhoea thermogenic.
(iv) Decrease Protein Synthesis.

Therapeutic Effect:- (i) Therapy after Menopause.
ii Post menoposal osteoporosis.
(iii) Contraceptive Pills.

Antiestrogen / Ovulation Stimulants:-

→ Clomiphene
→ Ethamoxy triphetal
→ Danazal.
→ Tamoxifen

÷ Natural Progestin ÷

Progesterone ÷ (21 carbon steroid) is derived from cholesterol

→ It was first isolated in 1929.

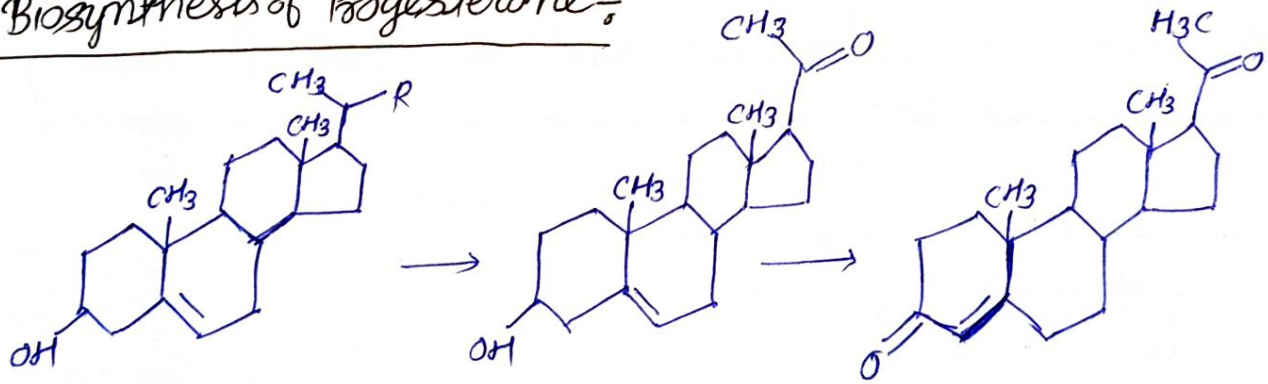
→ It is secreted by corpus luteum (10-20mg/day) in later half of menstrual cycle under influence of LH.

→ If ovum gets fertilized and implants - the blastocyst immediately starts producing chorionic gonadotropin which is absorbed and sustains the corpus luteum in early Pregnancy.

→ Secretion of Progesteron starts from Placenta from 2nd trimester till term.

→ Males also produces 1-5 gm progesterone per day from adrenals and testes.
(But Role in males is not known).

Biosynthesis of Progesterone ÷



Cholesterol → Pregnenolone → Progesteron.

R = $CH_2CH_2CH_2CH(CH_3)_2$

Synthetic Progestin's ÷

(i) **Progesterone Derivatives ÷** These are Pure Progestins they have weaker anti ovulatory action and are used primarily as adjutants to estrogens' for: HRT threatened abortions endometriosis.

(ii) 19 Nortestosterone derivatives:- They have weak estrogenic androgenic and anabolic action but have potent anti ovulatory action.

→ They are mainly used in combined contraceptive Pills. like Desogestrel and Norgestimate (Brodrug)

(1) Progesterone Derivatives
- → Medroxyprogesterone acetate.
- → Megestrol Acetate
- → Dydrogesterone
- → Hydroxyprogesterone caproate

(2) 19- Nortestosterone Derivative (OLD)
- → Norethindrone
- → Lynestrenol
- → Levonorgestrel (gonane)

(3) 19 Nortestosterone Derivative (New)
- → Desogestrel
- → Norgestimate
- → Gestodene.

Actions:- It prepares uterus for nidation and helps in maintance of pregnancy by preventing endometrial shedding decreases uterine motility and inhibiting immunological rejection of fetus. (T Cell function and cell mediated immunity.

Specific Action:- **Uterus:-** Secretory changes in estrogen primed endometrium (hyperemia, Tortuocity of glands and increased secretion)

→ Continued action of progesterone cause decidual changes in endometrium (Stroma enlarges, becomes spongy, glands atrophy and decreases sensitivity of myometrium to oxytocin.).

Cervix:- Converts watery secretion (Estrogen produced) to viscid, scanty and cellular which is hostile to sperm penetration.

Vagina:- Induces pregnancy like changes in the mucosa leukocyte infiltration of cornified epithelium.

Breast:- It causes proliferation of acini in the mammary gland. Acting along with estrogen which prepares the breast for lactation.

CNS:- May have sedative effect.

Body Temprature:- Causes slight (0.5C) rise in body temp.

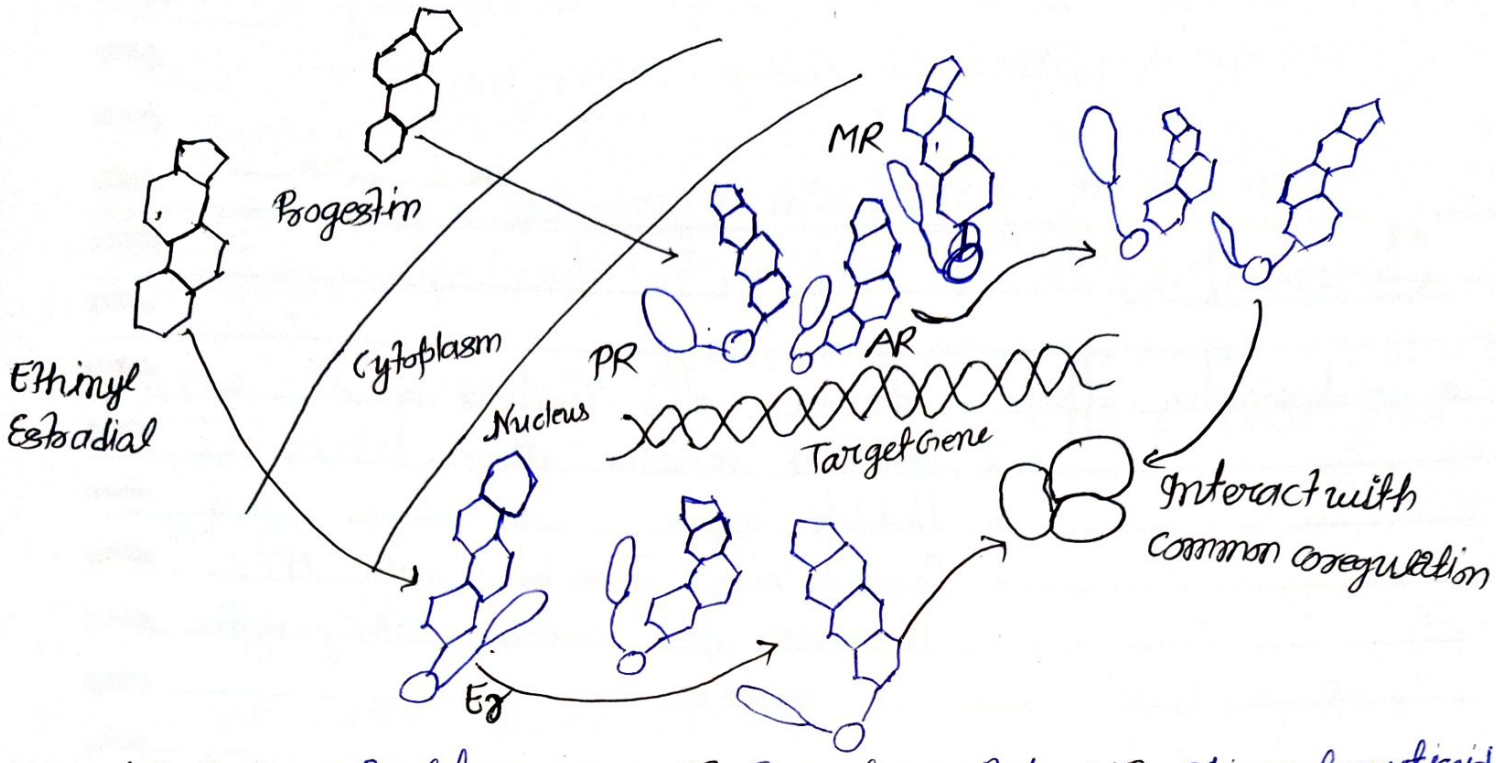
Respiration:- May stimulate respiration at high doses.

Metabalism:- Prolonged use of OCP impairs glucose tolerance. They also tend to raise LDL and lower HDL (that is 19-nortestosterone derivatives.)

Pituitary:- Progesterone is a weak inhibitor of Gonadotrophin secretion

:-Mechanism of Action:-

→ The progesterone receptors (PR) has limited distribution in the body confined mainly to the female genital tract, breast, CNS and pituitary upon hormone binding PR undergoes dimerization, attaches to progesterone responce element (PRE) of target genes and regulate transcription trough coactivators.



AR - Androgen Receptor PR - Progesterone Recp. MR - Mineralocorticoid receptor.
GR - Glucocorticoid Receptor. ER - Estrogen Receptor

∴ Pharmacokinetics ∴

→ Progesterone is orally inactive because of high first pass metabolism in liver.

→ Hence mostly given by I.M. in oily solution. I.M doses are rapidly cleared from plasma with a short t1/2 (5-7 min) and nearly completely degraded in liver (Major product pregnanediol excreted in urine as glucuronide and sulphate conjugates).

→ However the effects of progesterone lasts longer.

→ Micronized formulations has been developed for oral administration which contains micro fine particles of progesterone suspended in oil and dispensed in gelatin capsules

→ Its absorption occurs through lymphatics.

→ Most synthetic progestins are orally active, metabolised slowly, and have plasma $t_{1/2}$ btw 8–24 hours.

Adverse Effects :- → Breast engorgement, headache, rise in body temp, edema, esophageal reflex, acne and mood swings. may occur with higher doses.

→ Irregular bleeding (amenorrhoea) may occur if given continuously.

→ 19-nortestosterone derivatives lower Plasma HDL level there by may promote atherogenesis (Not seen in progesterone and its derivatives).

→ Long term use in HRT may increase risk of breast cancer

→ Blood sugar may rise and diabetes may be precipitated by long term use (levonorgestrel)

⇒ Intramuscular injections of progesterone are painful

⇒ If given in early pregnancy they can cause masculinization of female foetus or their congenital abnormalities.

% Uses :- (i) As Contraceptive :- Postcoital contraceptive (Mifepristone 600 mg within 72 hr) once a month contraceptive (Mifepristone - 200 mg 2 days after mid cycle)

(ii) Hormone Replacement therapy (HRT) :- Used in non hysterectomised post menopausal women estrogen therapy is supplemented with a progestin for 10–12 days each month to reduce the risk of endometrial carcinoma.

(iii) DUB (Dysfunctional Uterine bleeding): Progestin in large dose (Norethindrone 20-40 mg/day) Promptly stops the bleeding and keeps it abeyance as long as therapy is given.

⇒ Cyclic treatment regularizes and normalizes menstrual flow.

(iv) Threatened habitual abortion: A pure progestin without estrogenic or androgenic activity may show efficacy in preventing premature delivery in high risk pregnancy.

Preparation and Dose:

(1) Progesterone: 10 - 100 mg I.M OD.
"Progest, Proluton, Gestone, Naturogest, Ogest

(2) Hydroxyprogesterone Caproate: 250 - 500 mg I.M at 2-14 day intervals. Proluton Depot, Maintane Inj, Procaperm.

(3) Medroxyprogesterone Acetate: 5-20 mg OD-BD oral 50-150 mg I.M at 1-3 months interval.

→ Farlutal, Provera, Meprate, Modus, Depat-Provera.

(4) Dydrogesterone: 5-10 mg OD/TDS oral Duphaston.

(5) Norethindrone: 5-10 mg OD-BD oral, Primolutn Styptin, Regestrone, Norgest, Regestrone, HRT, Norela, HRT, Noristerat

(6) Lynestrenol (Ethinylestrenol): 5-10 mg OD oral, Orgametril.

(7) Allylestrenol: 10-40 mg/day Gestamin, Fetugard, Maintane, Profar.

(8) Levonorgestrel: 0.1-0.5 mg/day Duolution-L, Ovral

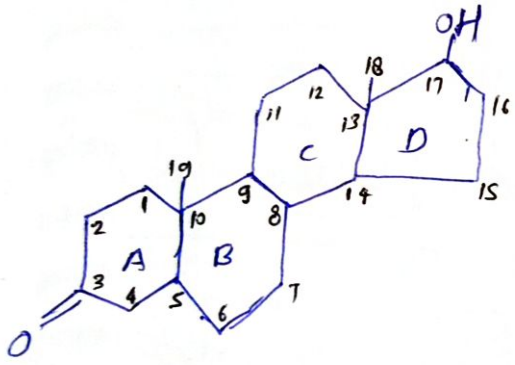
(9) **Desgestrel:** 150 micro g + Ethinylestrenol 30 micro g
Novelon 1 tab OD 3 week on 1 week of cyclic therapy

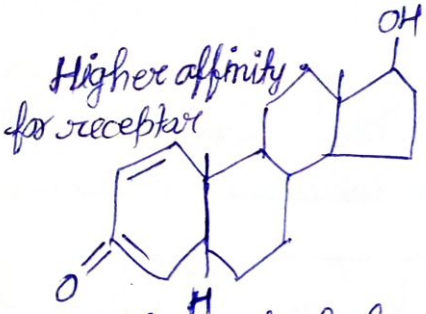
Androgens:

→ Includes testosterone. DHT. & Androstenedione.

⇒ Testosterone serves as a prohormone for -
→ Dihydrotestosterone (DHT)
→ Estradial.

(1) 95% of testosterone (8 mg/day) is Produced by Leyding cells after stimulation with LH.





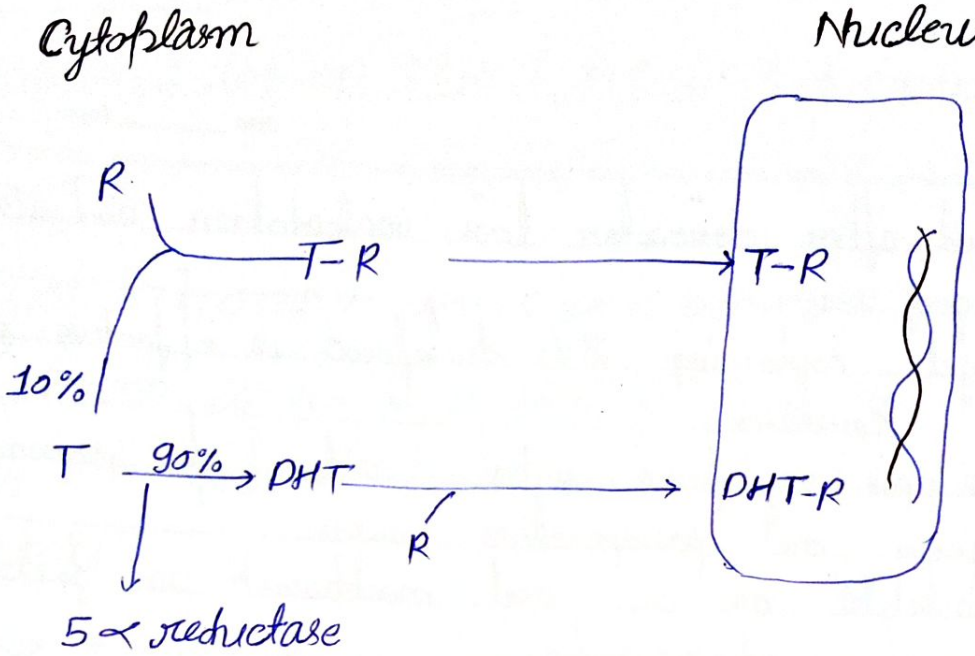
Testosterone —5α reductase→ Dihydrotestsferon. (DHT)

Tissue with 5αR are more sensitive to testosterone.

∴ Mechanism of action of steroid hormones:∴

→ Hormone enters cell by diffusion across plasma membrane
→ Bind to specific cytoplasmic receptor.
→ Translocation to nucleus.
→ Alteration in gene transcription.
→ Alteration in level of active mediator of effect.

Mechanism of Action :-



Action of Testosterone & DHT

Pharmacological Effects	Phamacologic Effects
→ Growth of genital in a body → Production of sperm → Growth of facial, Pubic and auxiliary hairs → Muscular development. → Growth of larynx and voice deepens. → Inhibition of bone growth → Thickening of skin, loss of s.c. fat. → Behavioral changes in men → Nitrogen retaining effect. → Erythropoietin secretion increased. → Increased LDL and decreased HDL.	→ Large doses of testosterone suppress gonadotropic secretion in adult males. → Androgens produce changes similar to male puberty in females. → Natural Androgens stimulates erythrocyte production. → Androgens increase protein synthesis / decreases protein breakdown (Anabolic effects) Effects last 1-2 months. → Anabolic effects are due to increase in nitrogen balance and retention of Phosphate, sulfur, K^+, Na^+, Cl^- and water

Pharmacokinetics:-

Absorption:- Undergoes high first pass metabolism. therefore i.m injections or synthetic preparations are used.

Transport:- Highly protein bound (98%) SHBG, Albumin)

Metabolism:- -By liver enzymes: Androsterone and etiocholanolone

→ Excretion by urine after conjugation.

→ Small quantity of oestrogen also produced from testosterone

Testosterone Preparation	Dose
Testosterone Aqueous Suspension	50-100mg / 2 Weeks.
Testosterone Esters - → Testo. Propionate. → Testo. Phenylpropionate → Testo. Cypionate → Testo - Enanthioate	 25-50mg / 3 times a week. 40-60mg / 1 or 2 Week. 100-200mg / 2 Weeks 250mg / 2 Weeks
Orally Active Preparations → Methyl testosterone tab. → Fluoxymesterone → Mesterolone.	
Transdermal Patches	2 Patches / day (Back, Abdomen, ~~thigh~~)
Implants	Wall of Abdomen / Thigh

Clinical Uses of Testosterone:

- Testicular failure: Primary & Secondary.
- Wasting syndromes - HIV, cancer, acute necotizing fasciitis
- Chronic illness, Burns.
- Osteoporosis and decreased muscle strength.
- Long term corticosteroid therapy.
- Pituitary dwarfism.
- Carcinoma of breast.
- Hereditary Angioneurotic oedema.
- Anaemia (Refractory)
- Menopausal syndrome.
- Ageing (Andropause (Male menopause or PADAM - (Partial Androgen Deficiency in the Ageing male fraity syndrome - easy tiring decrease of libido, mood disturbance, accelerated osteoporosis decreased muscle strength, and high susceptibility to disease.

Adverse effect of testosterone:

- Virilization (Female) Acne.
- Feminizing side effect (Male)
- Precocious Puberty and stunted growth
- Cholestatic Jaundice.
- Enlargement of Prostate.
- Atherosclerosis.
- Hepatic carcinoma.
- Oedema.
- Decreased spermatogenesis.
- Gynecomastia (Male breasts)
- Testicular Atrophy.

÷ Oral Contraceptive ÷

→ Oral contraceptive are those drugs which are used to prevent the pregnancy in female and helping in the down of Population.

→ In 1940 it was a first time to develope the method to inhibit the development of egg by using the progesteron hormone or sex hormone.

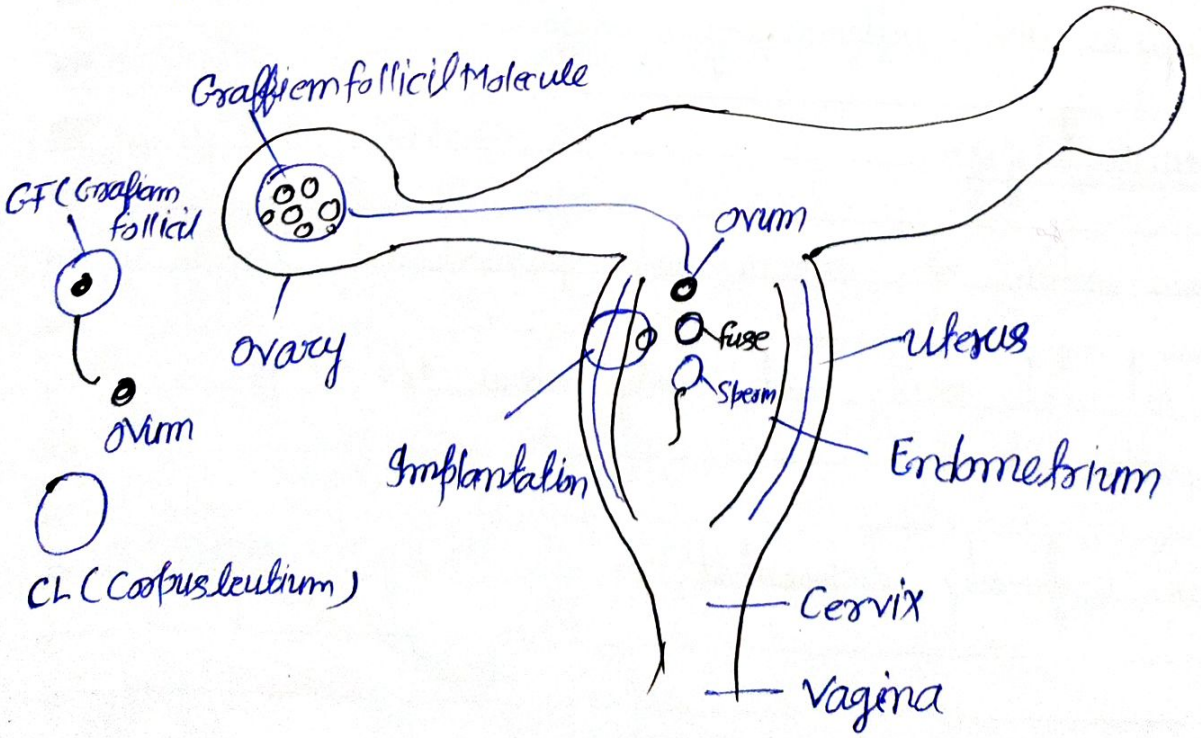
→ In 1944 the two scientist Bicken batch and Paulikovics has developed the method when the progesteron level is increase in our body then they decrease the rate of ovulation.

→ In 1955 first of all first family planning centre well developed. to aware the people about the population

→ In the 1960 to 1970 the Fertility control is become the popular things among the people.

→ The first contraceptive Pills are Ethiosterone which contain the active constituents Progesterone.

→ The first combination contraceptive pills ENOVID was developed



÷ Proposed Mechanism of Contraceptive Pills ÷

(1) Inhibiting ovulation.

(2) Modifying the cervical Mucus.

(3) Slow down the rate of Ovum Transport.

(4) Preventing the ovum maturation.

(5) Interfearing the Implantation

(6) By Decreasing the Spermatogenesis.

← Types of Oral Contraceptive →

(1) Combinational Preparation ⇒ This is the best and widely used method of oral contraceptives.

⇒ This contains the combination of two Hormones Estroges & Progesteron.

⇒ This drug is given 21 days in a month after the bleeding phase of the first weak from second to third weak this drug is always given.

⇒ Basically it contains the large dose of Progesteron which help in the inhibition of ovulation

(2) Sequential Preparation ÷ In the sequential Preparation drug is given for the 15 days in the three phases of Five days

⇒ First Five days in the bleeding time drug is given which contain only estrogen stop the bleeding and to maintaine the endometrium wall

⇒

→ The next five days the combination of Estrogen and Progesteron is given to mentaine the endometrium wall and reduce the Ovulation.

→ And the last five days it contain only Progesteron which inhibit the ovulation completely.

(3) Mini Pills or (Progestin Alone) :- This kind of Preparation contains only Progestron in a very small dose they are available in mini Pills. and they are given to the (Pregnent) leady entire whole months.

(4) Morning After or Postcoital Pills (or Estrogen Alone) →

→ This kind of drug are given after the intercourse within three days this contain the estrogen only and they help by the inhibiting of Zygote formation by implantation process.

⇒ They are always given in very emergency condition for the example to the rape.

(5) Vaginal Contraceptives or Spermicidal Agent :- These are the contraceptives Gels or Creams which are available in the semi solid form and they are applied by the female on their vaginal surfaces after the intercourse it punchers the or destroy the sperm so they are can't cause the pregnance.

(6) Interceptives or Abortifacients :- → Interceptive and abortifacients drug are very couseal used (And they are) in the very emergency condition.

⇒ They are used when the implantation is done and females becomes pregnant. and this is used within three month after the pregnant. When these drug is given then the increase estrogen level and decrease the Progesteron level so the endometrium wall is rapture and they comes out with the blood

And implants is also comes out and the Pregnancy is aborted.

(7) Progesterone blockers :- Progesteron blockers are drugs which is given after the implantation Process.

⇒ When the Progesterone level is decrease by the drug progesterone blockers. then the implantation do not get nuressment and nutrition so they becomes destray and comes out from the body.

(8) Depot Preparation :- (i) Once a month Preparation.

(ii) Intra Uterine device (IUD)

(iii) Bidegradable Sustain release Systems.

(iv) Non biodegradable subdermal implants & Vagenal rings.

(9) Condoms :- Condoms are generally used for the male they available for the female also.

⇒ They are used as a physical barrier.

⇒ They stop the entry of sperm into the vagina.

(10) Chemical contraceptive for men or Anti Androgens :-

→ They are used basically for the male and basically they destroy or Inhibit the formation of the spermatogenesis in the formation of sperm.

Drug Acting on Uterus

→ The Post Partum Haemorrhage (PPH) is a common things during the delivery.

→ When the blood loss after the delivery becomes more than half (500ml) an litre this is called Post Partum Haemorrhage.

→ And due to this the mortality rate of Pregnant mother is very high.

→ To reduce the blood loss during the delivery basically there are two kinds of drug is given this is called drug acting on uterus.

(i) Uterine Stimulants (Oxytocin / Uterotonics)

(ii) Uterine Relaxants (Tocolytics)

(1) Uterine Stimulants (Oxytocin / Uterotonics)

→ During the delivery of a Pregnant women the blood vessel release more blood into the uterus and due to this more loss of blood it will be lethal for the pregnant women.

→ So three kinds of drugs are geven which are basically works uterine constriction to avoid the PPH and Pre-mature birth.

(1) Oxytocin

(2) Ergotamin

(3) Prostaglandin

(1) Oxytocin :-

→ Oxytocin is major uterine stimulants drug basically this is hormone which release from the posterior Pituitary

⇒ The main function of oxytocin to increase the FOC (force of contraction of uterine muscle) and it helps delivery of the baby.

→ In 1909 oxytocin hormone identified by the scientist Henary Dale

→ The oxytocin hormone is release during the three time

→ (i) During sex

(ii) → During Birth

(iii) → During Lactation.

→ After the delivery Prolactin Hormone Prepare the milk inside the mamery gland but oxytocin help in the releasing of milk during the lactation.

(2) Ergot Alkaloid :-

→ Ergot is obtained from the clauisex Purpuria.

→ It is Alkaloidal in nature.

→ The main function of Ergotamine to increase the Frequency and duration of force of contraction in uterine muscle.

→ It also chelp in the avoiding the PPH during the delivery.

→ They are available in methylergovin injection in the I.V & I.M dose.

(3) Prostaglandins :-

→ Basically Prostaglandins also help in the uterine contraction and delivery of baby.

→ Basically the PG_E & PG_F series of Prostaglandins working in them

→ They help basically act two method -

→ by contraction of Uterous. as well by Relaxation of Cervix.

→ And they helps in delivery.

→ They also stop the PPM. two drugs basically available for the Prostaglandin in I.V form.

(i) Dinoprostone

(ii) carboprostal.

(2) Uterine Relaxants (Tocalytics)

→ They are basically give to stop the uterine contraction by relaxants the uterus and stop the premature birth of baby

→ They are basically selective β_2 Adrenoceptor Agonist. for

Ex - Ritodine

→ Solbutamol

→ Which supress Premature birth.

→ Some drugs which are given -

→ Indomethacine

→ Salbutamal

→ Terbutaline

→ Fenoterol

→ Nifedepine

→ MgSo4

→ Atosiban

→ Ritodine

Bioassa

Pharmacology

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Anurag Jaiswal

INTRODUCTION

What is a Bioassay?

- Comparative assessment of relative potency of a **test** compound to a **standard** compound on a living tissue.

- **Qualitative** identification & **Quantitative** measurement of the **amount of active principle** in pharmaceutical preparation or biological material.

- Measurement of conc. of a drug from magnitude of its biological effect.

PRINCIPLES OF BIOASSAY

- **Compare** potency of unknown substance with standard (including assessment of errors).

- Standard & test sample should have **same pharmacological effect & mode of action.**

PRINCIPLES OF BIOASSAY

- The test and standard should be compared using **a specified pharmacological technique.**

- Method selected should **be sensitive, reproducible & should minimize errors** d/t biological variations & methodology.

Types of Bioassays

There are three main types of bioassays (other than qualitative assays)

1. Direct Assays
2. Indirect Assays based upon quantitative responses
3. Indirect Assays based upon quantal responses („all or none")

Direct Assay

Doses of the standard and test preparations are sufficient to produce a specified response, and can be directly measured.

Indirect Assay

In indirect bio-assays the relationship between the dose and response of each preparation is first ascertained. Then the dose corresponding to a given response is obtained from the relation for each preparation separately

Quantal Assay

This response is in the form of „all or none" means no response or maximum response.
These can be biossayed by end point method.
Predetermined response is measured which is produced by threshold effect.
Quantal Responses are population response based on an all-or-nothing (0 or 1 – presence or absence) response such as death

$$\text{Concentration of Unkown} = \frac{\text{Dose of the Standard}}{\text{Dose of the Test}} \times \text{Concentration of Standard}$$

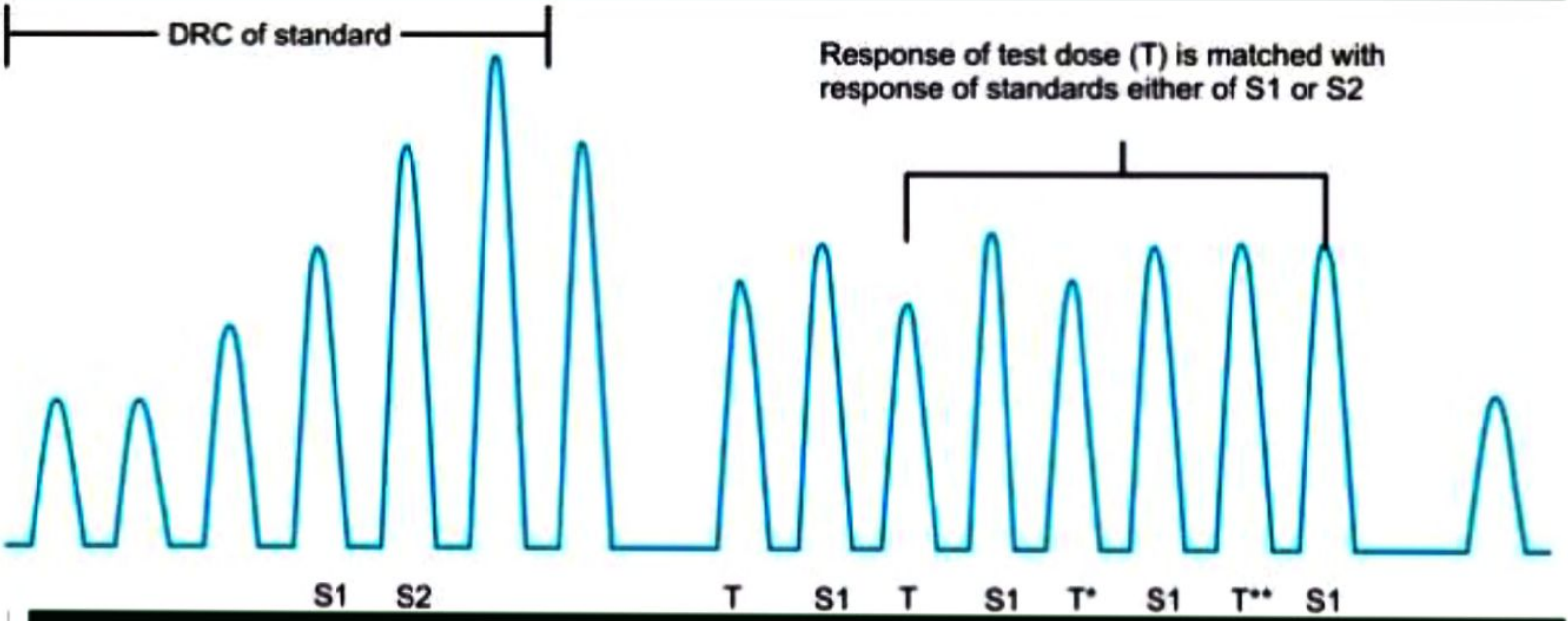
Graded Assay

It is proportional to the dose and response may lie between no response and maximum response . Graded Responses can be any type of measured responses in isolated tissues in particular, but also in whole animals. Such responses are infinitely graded and there are a large number of them. Examples include contractions of muscle, blood pressure, blood sugar concentrations, etc.

Matching Method

In this type of assay the test substance and the standard are applied and the responses obtained are matched by a trial and error process until they produce equal effects .
This may also limit to analytical dilution assay, as the assay involves the determination of the factor by which the test substance is diluted or concentrated in order to produce response that is equal to that of known amount of the standard drug

Matching Assay

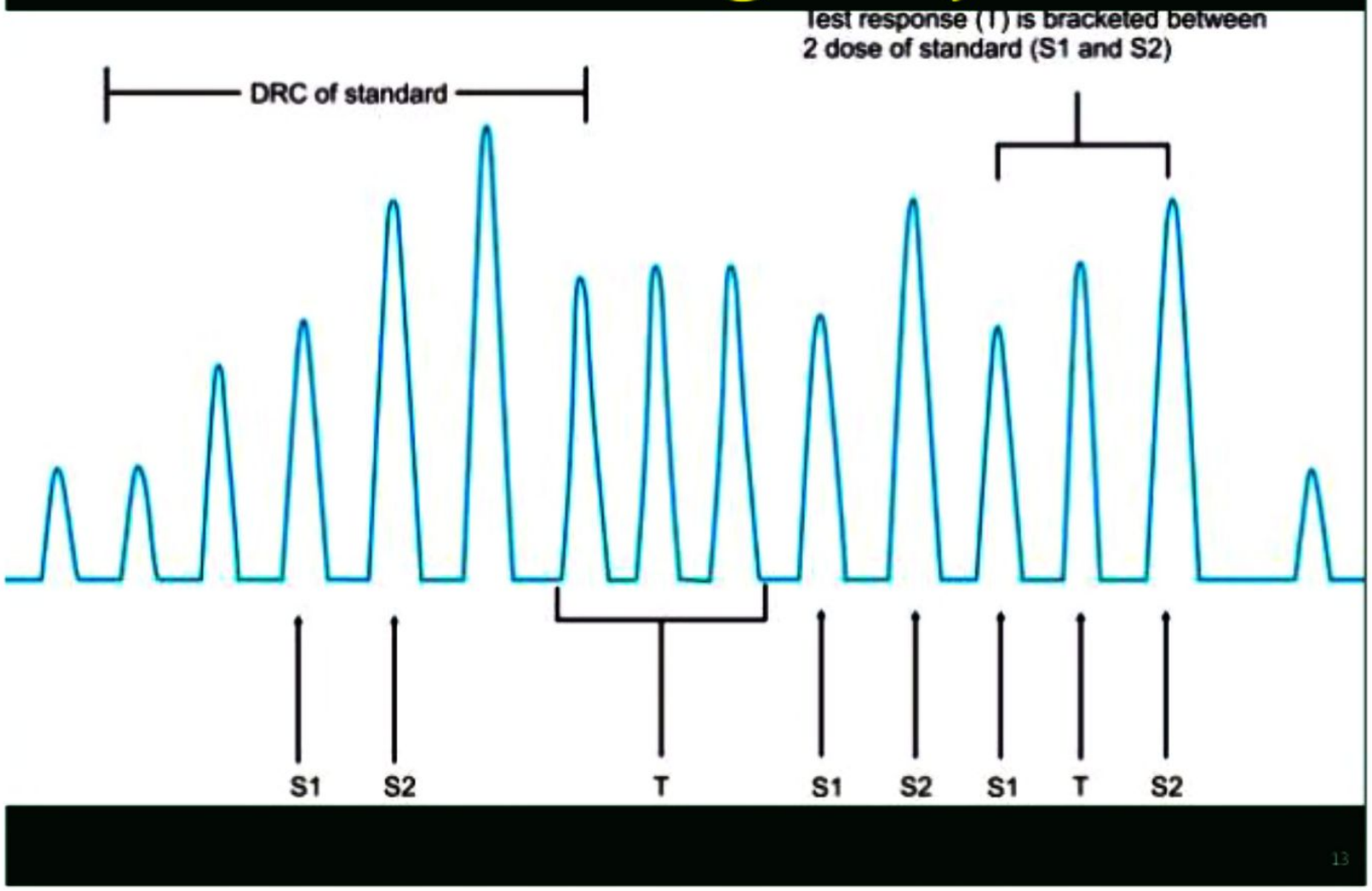


- **Adv**: Test DRC not reqd., small vol., fast.
- **Disadv**: Trial & Error method, poor precision.

Bracketing Method

Bracketing bioassay is performed by selecting two standard doses, which will give a close bracket on either side of the response produced by the unknown. The working dose of standard is first determined in the sensitive part of dose-response curve, that is, a dose that will approximately produce 50% of the maximal concentration. The dose of the standard drug is kept constant throughout the experiment, in order to have some idea about the change in the sensitivity of tissue with time.

Bracketing Assay



The standard drug is added at fixed intervals but alternating with the test so that each response produced by a dose of test substance is bracketed by responses produced by the dose of standard.

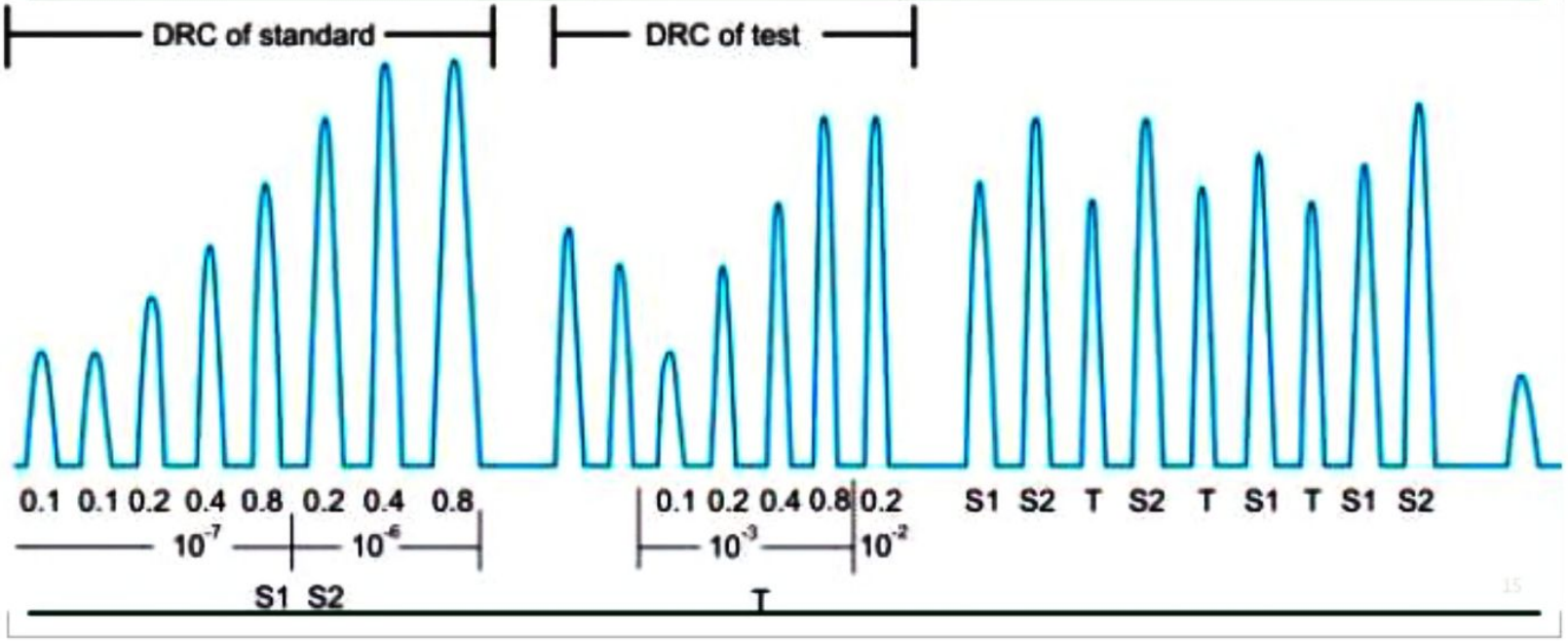
The response of test substance is bracketed between two responses of the standard. Close bracketing gives more accurate results.

Three Point Bioassay

In three point bioassay, the DRC of standard & test samples is first obtained from the responses due to graded doses. From the DRC of standard, two standard doses are selected in such a way that they have produced 25% & 50% of the maximal response respectively & are designated as S1 & S2. The responses of these doses lie on the steepest & straightest part (linear) of the curve

Three Point Assay

S_1	S_2	T
S_2	T	S_1
T	S_1	S_2



From the DRC of test sample one test is selected such that it gives a response which lies in between the two standard responses that is it gives a greater response than S1 & a smaller response than S2 & is designated as T

After selecting the standard & test doses, the bioassay is performed by recording the standard & test responses in randomized fashion as per Latin square design. The pattern of addition of doses is S1, S2, T; S2, T, S1 & T, S1, S2 in 3 successive cycles. The mean values of height of contraction for all the 3 doses are calculated and are used in plotting the graph so as to estimate the potency of the test sample.

The precision and reliability of this method is much better than matching and bracketing methods of bioassay & the sensitivity of the isolated tissue preparation is assessed prior to testing the unknown sample

Three Point Assay

- Mean responses of three sets taken.
- Potency ratio calculated.

$$M = \frac{T - S_1}{S_2 - S_1} \times \log \frac{s_1}{s_2}$$

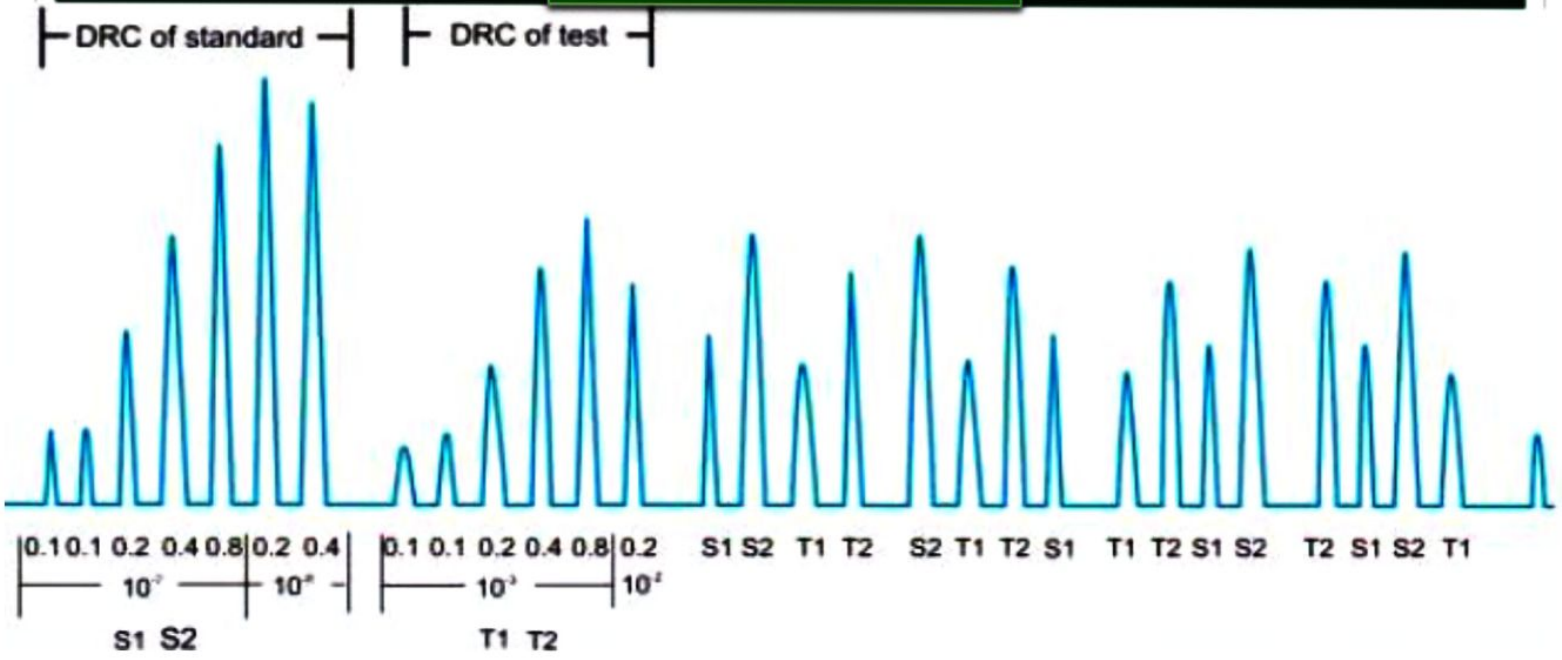
- Strength of test solution = $\frac{s_1}{t}$ x antilog M

Four Point Bioassay

The classic 2X2 parallel assay involves being able to measure parallelism where drugs acting through the same mechanism are expected to produce parallel dose-response curves

Four Point Assay

S_1	S_2	T_1	T_2
S_2	T_1	T_2	S_1
T_1	T_2	S_1	S_2
T_2	S_1	S_2	T_1



Four Point Assay

$$M = \frac{[T_1 - S_1 + T_2 - S_2]}{S_2 - S_1 + T_2 - T_1} \times \log \frac{s_2}{s_1}$$

$$T \text{ (concentration)} = \frac{s_1}{t_1} \times \text{antilog } M$$

Six Point & Eight Point Assay

Thank You

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