Program	**:**	**B. Pharmacy**
Semester	**:**	**V**
Subject /Course	**:**	**Pharmacology-II**
Subject/Course ID	**:**	**BP503T**
Module No.	**:**	**05**
Module Title	**:**	**Pharmacology of Drugs Acting on EndocrineSystem and Bioassay**
Course coordinator	**:**	**Ritu Kainth**
Mobile No.	**:**	**8847359620**
Email id	**:**	**ritukainth20@gmail.com**

Learning Outcome of Module-5

LO	Learning Outcome (LO)	Course Outcome Code
LO1	Describe hormones and their contribution to whole body homeostatic mechanisms.	BP503.4
LO2	Understand the chemical nature of different classes of anabolic steroids and androgens and how this determines their mechanism of action on target cells.	BP503.5
LO3	Understand the principles of feedback control for hormones release and its relevance for homeostasis.	BP503.5
LO4	Know about the anatomy of estrogens, progesterone and oral contraceptives.	BP503.5
LO5	Explain about the drugs used act on uterus.	BP503.5
LO6	Know about principles and applications of bioassay. Different types of bioassay.	BP503.6

Module Content Table

No.	Topic
1	Androgen and anabolic steroids
2	Estrogens, progesterone, and oral contraceptives
3	Drugs acting on uterus
4	Principles and applications of bioassay
5	Types of bioassay
6	Bioassay of insulin, oxytocin, vasopressin, ACTH, d-tubercuranie, digitalis, histamine and 5-HT

ANDROGENS AND ANABOLIC STEROIDS

INTRODUCTION

- Androgen is the hormones that control the building up of proteins and male secondary sex characteristics.
- Natural androgen is testosterone that is secreted mainly from the testis, adrenal cortex and also in ovary.
- Testosterone is the main natural androgen. It is synthesized mainly by the interstitial cells of the testis, and in smaller amounts by the ovaries and adrenal cortex.
- Androgen is formed in the foetal testis under the influence of maternal gonadotropin. It causes descent of the testis, later no androgen forms until puberty
- At puberty the hypophysial cells stimulate the laydig cells to produced androgen. It leads to the development of testis and secondary sexual characters
- Adrenal production of androgens is under the control of adreno corticotrophic hormone (corticotrophin). As for other steroid hormones, cholesterol is the starting substance. Dehydroepiandrosterone and androstenedione are important intermediates. They are released from the gonads and the adrenal cortex, and converted to testosterone in the liver.

Physiological Actions

- In general, the effects of exogenous androgens are the same as those of testosterone, and depend on the age and sex of the recipient.
- If administered to boys at the age of puberty, there is rapid development of secondary sexual characteristics maturation of the reproductive organs like prostrate, seminal vesicles and the external genitals is also stimulated.
- Life and fertility of spermatozoa is maintained
- Development of secondary sexual characters like appearance of moustache, beard, pubic and auxiliary hair etc.
- Growth of larynx, thickening of vocal cord and loudness of voice.
- The anabolic effects can be accompanied by retention of salt and water.
- The skin thickens and may darken, and sebaceous glands become more active (which can result in acne).

- Bony structure becomes more heavy and strong because of the stimulation by anabolic fraction of the testosterone.
- Muscular development is more in male compare to female because of the stimulation by anabolic fraction of the testosterone, which increased protein metabolism.
- Blood volumes and RBCs are more in male compared to female because of the stimulation by anabolic fraction of the testosterone.
- Water percentage is more in male since anabolic fraction stimulates Na+ and water retention.
- High temperature inhibits testicular activity. Libido is inspired in male.
- Physiological and behavioral changes like feeling of well-being and an increase in physical vigour, and may increase libido. Whether they are responsible for sexual behaviour as such is controversial, as is their contribution to aggressive behaviour.
- Administration of 'male' doses to women results in masculinisation, but lower doses (e.g. 300μg/day testosterone patches) restore plasma testosterone to normal female concentrations and improve sexual dysfunction in women following ovariectomy, without adverse effects.

Mechanism of Action

- ✓ In most target cells, testosterone works through an active metabolite, *dihydrotestosterone*, to which it is converted locally by a 5α-reductase enzyme.
- ✓ In contrast, testosterone itself causes virilisation of the genital tract in the male embryo and regulates LH/ICSH production in anterior pituitary cells.
- ✓ Testosterone and dihydrotestosterone modify gene transcription by interacting with intracellular receptors.

ADME:

It's well absorbed when given by oral route, undergoes metabolism in liver and hence ineffective therapeutically.

Therapeutic uses:

- ✓ Rejuvinate testis and in impotance to increased testicular secretion
- ✓ It prevents body atrophy in old people.
- ✓ Use in severe trauma and prolonged illness.
- ✓ To overcome osteoporosis in old people.

- ✓ In women testosterone can be used to produced symptomatic relief from breast cancer
- ✓ It is also used to check uterine bleeding in menorrhagia.

ADR:

- ✓ Liver damage, decreased release of gonadotropin hormones, increased salt and water retention leads to edema.
- ✓ In female- produced acne, masculisation.

Preparations:

- ✓ Testosterone itself can be given by subcutaneous implantation or by transdermal patches.
- ✓ Various esters (e.g. enanthate and proprionate) are given by intramuscular depot injection. Testosterone undecanoate and mesterolone can be given orally.

ANABOLIC STEROIDS

- ❖ Androgens can be modified chemically to alter the balance of anabolic and other effects. Such 'anabolic steroids' (e.g. **nandrolone**) increase protein synthesis and muscle development, but clinical use (e.g. in debilitating disease) has been disappointing.
- ❖ They are used in the therapy of aplastic anaemia, and (notoriously) abused by some athletes. Unwanted effects are described above, under *Androgens*. In addition, cholestatic jaundice, liver tumours and increased risk of coronary heart disease are recognised adverse effects of high-dose anabolic steroids.
- ❖ Androgens and the hormonal control of the male reproductive system.
- ❖ Gonadotrophin-releasing hormone from the hypothalamus acts on the anterior pituitary to release both follicle-stimulating hormone, which stimulates gametogenesis, and luteinising hormone (also called interstitial cell-stimulating hormone), which stimulates androgen secretion.
- ❖ The endogenous hormone is testosterone; intramuscular depot injections of testosterone esters are used for replacement therapy.
- ❖ Mechanism of action is via intracellular receptors.
- ❖ Effects depend on age/sex, and include development of male secondary sexual characteristics in prepubertal boys and masculinisation in women.

Classification

1. **Derivatives of testosterone:** Nondrolone phenyl propionate, Nandrolone decanloate

2. **Derivatives of methyl testosterore:** Oxymetholone, Oxondrolone, Stanozolol, Methly testosterone.

Pharmacological Actions

Protein metabolism: They promote protein metabolism. This manifests as increased in muscle mass and body weight.

Anti catabolic effects:

The catabolic effects of glucocorticoids are counter − acted and a positive nitrogen balance is produced.

Miscellaneous: Progestational activity and decreased in bone resorption which prevents osteoporesis.

Therapeutic Uses

- In chronic illness to accelerate rebuilding of tissues.
- To promote growth in hypogonaldal children and pituitary dwarfs.
- Breast cancer in female.
- Androgens (**testosterone** preparations) as hormone replacement in:
- Male hypogonadism due to pituitary or testicular disease
- Hyposexuality following ovariectomy (e.g. 300µg/day patches).

Antiandrogens:

- Both *oestrogens* and *progestogens* have antiandrogen activity, oestrogens mainly by inhibiting gonadotrophin secretion and progestogens by competing with androgens in target organs. **Cyproterone** is a derivative of progesterone and has weak progestational activity. It is a partial agonist at androgen receptors, competing with dihydrotestosterone for receptors in androgen-sensitive target tissues.
- Through its effect in the hypothalamus, it depresses the synthesis of gonadotrophins. It is used as an adjunct in the treatment of prostatic cancer during initiation of GnRH treatment.
- It is also used in the therapy of precocious puberty in males, and of masculinisation and acne in women.
- It also has a central nervous system effect, decreasing libido, and has been used to treat hypersexuality in male sexual offenders.

- **Flutamide** is a non-steroidal antiandrogen used with GnRH in the treatment of prostate cancer.
- Drugs can have antiandrogen action by inhibiting synthetic enzymes.
- **Finasteride** inhibits the enzyme (5α-reductase) that converts testosterone to dihydrotestosterone, which has greater affinity than testosterone for androgen receptors in the prostate gland.
- Finasteride is well absorbed after oral administration, has a half-life of about 7 hours, and is excreted in the urine and faeces.
- It is used to treat benign prostatic hyperplasia, although α1-adrenoceptor antagonists, **terazosin** or **tamsulosin**, are more effective (working by the entirely different mechanism of relaxing smooth muscle in the capsule of the prostate gland). Surgery is the preferred option (especially by surgeons).

ADME:

- If given orally, testosterone is rapidly metabolised in the liver. It is therefore usually injected.
- Virtually all testosterone in the circulation is bound to plasma protein-mainly to the sex steroid-binding globulin.
- The elimination half-life of free testosterone is short (10-20 minutes). It is inactivated in the liver by conversion to androstenedione.
- This has weak androgenic activity in its own right and can be reconverted to testosterone, although approximately 90% of testosterone is eliminated as metabolites rather than the parent compound.
- Synthetic androgens are less rapidly metabolized, and some are excreted in the urine unchanged.

Therapeutic uses:

- Antiandrogens (e.g. **flutamide, cyproterone**) are used as part of the treatment of prostatic cancer.
- 5α-Reductase inhibitors (e.g. **finasteride**) are used in benign prostatic hypertrophy.

ADR:

- Cholestic jaundice, Liver damage, Sodium and water retention on prolonged use.

- Unwanted effects of androgens include eventual decrease of gonadotrophin release, with resultant infertility, and salt and water retention leading to oedema. Adenocarcinoma of the liver has been reported.
- Androgens impair growth in children (via premature fusion of epiphyses), cause acne, and lead to masculinisation in girls.
- Adverse effects of testosterone, replacement and monitoring for these are reviewed by Rhoden & Morgentaler.

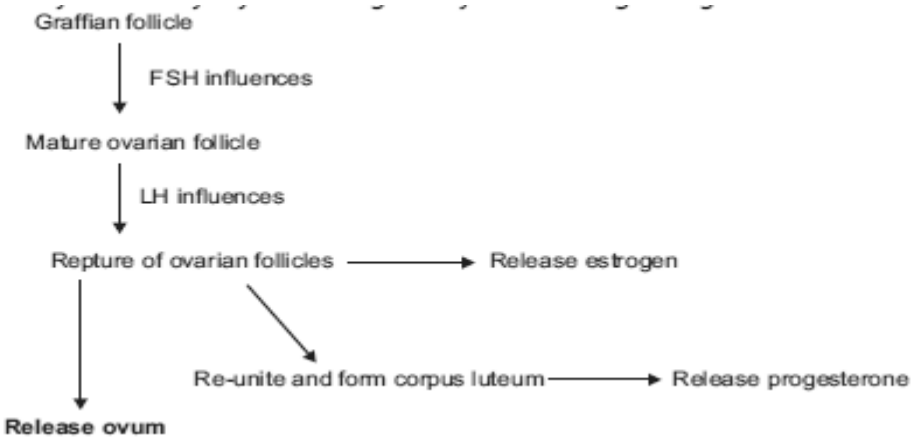
ORAL CONTRACEPTIVES

Introduction

Contraceptives are the drugs which prevent contraception since they control fertility; they are also called anti fertility drugs.

General physiology of human reproduction

Ovulation is the key event in human reproductive cycle. It usually occurs on the 14^{th} ± 2 days of 28 days cycle. During this cycle following changes occurs:



Release Ovum:

- This released ovum enters into the fallopian tube and gets fertilized by the sperm.
- The fertilized ovum gets converted into blastocyst.
- This reaches the uterine cavity and gets implanted by 21st to 23rd day of the cycle.
- The implantation completed by 35th day.
- Oral contraceptives are the hormonal preparation/pills use to prevent conception, fertilized ovum.

- During pregnancy ovarian hormonal levels are very high and they prevent ovulations. So ovulation can be prevented either by estrogen/progesterone/both.
- So when ovulation is prevented, pregnancy is automatically prevented.
- Oral contraceptives contain estrogen/progesterone/both.
- Oral contraceptive pills are prescribed medications used to prevent pregnancy.
- Also used to reduce menstrual cramps and anemia.

Oral contraceptive

- The combined oral contraceptive pill is extremely effective, at least in the absence of intercurrent illness and of treatment with potentially interacting drugs.
- The oestrogen in most combined preparations (second-generation pills) is **ethinylestradiol**, although a few preparations contain **mestranol** instead.
- The progestogen may be **norethisterone**, **levonorgestrel**, **ethynodiol**, or-in 'thirdgeneration' pills-**desogestrel** or **gestodene**, which are more potent, have less androgenic action and cause less change in lipoprotein metabolism, but which probably cause a greater risk of thromboembolism than do second-generation preparations.
- The oestrogen content is generally 20-50µg of ethinylestradiol or its equivalent, and a preparation is chosen with the lowest oestrogen and progestogen content that is well tolerated and gives good cycle control in the individual woman.
- This combined pill is taken for 21 consecutive days followed by 7 pill-free days, which causes a withdrawal bleed.
- Normal cycles of menstruation usually commence fairly soon after discontinuing treatment, and permanent loss of fertility (which may be a result of early menopause rather than a long-term consequence of the contraceptive pill) is rare.

MOA:

- Oestrogen inhibits secretion of FSH via negative feedback on the anterior pituitary, and thus suppresses development of the ovarian follicle.
- Progestogen inhibits secretion of LH and thus prevents ovulation; it also makes the cervical mucus less suitable for the passage of sperm.
- These also modify the cervical mucosa, secretion become thick and slowing down sperm penetration and prevent implantation.

- Oestrogen and Progestogen act in concert to alter the endometrium in such a way as to discourage implantation.
- They may also interfere with the coordinated contractions of cervix, uterus and fallopian tubes that facilitate fertilization and implantation.
- Potential unwanted and beneficial effects of the combined pill.

Common ADR:

- Weight gain, owing to fluid retention or an anabolic effect, or both.
- Mild nausea, flushing, dizziness, depression or irritability.
- Skin changes (e.g. acne and/or an increase in pigmentation).
- Amenorrhoea of variable duration on cessation of taking the pill.
- Mastalgia, migraine, chlosma, Increased vaginal secretion, Hypertension.
- Venous thrombo embolism, Decreased HDL, Bleeding irregularities.

Types of Oral Contraceptive

1. Combined pills
2. Progesterone only pills
3. Post coital (emergency pills)
4. Long acting progesterone only pills
5. Mini pills
6. Male pills

1. Combined Pills:

- They are daily medications containing two hormones, estrogen and progesterone.
- Estrogens- ethinylestradiol, mestranol
- Progesterone- levonorgestrol, norethisterone, ethinodiol
- Is taken for 21 days (from 5th day to 21st of the 28 days cycle) a month and the last 7 days are pill free days.
- Menstruation starts after medication is over.

Benefits:

- Decreases menstrual symptoms like irregular periods and inter menstrual bleeding.
- Decrease in benign breast diseases, uterine fibroids, functional cysts.
- Hypertension and increased risk of breast cancer.

ADR:

- Weight gain.
- Nausea, flushing, dizziness.
- Skin changes like acne or pigmentation.
- Amenhorrea.

Indications where estrogen must be avoided:

- History of blood clot disorders.
- History of stroke or heart attack.
- Severe hypertension.
- Diabetes that relates to blood vessel disorders.
- Poorly controlled diabetes.
- Severe headaches (migraine).
- Breast cancer.
- Liver cancer.

2. Progesterone only Pills:

- This pill is also called mini pills.
- This pill is given monthly once and it contains norgesterol.
- Used in cases where estrogen is contraindicated.
- Reasons are venous thrombosis, old age, smoking, Increased Blood pressure.
- Contraceptive effect is not so good as in combined pills.
- Mechanism of action is by causing alteration in the cervical mucus, making it inhospitable to sperms.
- Adverse reactions include irregular bleeding, weight gain and hair loss.

3. Post Coital Emergency Pills:

- Taken after unprotected intercourse to avoid pregnancy.
- Mostly contains Levonorgestrol or levonorgestrol along with estrogen (di ethyl stilbestrol).
- Must be taken within 72 hours of unsafe intercourse and reduces risk of pregnancy 75%.

Acts by:

- Blocking ovulation.
- Altering mucus in cervix.

- Changing endometrium.

ADR:

- Nausea and vomiting so taken with domperidone.
- Dizziness.
- Fatigue.
- Headache.
- Breast tenderness.
- Bleeding between periods or heavier menstrual bleeding.
- Lower abdominal pain or cramps.

4. Long acting Progesterone only pills:

- Used for a duration of 2-5 months.
- Mostly progesterone alone used or Medroxyprogesterone used.
- Medroxy progesterone given intra muscular.
- Progesterone implanted subcutaneously in biodegradable capsules.
- Is also given as intrauterine device that can show action up to 5 years.
- Effective and safe.
- Adverse effects include irregular bleeding and headache.

5. Sequential Pill:

Ethinyl estradiol is given from 5th day to 20th day and then combination of estrogen and progesterone given from 21st to 25th day of 28 days menstrual cycle

ADR:

- Weight gain.
- Nausea, Vomiting, dizziness.
- Skin changes like acne or pigmentation.

6. Mini Pill:

- These pills contain only progesterone and given once in a month.
- This contain norgesterol

ADR:

- Nausea and vomiting so taken with domperidone.
- Dizziness.
- Fatigue.

- Headache.
- Breast tenderness.
- Bleeding between periods or heavier menstrual bleeding.
- Lower abdominal pain or cramps.

7. Male Pill:

- Male contraceptives or male are methods of preventing pregnancy that primarily involve the male physiology.
- Most commonly used male contraceptive methods are condoms, withdrawal method or vasectomy.
- Pills are rarely available for men.
- Gossypol, an extract of cotton, has been studied as a male contraceptive pill. It decreases sperm production; however this is permanent in 20% of people.

OESTROGENS

Introduction

- Oestrogens are synthesised by the ovary and placenta, and in small amounts by the testis and adrenal cortex.
- As for other steroids, the starting substance for oestrogen synthesis is cholesterol. The immediate precursors to the oestrogens are androgenic substancesandrostenedione or testosterone.
- There are three main endogenous oestrogens in humans: *oestradiol*, *oestrone* and *oestriol*. Oestradiol is the most potent and is the principal oestrogen secreted by the ovary.
- The mass ovary contains ovarian follicle, the follicles are about 40,000 in number they are formed during foetal life.
- About 400 of them developed into adult life, the rest get degenerated, but all the follicels are lost at menopause.
- Ovulation occurs due to the rupture of ovarian follicle , this is stimulated by LH of anterior pituitary. After discharging the ovum, the rest of the follicle in the ovary form the corpus luteum.
- Estrogen are produced by the developing follicle , progesterore are produced by corpus lutem.

Physiological Roles

- ✓ Effects of exogenous oestrogen depend on the state of sexual maturity when the oestrogen is administered.
- ✓ In primary hypogonadism: oestrogen stimulates development of secondary sexual characteristics and accelerates growth.
- ✓ Puberty changes in female such as appearance of pubic and auxillary hair, development of breast.
- ✓ Development and growth of Vagina, uterus, fallopian tube and ovaries. Growth of uterus during pregnancy.
- ✓ The development of uterus endomatrium during proliferative stage of menstrual cycle depend on the secretions of estrogen from the ovaries.
- ✓ Stimulates protein and fat metabolism and growth of skeletal muscles.
- ✓ Libido is inspaired and metabolic changes like, Na+ and water retension.
- ✓ In adults with primary amenorrhoea: oestrogen, given cyclically with a progestogen, induces an artificial cycle.
- ✓ In sexually mature women: oestrogen (with a progestogen) is contraceptive.
- ✓ At or after the menopause: oestrogen replacement prevents menopausal symptoms and bone loss. Oestrogens have several metabolic actions, including mineralocorticoid (retention of salt and water) and mild anabolic actions.
- ✓ They increase plasma concentrations of high-density lipoproteins, a potentially beneficial effect that may contribute to the relatively low risk of atheromatous disease in premenopausal women compared with men of the same age.
- ✓ Oestrogens increase the coagulability of blood, and increase the risk of thromboembolism. This effect is dose-related.

MOA:

- ✓ As with other steroids, oestrogen binds to type 4 nuclear receptors. There are at least two types of oestrogen receptor, termed *ER*α; and *ER*β, the roles of which are currently being investigated using mice in which the gene coding one or other of these has been 'knocked out' .

- ✓ Binding is followed by interaction of the resultant complexes with nuclear sites and subsequent genomic effects-either gene transcription (i.e. DNA-directed RNA and protein synthesis) or gene repression (inhibition of transcription
- ✓ In addition to these 'classic' intracellular receptors, some oestrogen effects, in particular its rapid vascular actions may be initiated by interaction with membrane receptors.
- ✓ Acute vasodilatation caused by 17-β-oestradiol is mediated by nitric oxide, and a plant-derived (*phyto*-) oestrogen called **genistein** (which is selective for ERβ, as well as having quite distinct effects from inhibition of protein kinase C) is as potent as 17- β-oestradiol in this regard.
- ✓ Oestrogen receptor modulators (receptor-selective oestrogen agonists or antagonists) are mentioned briefly immediately below this section.

Classifications

Natural estrogen: Estradiaol, Estrone, Estriol

Semisynthetic estrogens: Ethinyl estradiaol, Mestranol, Quinestrol

Synthetic estrogens: Diethylstillbestrol, Methallenosstrol, Chlorotrianisene

Non steroidal agents with estrogenic activity: Hexestrol, Dienestrol, Benzestrol, Methallenoestril.

Preparations

- ✓ Many preparations (oral, transdermal, intramuscular, implantable and topical) of oestrogens are available for a wide range of indications.
- ✓ These preparations include natural (e.g. **estradiol**, **estriol**) and synthetic (e.g. **mestranol**, **ethinylestradiol**, **stilbestrol**) oestrogens.
- ✓ Oestrogens are presented either as single agents or combined with progestogen.

Anti-oestrogens

Replacement therapy for:

- Primary ovarian failure (e.g. Turner's syndrome).
- Secondary ovarian failure (menopause) for flushing, vaginal dryness and to preserve bone mass.
- Contraception.
- Prostate and breast cancer (these uses have largely been superseded by other hormonal manipulations)

- To treat oestrogen-sensitive breast cancer (tamoxifen).
- To induce ovulation (clomiphene) in treating infertility.

ADME:

- ✓ Natural as well as synthetic oestrogens are well absorbed in the gastrointestinal tract, but after absorption the natural oestrogens are rapidly metabolised in the liver, whereas synthetic oestrogens are degraded less rapidly.
- ✓ There is a variable amount of enterohepatic cycling, which forms the basis for drug interaction, because broad-spectrum antibiotic use alters bowel flora and can thereby render oral contraception ineffective. Most oestrogens are readily absorbed from skin and mucous membranes.
- ✓ They may be given topically in the vagina as creams or pessaries for local effect. In the plasma, natural oestrogens are bound to albumin and to a sex steroid-binding globulin.
- ✓ Natural oestrogens are excreted in the urine as glucuronides and sulfates.

Therapeutic Uses:

- ✓ To induces menses in primary and secondary amenorrhoea.
- ✓ To reduced disturbing menopausal syndrome.
- ✓ Post menopausal osteoporosis.
- ✓ Acne and Hirsutism.
- ✓ Oral contraceptives.

ADR:

- ✓ Tenderness in the breasts, nausea, vomiting, anorexia, retention of salt and water with resultant oedema, and increased risk of thromboembolism.
- ✓ Used intermittently for postmenopausal replacement therapy, oestrogens cause menstruation-like bleeding.
- ✓ Oestrogen causes endometrial hyperplasia unless given cyclically with a progestogen. When administered to males, oestrogens result in feminisation.
- ✓ Oestrogen administration to pregnant women can cause genital abnormalities in their offspring. Carcinoma of the vagina was more common in young women whose mothers were given stilbestrol in early pregnancy in a misguided attempt to prevent miscarriage.

Oestrogen Receptor Modulator:

Raloxifene, a 'selective oestrogen receptor modulator', has antioestrogenic effects on breast and uterus but oestrogenic effects on bone, lipid metabolism and blood coagulation.

- ✓ It is used for prevention and treatment of postmenopausal osteoporosis and reduces the incidence of oestrogen receptor-positive breast cancer, although its role in therapy of breast cancer is undefined. Unlike oestrogen, it does not prevent menopausal flushes.

Antioestrogens:

- ✓ Antioestrogens compete with natural oestrogens for receptors in target organs.

Tamoxifen has antioestrogenic action on mammary tissue but oestrogenic actions on plasma lipids, endometrium and bone.

- ✓ It produces mild oestrogen-like adverse effects consistent with partial agonist activity.
- ✓ The tamoxifen-oestrogen receptor complex does not readily dissociate, so there is interference with the recycling of receptors. Tamoxifen up-regulates transforming growth factor-β, decreased function of which is associated with the progression of malignancy, and which has a role in controlling the balance between bone-producing osteoblasts and bone-resorbing osteoclasts.

Clomiphene inhibits oestrogen binding in the anterior pituitary, so preventing the normal modulation by negative feedback and causing increased secretion of GnRH and gonadotrophins.

- ✓ This results in a marked stimulation and enlargement of the ovaries and increased oestrogen secretion.
- ✓ The main effect of their antioestrogen action in the pituitary is that they induce ovulation. It is used in treating infertility caused by lack of ovulation.
- ✓ Twins are common, but multiple pregnancy is unusual.

PROGESTERONE

Introduction

- ✓ It is secreted from corpus luteum from the healing scar of the ovary after ovulation.
- ✓ It is synthesized in placenta, adrenal and testes
- ✓ It is secreted during the last month of pregnancy. It is an important intermediate in the synthesis of steroids

- ✓ Progesterone inhibits ovulation, advance pregnancy, stabilies uterus and enlargement of breast.
- ✓ The natural progestational hormone (*progestogen*) is progesterone . This is secreted by the corpus luteum in the second part of the menstrual cycle, and by the placenta during pregnancy. Small amounts are also secreted by testis and adrenal cortex.

MOA:

Progestogens act, as do other steroid hormones, on nuclear receptors. The density of progesterone receptors is controlled by oestrogens.

Classifications

Natural: Progesterone.

Derivatives of progesterone: Hydroxyprogesterone, Dehydrogesterone, Methoxyprogesterone.

Derivatives of testosterone: Ethisterone, Dimethisterone.

Derivatives of 19-nor testosterone: Norethisterone, Norethynodrel, Norgestrol, Lynesterol.

Preparations:

There are two main groups of progestogens.

- ✓ The naturally occurring hormone and its derivatives (e.g. **hydroxyprogesterone**, **medroxyprogesterone**, **dyhydrogesterone**). Progesterone itself is virtually inactive orally, because after absorption it is metabolised in the liver, and hepatic extraction is nearly complete.
- ✓ Other preparations are available for oral administration, intramuscular injection, or administration via the vagina or rectum.
- ✓ Testosterone derivatives (e.g. **norethisterone**, **norgestrel** and **ethynodiol**) can be given orally. The first two have some androgenic activity and are metabolised to give oestrogenic products.
- ✓ Newer progestogens used in contraception include **desogestrel** and **gestodene**; they may have less adverse effects on lipids than ethynodiol and may be considered for women who experience side effects such as acne, depression or breakthrough bleeding with the older drugs.
- ✓ However, these newer drugs have been associated with higher risks of venous thromboembolic disease.

Physiological Actions

- ✓ Premenstrual stimulation of estrogens and preparations of the endomatrium for menstrual cycle. Menstrual occurs when progesterone level falls.
- ✓ Pregnancy is sustained because of progesterone secretion.
- ✓ It neutralizes Oxytocin of pituitary and protect pregnancy by preventing uterine contraction.
- ✓ During pregnancy menstruation is inhibiting and development of breast are due to progesterone.
- ✓ Birth passage is relaxed by progesterone and so its widen to facilitate birth of baby.
- ✓ Protein metabolism is decreased with progesterone.
- ✓ Estrogen-progesterone in combination may be synergestic or opposite, competitive depend upon the stage of in the sex life of a women.
- ✓ Progesterone acts, in turn, on oestrogen-primed endometrium, stimulating the *secretory phase* of the cycle, which renders the endometrium suitable for the implantation of a fertilised ovum. During this phase, cervical mucus becomes more viscous, less alkaline, less copious and in general less welcoming for sperm.
- ✓ Progesterone exerts negative feedback on hypothalamus and pituitary, decreasing the release of LH.
- ✓ It also has a thermogenic effect, causing a rise in body temperature of about 0.5°C at ovulation, which is maintained until the end of the cycle.
- ✓ If implantation of the ovum does not occur, progesterone secretion stops, triggering menstruation.
- ✓ If implantation does occur, the corpus luteum continues to secrete progesterone, which, by its effect on the hypothalamus and anterior pituitary, prevents further ovulation.
- ✓ The chorion (an antecedent of the placenta) secretes *human chorionic gonadotrophin* (*HCG*), which maintains the lining of the womb during pregnancy.
- ✓ For reasons that are not physiologically obvious, HCG has an additional pharmacological action in stimulating ovulation.
- ✓ As pregnancy proceeds, the placenta develops further hormonal functions and secretes a gamut of hormone variants (often with post-translational modifications), including *gonadotrophins* as well as *progesterone* and *oestrogens*. Progesterone secreted during

pregnancy controls the development of the secretary alveoli in the mammary gland, while oestrogen stimulates the lactiferous ducts.

- ✓ After parturition, oestrogens, along with *prolactin* are responsible for stimulating and maintaining lactation, whereas high doses of exogenous oestrogen suppress this.
- ✓ Progesterone controls the later secretory phase, and has negative feedback effects on both hypothalamus and anterior pituitary.
- ✓ If a fertilized ovum is implanted, the corpus luteum continues to secrete progesterone.
- ✓ After implantation, human chorionic gonadotrophin from the chorion becomes important, and later in pregnancy progesterone and other hormones are secreted by the placenta.

ADME:

- ✓ Injected progesterone is bound to albumin, not to the sex steroid-binding globulin. Some is stored in adipose tissue.
- ✓ It is metabolized in the liver, and the products, pregnanolone and pregnanediol, are conjugated with glucuronic acid and excreted in the urine.

Therapeutic uses:

- ✓ To prevent the threatened operation in the first three month of pregnancy
- ✓ Recession of endomatrium cancer occurs if large dose of progesterone is administered
- ✓ To control bleeding during and after delivery
- ✓ Use as replacement therapy during irregular bleeding.
- ✓ As contraceptives:
- With oestrogen in *combined oral contraceptive pill*.
- As injectable or implantable progesterone-only contraception.
- As part of an intrauterine contraceptive system.
- Progesterone with high dose of estrogen use as contraceptives.
- As progesterone-only contraceptive pill.
- ✓ Use in cystic fibrosis.
- ✓ If high dose of progesterone with lower dose of estrogen administered in first week of menstrual cycle it help in conceive.
- ✓ It is a pill of contraception, a pill to conceive, depend upon time of administration.
- ✓ In *endometrial carcinoma*; use in breast and renal cancer has declined.

ADR:

- ✓ Nausea, breast discomfort, headache, fatigue, mental depression and rarely liver damage.
- ✓ Weak androgenic actions, acne, fluid retention, weight change, depression, change in libido, breast discomfort, premenstrual symptoms, irregular menstrual cycles and breakthrough bleeding.
- ✓ There is an increased incidence of thromboembolism.
- ✓ Poorly validated uses have included various menstrual disorders.

Progestogens and Antiprogestogens

- Medical termination of pregnancy: **mifepristone** (partial agonist) combined with a prostaglandin (e.g. **gemeprost**).
- The endogenous hormone is progesterone. Examples of synthetic drugs are the progesterone derivative **medroxyprogesterone** and the testosterone derivative **norethisterone**.
- Mechanism of action involves intracellular receptor/altered gene expression, as for other steroid hormones. Oestrogen stimulates synthesis of progesterone receptors, whereas progesterone inhibits synthesis of oestrogen receptors.
- Main therapeutic uses are in oral contraception and oestrogen replacement regimens, and to treat endometriosis.
- The antiprogestogen **mifepristone**, in combination with prostaglandin analogues, is an effective medical alternative to surgical termination of early pregnancy.

Antiprogestogens

- Mifepristone is a partial agonist at progesterone receptors. It sensitises the uterus to the action of prostaglandins. It is given orally and has a plasma half-life of 21 hours.
- Mifepristone is used, in combination with a prostaglandin (e.g. gemeprost; see below), as a medical alternative to surgical termination of pregnancy.

DRUGS ACTING ON THE UTERUS

Introduction

- Drugs acting on uterus are uterine stimulants and uterine relaxants.
- These drugs directly can affect endomatrium amd myomatrium. Important drugs affecting endomatrium and myomatrium are estrogens and progesterone.

- Myomatrium receives both sympathetic and parasympathetic innervations. So autonomic drugs can affect motility.
- Drugs affecting directly and indirectly moderately affect the normal and gestational status of uterus.

UTERINE STIMULANTS

These drugs increased uterine motility so they are called Ecbolics/ Abortifacients/ Oxytocics.

Oxytocin

- Oxytocin is a powerful hormone. Oxytocin's level increases when, we hug, or kiss someone. It plays roles especially in sexual reproduction, the most common situations are before and after childbirth.
- It is released in large amounts after distension of the cervix and uterus during labor, facilitating birth, maternal bonding, and after stimulation of the nipples, breastfeeding.
- This hormone affects orgasm, social recognition, pair bonding, anxiety, and breastfeeding. That's why sometimes it is called the "love hormone". Oxytocin is produced in the hypothalamus. The myoepithelial cells of the breast,which surround the alveoli of the mammary gland, and the smooth muscle cells of the uterus.
- Oxytocin is controlled by a positive feedback mechanism where release of the hormone causes an action which stimulates more of its own release. Whencontraction of the uterus starts, for example, oxytocin is released which stimulates more contractions and more oxytocin to be released. In this way, contractions increase in intensity and frequency.
- There is also a positive feedback involved in the milk-ejection reflex. When a baby sucks at the breast of its mother, the stimulation leads to oxytocin secretion into the blood which then causes milk to be let down into the breast. Oxytocin is also released into the brain to help stimulate further oxytocin secretion. These processes are self-limiting; production of the hormone is stopped after the baby is delivered or when the baby stops feeding. Oxytocin is a mammalian hormone that has many functions, the most notable having to do with pregnant or lactating mammals. In this capacity, some of the hormone's main function is preparing a female's body for childbirth. Oxytocin as feed inhibitor, maintaining homeostasis in consummatory behavior.
- The structure of the hormone is very similar to that of vasopressin, also a nonapeptide with a sulfur bridge, whose sequence differs from oxytocin by two amino acids.

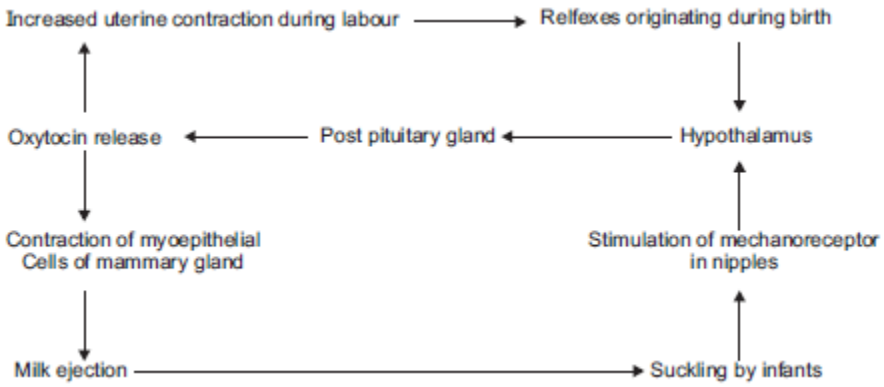
Classifications:

1. **Posterior Pituitary Hormone:** Oxytocin.
2. **Ergot Alkaloids:** Ergometrine, Methyl Ergometrine.
3. **Prostaglandin:** PGE2, PGF2α, Misoprostol.
4. **Miscellaneous:** Quinine, Ethacridine.

Oxytocin means: OXYS-Quick; TOKOS-Child Birth.

It is a nonapeptide hormone derived from paraventricular nucleus of hypothalamus and release from posterior pituitary gland along with ADH.

MOA



Flowchart of Mechanism and Pharmacological actions of Oxytocin and factors controlling Oxytocin release:

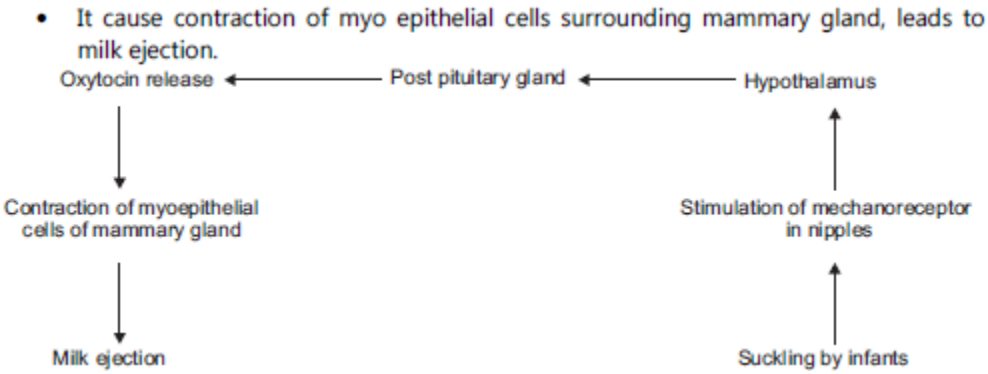
- Oxytocin acts on Oxytocin receptors which is mediated by:
- It acts through IP3 /DAG system and increased influx of Ca2+ release, which cause contraction.
- Caused depolarization.
- Increased PG synthesis and release by endomatrium which causes contraction.
- All the above actions together causes uterine contraction.

Pharmacological Actions:

Uterus:

- Oxytocin increases the force and frequency of uterine contraction.
- Basal tone of uterus is also increased at high dose.
- At low dose there is relaxation in between the contraction.
- Increased uterus contraction is due to heightened electrical activity of myomatrium.

Breast:



CVS:

Promotes Oxytocin release, which leads to vasodilatation and decreased BP.

Kidney:

High dose decreases urine output.

ADME:

Being peptide is inactivated by oral route of administration. So it is administered by parenteral/intranasal route. Rapidly degraded in liver and kidney by oxytinase enzyme and excreted in urine.

Therapeutic activity:

- Induction of labour.
- In uterine inertia.
- Postpartum hemorrhage.
- Cesearean section.
- Abortion.
- Breast engorment and for milk ejection.

ADR:

- Due to powerful contraction during delivery it may cause damage of soft tissue of mother and baby.
- Asphyxia and Death.

Ergometrine

MOA:

- It acts through IP3 /DAG system and increased influx of Ca2+ release, which cause contraction.

- Caused depolarization.
- Increased PG synthesis and release by endomatrium which caused contraction.
- All the above actions together caused uterine contraction.

Pharmacological Actions:

Uterus:

- It increases the force and frequency of uterine contraction.
- Basal tone of uterus is also increased at high dose.
- At low dose there is relaxation in between the contraction.
- Increased uterus contraction is due to heightened electrical activity of myomatrium.

CVS:

Weaker vasoconstrictor, cause insignificant raise in BP.

GIT:

At high dose increased GIT peristalysis.

ADME:

Well absorbed and distributed well, Metabolised in liver and excreted in urine.

Therapeutic Uses:

- Management of third stage of pregnancy.
- To control and prevent post partum hemorrhage.
- To ensure normal ovulation.

ADR:

Due to powerful contraction during delivery it may cause damage of soft tissue of mother and baby.

UTERINE RELAXANTS

- The primary role of uterine relaxants is to prevent pre-mature delivery or labour that starts before the end of 37th week of gestation.
- Premature labour can be developed spontaneously or may follow genetically predisposed early rupture of foetal membrane.
- Tocolytics agents either acts directly to suppress myoepitheliam smooth muscles contraction by decreasing intracellular Ca2+ concentrations and reducing the efflux of Ca2+ on muscles contraction.

- Or they may act indirectly by inhibiting the synthesis or release or receptor actions of PG or Oxytocin.
- These drugs also used to delay labour, arrest threatened abortion or to treat dysmenorrheal.
- Suppression of labour also important to allow foetus to mature to initiate glucocorticoids therapy to mother, so that lungs of new born baby get sufficient time for maturation and neonatal respiration distress can be reduced.
- Tocolytics agents use for this pupose are β adrenorecepter agonists, magnesium sulphate, Ca2+ channel blocker, hydroxyl progesterone, PG synthesis inhibitors and Oxytocin receptor antagonists.

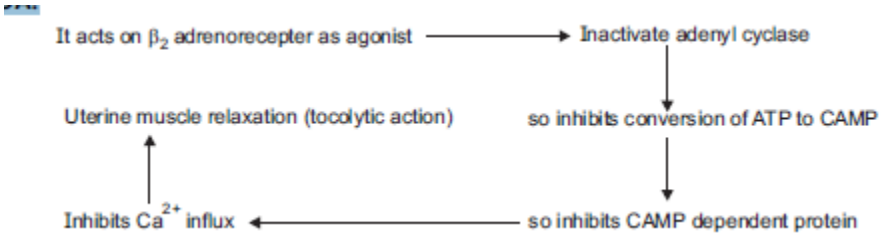
Classifications:

Selective β adrenoreceptor agonists- Ritodrine, Salbutamol, Terbutaline, Orciprenaline, Isoxsuprine.

Calcium Channel blocker: Verapamil, Nifedipine, Nicardipine, Amlodipine.

Ritrodine:

MOA:



Therapeutic uses:

Uterine relaxants.

ADR:

Hypotension, Bradycardia, Arrthymia, Pulmonary edema, Hypokalamia, Hyperglyemia.

Calcium Channel Blockers:

MOA:

- Calcium Channel Blockers blocks L-Type of voltage gated calcium channel in myomatrium.

↓

Inhibits Calcium influx

↓

Uterine muscle relaxation (Tocolytic action)

Therapeutic uses:

Uterine relaxants.

ADR:

Hypotension, Bradycardia, Foetal hypoxia.

Magnesium Sulphate:

MOA:

- It directly uncouple the excatation coupling and inhibits cellular action potential.
- Cause relaxation of uterine muscles, tocolytic action.
- It is preferred over β2 agonists if the patients is having cardiac and hyperthyroidism problem.

Therapeutic uses:

Uterine relaxants.

ADR:

Feeling of warmth, sweating, flushing, dry mouth, nausea, headache, palpitation.

BIO-ASSAYS GENERAL ASPECTS

Introduction

- Bioassays are procedures by which the potency or the nature of the substance is estimated by studying its effects on living matter. The basic principle of such assays is to compare how much of a sample being tested produces the same biological effect as a given quantity of a standard preparation. They are generally carried out by using either intact animals; as in the case of bio-assay of insulin by using rabbits or mice, bio-assay of digitalis by using guinea pigs or pigeons; or isolated animal organs or tissues as in the case of bio-assay of histamine by using guinea pig ileum, bio-assay of acetyl choline by using frog's rectus abdominus muscle preparation.
- Bio-assay can be defined as a procedure for determining the quantitative relationship between the dose of a drug and the magnitude of biological response it evokes; or the determination of the potency or concentration of a biologically active drug of physical, chemical or biological origin by using a biological indicator. The biological indicator could be a whole animal like frog, mouse, rat, guinea pig, cat, dog etc., or part of an animal like isolated heart, strip of stomach, uterus, ileum, jejunum, colon, diaphragm, rectus abdominus muscle etc., or blood cells or microorganisms.

Unlike the physical and chemical methods, the bioassays are always comparative. The effect of the test substance is compared with that of the reference standard.

RESERVATIONS OF BIOASSAYS

Bioassay procedures are generally employed:

- When a chemical assay for the substance is not available or the substance gets inactivated by interacting with the chemicals as the case with hormones.
- When the quantity of the sample is too small.
- When the bioassay is more sensitive than the chemical assay.
- To estimate the concentrations of active principles present in the tissue extracts such as the endogenous mediators like Ach, 5-HT, PGs etc.
- To measure the drug toxicity.
- To measure the pharmacological activity of a new or chemically unidentified substance.

Bioassays are also essential in the development of new drugs. In the pre-clinical assessment of a new compound, the biological activity is compared with that of a known (standard) compound using appropriate test systems. In such studies the tests must be simple, reproducible and economical. Biological assessment of a new compound generally consists of carrying out a battery of such assays and based on these tests, constructing a profile of activity. Clinical testing of drugs is guided by such profile of activity generated in animals.

USE OF STANDARDS

Bioassays are designed to measure relative potency of two preparations usually a standard and an unknown. Use of a standard substance for comparison also helps in solving problems arising out of biological variations. The observed response of the unknown would be always relative to the effect that is produced by a standard substance. The standard substance is a pure substance and in official bioassays it refers to Pharmacopeial standards. In case of hormones, biological products and vaccines it is often necessary to establish the standard response of the standard substance against which unknown samples are calibrated.

PRINCIPLES

- Burn and Dale enunciated certain principles for conducting bioassays. These include,
- All bioassays must be comparative and compared against a standard drug or preparation. This is to overcome errors due to biological variation.

- The standard and the new drug should be identical to each other, so that their dose-response curves will have the same slope and would be parallel to each other, i.e., the potency ratio would be constant all along the response levels.
- The method for comparing the unknown and the standard should preferably test the therapeutic property of the drug. Ideally, an analgesic is tested for analgesic activity and an anti-convulsant for anti-convulsant activity. However, this is not always convenient or possible to do so. For e.g., For estimating digitaloid drugs, the cardiac arrest in pigeon or guinea pig is used as the end point; which is the toxic effect of the drug but not the therapeutic effect.
- The method should eliminate all possible errors and allow an estimation of the error due to biological variation in different animals/persons at any one time and in the same animal/person at different times. Precautions should be taken to minimize errors due to biological variations. These include the selection of suitable animals/preparations and also the selection of the experimental conditions.
- The results of the test should be subject to statistical analysis to minimize errors due to biological variation.

BIOLOGICAL VARIATION

Biological variation means that no two-test preparations can be expected to give identical results and that the same preparation at some other point of time may be expected to react differently. In all biological methods, the interactions between the chemicals and the biological systems are observed. One of the characteristics of the biological systems is that they are continually changing and therefore are never the same. A number of factors have been found which alter the responsiveness of the isolated biological systems as well as the whole animals. These include,

- Environment
- Temperature
- Diet
- Solvent
- Species
- Strain
- Sex
- Age

- Weight
- Season and
- Inexperience.

CLASSIFICATION

Bioassays may be broadly classified into 2 types depending upon the type of responses recorded, i.e.

- ✓ Quantal
- ✓ Graded

(A) Quantal Bioassays:

The quantal bioassay is an all or none phenomenon. In this form of bioassay at least 2 groups of animals are employed. The response is either positive or negative, i.e., there is no intermediate response. For e.g,

- Insulin induced hypoglycemic convulsive reaction, i.e., an animal receiving insulin either show convulsions or does not;
- The animal receiving a dose of a drug, as in toxicity studies, either dies or does not die.

This method of bioassay is not very accurate, but can be employed for the following cases:

- Comparison of threshold responses.
- Comparison of ED50 or LD50.

$$\text{Concentration of test substance} = \frac{\text{Threshold dose of Std.}}{\text{Threshold dose of test}} \times \text{Concentration of Std.}$$

$$\text{Concentration of test substance} = \frac{ED_{50}/LD_{50} \text{ of Std.}}{ED_{50}/LD_{50} \text{ of Test}} \times \text{Concentration of Std.}$$

(B) Graded Bioassays:

- ✓ Graded responses are those that are measured by continuous variables such as weight, body temperature, blood glucose level, blood pressure, the number and strength of contractions of heart, respiratory rate, the extent to which an isolated tissue contracts or relaxes etc., The graded responses may be assessed by using either the whole animal or a part of the animal.
- ✓ The graded bioassays are based on the observations that there is a proportionate increase in the observed response with a subsequent increase in the concentration or dose. Then the test responses are compared with that of the standard. The parameters employed in

such bioassays are based on the nature of the effect of the substance that is expected to produce. For e.g., contraction of a smooth muscle preparation (guinea pig ileum) for assaying histamine; the study of blood pressure response in case of adrenaline.

- ✓ This type of assay gives almost identical results. The choice of the assay depends upon,
  - Precision of assay demands.
  - Quantity of the sample available.
  - Availability of experimental animals.
- ✓ The various methods of graded bioassays are,

1. Matching Dose Bioassay:

It is the simplest form of all graded bioassays and involves no calculations. In this type of bioassay, the response of the standard substance is recorded first and then the response of the test substance is tried to match with that of the standard by a trial and error process, until they produce equal effects. It is also called as the *analytical dilution assay* as the assay involves the determination of the factor by which the test substance is either diluted or concentrated in order to produce a response that is equal to that of a known amount of the standard drug. A corresponding concentration of the test substance is then calculated.

This assay is generally employed when the ample amount of sample is available.

Since the assay does not involve the recording of CRC, the sensitivity of the preparation is not taken into consideration.

Advantages:

The assay does not depend on the assumption of a dose response relationship.

Disadvantages:

- Purely subjective method.
- Inefficient as preliminary effects are not utilized in final assessment.
- Lot of experimental errors, which cannot be determined.
- A crude method and not the exact method of determining the potency of a drug.
- Precision and reliability are poor.

Bio assay of ach by matching

Bath vol 20 ml, tension 1 gm, Magnification 1 : 10,

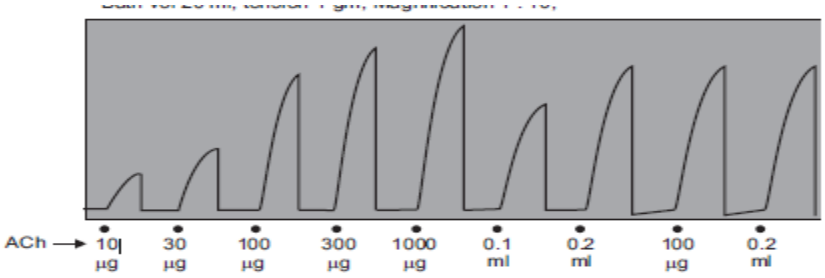


Fig. 24.1: Bioassay of Ach by matching method

2. Bracketing Dose Bioassay:

- ✓ It is also a simple assay procedure, which is employed when the test sample is very small. In this method, the response due to a constant dose of the test substance is bracketed between the greater and the smaller responses due to varying doses of standard substance that provides the closest bracket. Initially, two responses of the standard substance are taken. The doses are adjusted such that one is giving response of approximately 20% and the other 70% of the maximum. The response of unknown, which lies in between the two responses of standard doses, is taken. The panel is repeated by increasing or decreasing the doses of the standard till all three equal responses are obtained/ a closest bracket is provided for the test response by the two standard responses.
- ✓ In the end, the responses due to the double doses of the standard and the test are taken which should be equal. Concentration of the test sample can be determined as follows:

$$\text{Concentration of unknown} = \frac{\text{Dose of Standard}}{\text{Dose ot Test}} \times \text{Concentration of Standard}$$

This method has following limitations:

- It occupies a larger area of the drum as far as tracings are concerned.
- There are chances of errors that one cannot determine.
- It does not give any idea of dose-response relationship.
- The precision and reliability of this assay procedure is poor.

However, this method is particularly useful when the sensitivity of the preparation is not stable.

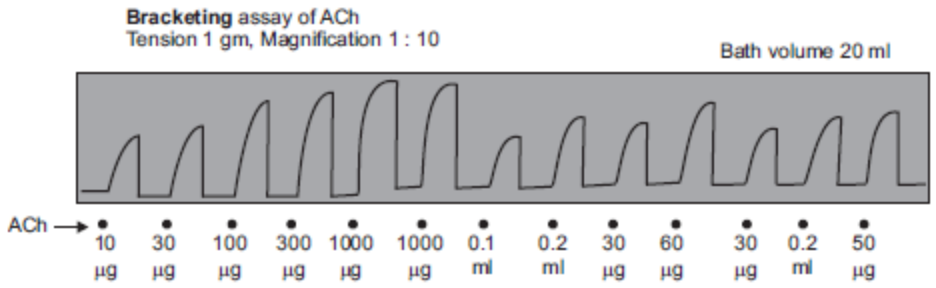


Fig. 24.2: Assay of Arch by bracketing method

3. Interpolation Method:

- ✓ This method is based on the assumption of dose-response relationship. At first, the concentration response curve due to graded doses of a standard substance followed by the dose response curve of the test substance is recorded. Then two standard doses and one test dose are selected from the respective DRCs such that they lie on the linear portion of the DRC. The test dose is selected in such a way that its response is greater than that of smaller dose of standard and is lesser than that of larger dose of the standard. Bioassay is then carried out with the selected standard and test doses in 2-3 cycles. The height of contraction of all the standard and test doses in the bioassay is measured. Then, a log DRC is plotted with the mean values of the standard responses in the bioassay and the dose of the standard producing the same response as produced by the test sample is directly read from the graph and the concentration of the test sample is determined.
- ✓ It is a simple method and chances of errors are less if the sensitivity of the preparation is not changed. The precision and reliability of the assay is much better as compared to the earlier methods as the sensitivity of the preparation is assessed prior to testing the unknown sample.

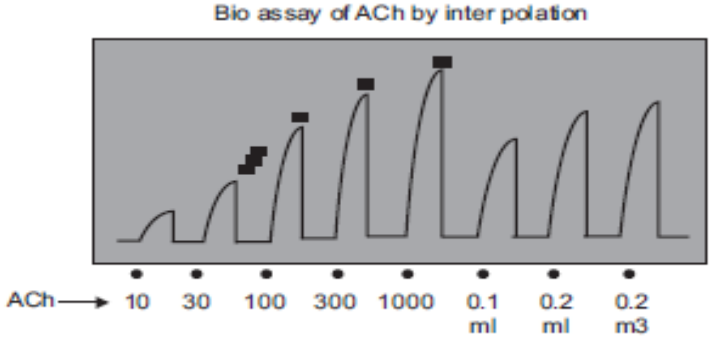


Fig. 24.3

4. Multiple Point Bioassays:

- ✓ The various types of multiple point bioassays, which are also based on doseresponse relationship, are:
  - 2-point bioassay
  - 3-point bioassay
  - 4-point bioassay
  - 6-point bioassay
  - 8-point bioassay
- ✓ In these bioassays, the responses are repeated several times and the mean of each is taken. Thus, chances of error are minimized in these methods. The sequence of responses is recorded as per the Latin square method of randomization in order to avoid any bias.

(A) 2-Point Bioassay:

Any one dose that produces from minimal to maximal response of the standard and the test substances are taken from the DRC and then their % of response is calculated.

$$\text{Concentration of Test Substance} = \frac{\%\ \text{Response of Standard}}{\%\ \text{Response of Test}} \times \text{Concentration of Standard}$$

However, this is not used practically.

(B) 3-Point Bioassay:

- ➢ This method is also based on the assumption of dose-response relationship. At first, the concentration response curve due to graded doses of a standard substance followed by the dose response curve of the test substance is recorded. Then, two standard doses and one test dose are selected from the respective DRCs such that they lie on the straightest and steepest part of the DRC. The test dose is selected in such a way that its response is greater than that of smaller dose of standard and is lesser than that of larger dose of the standard.
- ➢ Bioassay is then carried out with the chosen standard and the test doses in 3 successive cycles as per the Latin square design. The responses are recorded in the order of
  - S1, S2, T;
  - S2, T, S1 and
  - T, S1, S2.
- ➢ The height of contraction of all the standard and test doses in the bioassay is measured. Then, a log DRC is plotted with the mean values of the standard responses in the

bioassay and the dose of the standard producing the same response as produced by the test sample is directly read from the graph and the concentration of the test sample is determined. The concentration of the unknown can also be determined mathematically as follows:

$$\text{Concentration of unknown} = \frac{n_2}{T} \times \text{antilog}\left[\frac{T\text{-}S_1}{S_2 - S_1} \times \log\frac{n_2}{n_1}\right] C_s$$

Where n1 = Lower Standard dose

n2 = Higher Standard dose

t = Test dose

S1 = Response of n1

S2 = Response of n2

T = Response of t

Cs = Concentration of Standard.

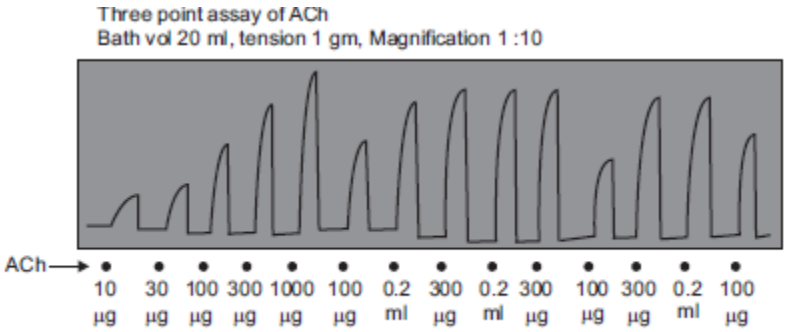


Fig. 24.4

(C) 4-Point Bioassay:

- This method is almost similar to the 3-point bioassay, but in this method 2 doses of the standard and the 2 doses of the test are used. The selection of the standard doses should be such that they lie on the linear portion of the CRC and also the ratio between the smaller to greater dose should be preferably 1:2. The selection of the test doses is done by hit and trial method so that the responses fall on the linear part of the curve. By employing the Latin square design, the responses of the chosen standard and the test doses is recorded in 4 successive cycles, in the order of
  - S1, S2, T1, T2;
  - S2, T1, T2, S1;
  - T1, T2, S1, S2 and

- T2, S1, S2, T1.

- The height of contraction of all the standard and the test doses in the bioassay is measured and their mean values are calculated. The concentration of the test sample can be determined by graphical method as well as mathematically by employing the following formula:

$$\text{Concentration of unknown} = \frac{n_1}{t_1} \times \text{antilog}\left[\frac{(S_1 + S_2) - (T_1 + T_2)}{(S_2 + T_2) - (S_1 + T_1)}\right] \times \log \frac{n_2}{n_1}$$

Where, n1 = Lower Standard dose

n2 = Higher Standard dose

t1 = Lower Test dose

t2 = Higher Test dose

S1 = Response of n1

S2 = Response of n2

T1 = Response of t1

T2 = Response of t2

- The precision, reliability and reproducibility of this assay method is very high. Hence, it is the most commonly used bioassay for the estimation of the concentration of active substances present in the biological fluids.

Advantages:

- A chemical assay finds out only the amount of active substance present in a given sample where as the bioassay measures the actual biological activity of the active substance.
- Bioassay measures small traces of compound too.
- Bioassay can establish the biological activity of a substance even when its chemical identity is not known.
- Sensitivity of a bioassay is greater than a chemical assay.
- Chemically unstable drugs can be conveniently assayed by a bioassay.
- The active and the inactive isomers present in a racemic mixture can be easily distinguished by a bioassay.

Disadvantages:

- Complicated set up.
- Expensive.

- Time consuming.
- Too laborious.
- Requires skilled labor.
- The effect observed in animals may not be observed in humans.
- The quantitative accuracy of a bioassay usually falls considerably below that attainable with most chemical assays.

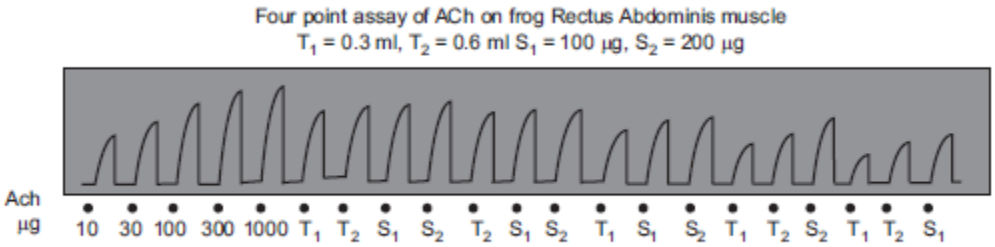


Fig. 24.5

Applications:

- Drugs, which are primarily of natural origin, are usually assayed by biological methods.
- When there is no suitable chemical assay is available for certain drugs such as insulin, oxytocin etc., and then bioassay is the only choice to estimate them.
- Bioassay is the only choice of analysis for some substances when the chemical assay is not a valid indication of their biological activity.
- Bioassays are useful to standardize those drugs that are composed of a complex mixture of substances of varying structure and activity. E.g., Digitalis.
- Bioassays are helpful when the purification of the crude drug sufficient for the performance of a chemical assay is not possible or practical. E.g., Vitamin D from irradiated oils.
- Bioassays are employed to find out the LD50 and ED50 of a drug under investigation.

BIOLOGICAL ASSAY OF INSULIN

Standard Preparation and Unit: It is pure, dry and crystalline insulin. One unit contains 0.04082 mg. This unit is specified by Ministry of Health, Government of India and is equivalent to international unit.

Preparation of Standard Solution: Accurately weigh 20 units of insulin and dissolve it in normal saline. Acidify it with HCl to pH 2.5. Add 0.5% phenol as preservative. Add 1.4% to

1.8% glycerin. Final volume should contain 20 units/ml. Store the solution in a cool place and use it within six months.

Preparation of Test Sample Solution: The solution of the test sample is prepared in the same way as the standard solution.

Rabbit Method

Selection of Rabbits: They should be healthy, weighing about 1800-3000 gm. They should then be maintained on uniform diet but are fasted for 18 hrs. before assay. Water is withdrawn during the experiment.

Standard and Sample Dilutions: These are freshly prepared by diluting with normal NaCl solution so as to contain 1 unit/ml. and 2 units/ml.

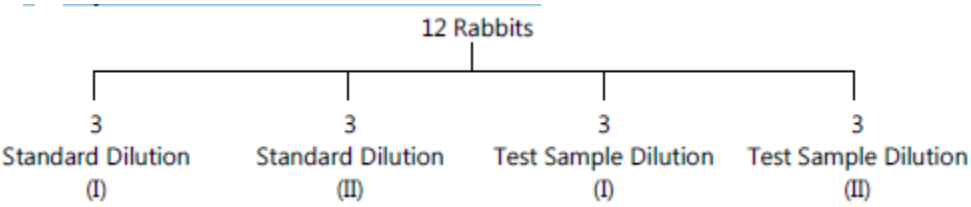
Doses: The dose which can produce suitable fall in blood sugar level is calculated for the standard.

Principle: The potency of a test sample is estimated by comparing the hypoglycemic effect of the sample with that of the std. preparation of insulin.

Experimental Procedure: Animals are divided into 4 groups of 3 rabbits each. The rabbits are then put into an animal holder. They should be handled with care to avoid excitement.

First part of the Test: A sample of blood is taken from the marginal ear vein of each rabbit. Presence of reducing sugar is estimated per 100 ml. of blood by a suitable chemical method. This concentration is called 'Initial Blood Sugar Level'.

- The four groups of rabbits are then given sc. injections of insulin as follows:
- Any other suitable method can also be used.



- From each rabbit, a sample of blood is withdrawn up to 5 hrs. at the interval of 1 hr. each. Blood sugar is determined again. This is known as 'Final Blood Sugar Level'.
- Second part of the test (Cross over test): The same animals are used for the second part. The experiment can be carried out after one week.
- Again they are fasted and initial blood sugar is determined. The grouping is reversed, that is to say, those animals which received the standard are given the test and those which

received the test are now given the standard. Those animals which received the less dose of the standard are given the higher dose of the test sample and vice-versa. This test is known as 'Twin Cross Over Test'.

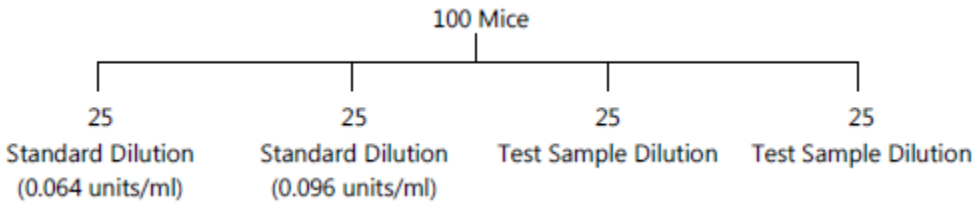
Mouse Method

Mice show characteristic convulsions after s.c. injection of insulin at elevated temperatures. The percentage convulsions produced by the test and standard preparations are compared.

Experimental Procedure: Minimum 100 mice weighing between 18-22gm of the same strain are used. They should be maintained on constant diet. They should be fasted 18 hrs. prior to the experiment.

Standard and Sample Dilutions: Dilutions are prepared with sterile saline solution, so as to contain 0.064 units/ml. (std dilution I) and 0.096 untis/ml. (std. dilution II). Similarly, test sample solutions are also prepared.

Mice are divided into four groups each containing 25 mice and insulin is injected s.c. as follows:



Mice are put in an air incubator at 33oC and observed for one and a half hr. The mice which convulse or die are taken out of the incubator and observed. These mice usually convulse severely but failure of the animal to upright itself when placed on its back, should as well be considered as convulsion.

Rat Diaphragm Method

Sprague Dawley rats weighing 70–100 g are used. The animals are sacrificed during anesthesia and the diaphragms still attached to the rib cages are carefully removed, released from the rib cages and adhering connective and fat tissues, washed in PBS, spread out and divided into two equal pieces as described by Muller and coworkers (1994). For assaying the effects of insulin/compounds/drugs, the hemidiaphragms are incubated in KRH buffer gassed with carbogen (95% O2/5% CO2) in the presence of 5 mM glucose.

Epididymal fat pad of rats:

- Insulin-like activity can be measured by the uptake of glucose into fat cells. Adipose tissue from the epididymal fat pad of rats has been found to very suitable.

- The difference of glucose concentration in the medium after incubation of pieces of epididymal rat adipose tissue or measured oxygen consumption in Warburg vessels, Radiolabelled 14C glucose, the 14CO2 is trapped and counted.
- The concentration is determined by immuno-assay.

BIOLOGICAL ASSAY OF OXYTOCIN

Principle: The potency of oxytocin is determined by comparing its activity with that of the Standard Preparation of oxytocin under the conditions of a suitable method of assay.

Standard Preparation: The Standard Preparation is the 4th International Standard for Oxytocin, established in 1978, consisting of freeze-dried synthetic oxytocin peptide with human albumin and citric acid (supplied in ampoules containing 12.5 Units), or another suitable preparation the potency of which has been determined in relation to the International standard.

Method A:

By depression of the blood pressure in chicken:

- Anaesthetise a young healthy adult cockerel weighing 1.2 to 2.3 kg with an anaesthetic that will maintain a 18 prolonged and constant high blood pressure.
- Expose the gluteus primus muscle in one thigh and cut and retract it to reveal the popliteal artery and crural vein.
- Cannulate the popliteal artery and record the blood pressure on a suitable recorder calibrated for use over a linear range. Cannulate the crural or brachial vein.
- Immediately before use prepare a solution of the Standard Preparation in saline solution so that the volume to be injected is between 0.1 ml and 0.5 ml. Record the blood pressure responses to the injection into the cannulated vein of two doses of this solution; the doses should be such as to produce clearly discriminated, precipitous, submaximal decreases in blood pressure; the required doses normally lie between 20 and 100 milli Units. The interval between injections should be constant and lie between 3 and 10 minutes depending on the rate at which the blood pressure returns to normal. Immediately before use dilute the preparation being examined with saline solution so as to obtain responses similar to those obtained with the Standard Preparation. The ratio between the two doses of the preparation being examined should be the same as that between the two doses of the Standard Preparation and this ratio should be kept constant throughout the assay. The

two doses of the Standard Preparation and the two doses of the preparation being examined should be given according to a randomised block or a

- Latin square design and at least six responses to each should be recorded. If the animal rapidly becomes insensitive to the repeated injections of the solutions another animal must be used. Measure all the responses and calculate the result of the assay by standard statistical methods.

Method B:

By contraction of the rat uterus:

- Inject 100 mg of oestradiol benzoate intramuscularly into a female rat weighing 120 to 200 g 18 to 24 hours before the assay. Immediately before the assay confirm by vaginal smear that the rat is in oestrus or preoestrus. Kill the rat and suspend one horn of the uterus in a bath containing a solution of the following composition:
  - Composition (% w/v)
  - Sodium chloride 0.662
  - Potassium chloride 0.045
  - Calcium chloride 0.007
  - Sodium bicarbonate 0.256
  - Disodium hydrogen phosphate 0.029
  - Sodium dihydrogen phosphate 0.003
  - Magnesium chloride 0.010
  - Dextrose 0.050
- Maintain the bath at a temperature of 32°C or at some other suitable temperature at which spontaneous contractions of the uterus are abolished and the preparation maintains its sensitivity. Oxygenate the solution with a mixture of 95% of oxygen and 5% of carbon dioxide and record the contractions of the muscle using a suitable instrument giving a linear response (for example an isotonic lever with a load not exceeding 2 g). Record the contractions produced by the addition to the bath of two doses of the Standard Preparation suitably diluted with the above solution. The doses should be such as to produce clearly discriminated, submaximal contractions; the required doses normally lie between 10 and 50 micro Units per ml of bath liquid. When maximal contraction has

been reached, replace the bath liquid by a fresh solution. The doses should be added at regular intervals of 3 to 5 minutes depending upon the rate of recovery of the muscle.

- Dilute the preparation being examined so as to obtain responses on the addition of two doses similar to those obtained with the Standard Preparation. The ratio between the two doses of the preparation being examined should be the same as that between the two doses of the Standard Preparation and this ratio should be kept constant throughout the assay.
- The two doses of Standard Preparation and the two doses of the preparation being examined should be given according to a randomized block or a Latin square design and at least six responses to each should be recorded.
- Measure all the responses and calculate the result of the assay by standard statistical methods.

Method C:

By measurement of milk-ejection pressure in a lactating rat:

- Select a lactating rat, in the third to twenty-first day after parturition and weighing about 300 g, separate it from the litter and 30 to 60 minutes later anaesthetise (for example, by the intraperitoneal injection of a solution of Pentobarbitone Sodium). Tie the rat to an operating table, maintained at 37°C by its hind legs leaving the front legs free.
- Cannulate the trachea with a short polyethylene tube of internal diameter about 2.5 mm in such a manner so as to ensure a free airway; apply artificial respiration only if necessary. Cannulate an external jugular or femoral vein with a polyethylene tube of internal diameter about 0.4 mm which is filled with saline solution and closed with a pin. Shave the skin surrounding the inguinal and abdominal teats and excise the tip of one teat, preferably the lower inguinal teat. Insert a polyethylene tube of internal diameter about 0.3 mm and external diameter about 0.6 mm, to a depth sufficient to obtain appropriate measurement of pressure (3 to 10 mm depth), into the primary teat duct which opens onto the cut surface and tie firmly in place with a ligature.
- Connect this cannula with a suitable strain gauge transducer (such as that used for recording arterial blood pressure in the rat) and fill the whole system with a 3.8% w/v solution of sodium citrate or saline solution containing 50 Units of heparin sodium per ml to prevent clotting of milk.

- After cannulation, inject a small volume (0.05 to 0.2 ml) of this solution into the teat duct through the transducer to clear the milk from the tip of the cannula. (This procedure may be repeated during the assay should obstruction arise from milk ejected into the cannula). Clamp the strain gauge so that a slight tension is applied to the teat and its natural alignment is preserved and connect the gauge to a potentiometric recorder adjusted to give fullscale deflection for an increase in milk-ejection pressure of about 5.3 kPa. Inject all solutions through the venous cannula using a 1-ml syringe graduated in 0.01 ml and wash them in with 0.2 ml of saline solution.

Prepare a solution of the Standard

- Preparation and a solution of the preparation being examined in saline solution so that the volume to be injected is between 0.1 ml and 0.4 ml. Choose two doses of the Standard Preparation such that the increase in milk-ejection pressure is about 1.35 kPa for the lower dose and about 2.7 kPa for the higher dose. As an initial proximation, a lower dose of between 0.1 and 0.4 milliUnit and an upper dose of 1.5 to 2 times this amount may be tried. Choose two doses of the preparation being examined with the same inter-dose ratio, matching the effects of the doses of the Standard Preparation as closely as possible. Inject the four doses (two doses of the Standard Preparation and two doses of the preparation being examined) at intervals of 3 to 5 minutes. The two doses of Standard Preparation and the two doses of the preparation being examined should be given according to a randomised lock or a Latin square design and at least four responses to each should be recorded. Measure all the responses and calculate the result of the assay by standard statistical methods.

BIOASSAY OF DIGITALIS

Principle: Potency of the test sample is compared with that of the standard preparation by determining the action on the cardiac muscle. Any other equivalent method, which gives results similar to those obtained by this method as also valid.

Standard Preparation and Units: The standard preparation is a mixture of dried and powdered digitalis leaves (1 unit = 76 mg.)

Preparation of Extracts: Exact amount of the powder is extracted with dehydrated alcohol in a continuous extraction apparatus for six hours. The final extract should contain 10 ml. (5 ml.

alcohol + 5 ml. water) per 10 g. of digitalis powder. It should be stored in between 5˚C and –5℃.

1. Guinea–pig Method (End point method):

- Standard and test sample extracts are diluted with normal saline in such a way that 1 g of digitalis powder is diluted to 80 ml. A guinea pig is anaesthetized with a suitable anaesthetic. It is dissected on the operation table.
- The jugular vein is traced out by removing adhering tissues and cannulated by means of venous cannula. A pin is inserted in the heart, such that it gets inserted in the apex of the heart. In this way, we can observe the heart beats by up and down movements of the pin.
- The injection is continued through venous cannula until the heart is arrested in systole. The amount of extract required to produce this effect is taken as the lethal dose of the extract.
- Another set of 19 animals of the same species are used for this experiment and the average lethal dose is determined.
- It is not necessary to determine the lethal dose of the std. during each time of the experiment. But it should be occasionally checked.
- The lethal dose of the test sample is determined in a similar way using minimum 6 guinea–pigs of the same strain.
- The potency of the test sample is calculated in relation to that of the std. preparation by dividing the average lethal dose of the sample to the test and expressed as units per gram.

2. Pigeon Method:

- Minimum 6 pigeons are used for testing each sample. They should be free from gross evidence of disease or emaciation.
- The weight of the heaviest pigeon should not exceed twice the weight of the lightest pigeon. Food is withheld 16-28 hours before the experiment. Pigeons are divided on the basis of their sex, weight and breed, into two groups.
- They are anaesthetized with anaesthetic ether. One side of the wing is dissected and the alar vein is cannulated by means of a venous cannula. Dilutions are made with normal saline. Average lethal dose of each sample is determined; results are tabulated and calculated as per guinea pig method.

- The lethal dose per kg. of body weight is determined for each pigeon. The potency of the test sample is determined by dividing the mean lethal dose of standard by the mean lethal dose of the test sample.
- In pigeons, stoppage of heart is associated with a characteristic vomiting response called 'emesis'. The milk from the crop sac of pigeons is being ejected out. This may be taken as the end point response of digitalis.

BIOASSAY OF D-TUBOCURARINE

Rabbit Head-drop Method

Principle: d-Tubocararine hydrochloride is injected into the marginal vein of a rabbit's ear till the rabbit's neck muscles are relaxed such that the animal cannot hold its head up. The total amount of test sample required to produce the endpoint is compared with the total amount of the standard sample required to produce similar endpoint.

Selection of Rabbits: Rabbits weighing 2 kg are used. Animals should be free from disease, obtained from a healthy colony and should be accustomed with the experimental procedure.

Experimental Procedure: Each rabbit is placed in a holder with its head protruding outside. The head should be freely movable. Minimum 8 rabbits are used. They are divided into two groups each containing 4 rabbits. First group will receive standard sample and the second group will receive the sample under test. d-Tubocurarine solution is injected at a constant speed by infusion apparatus through the marginal vein.

(i) i.v. inj. of d-tubocurarine.

(ii) Head drop after injection.

Injection should be given at a rate of 0.4 ml/min and should take about 10 min. Infusion is continued till the rabbit will not be in a position to hold its head erect or there will be no response by focusing light on the eyes and the neck gets elongated and toneless.

Suitable dose of d-tubocurarine is 0.012% w/v in saline. Rabbits recover immediately from the effect of curarization. During the expt. there is a possibility or respiratory embarrassment which is treated by injecting neostigmine methyl sulphate (0.05 mg.) and atropine sulphate immediately through the marginal ear vein.

Cross-over test is carried out to minimise biological error due to animal variation. Those rabbits which received the standard sample on the first day will be given test sample on the second day

of expt. and vice versa. Mean dose which produces head drop of the test sample is compared with the mean dose of standard preparation.

BIOASSAY OF HISTAMINE

Bioassay of Histamine using guinea-pig Ileum:

Drugs:

Standard histamine solution (St.) (10 µg/ml)

Test histamine solution (T)

Procedure:

- A clean strip of the ileum is placed in freshly prepared Tyrode's solution. A small segment (3cm) of ileum is cut; a thread is passed through the lumen & the wall at each end of the segment with the aid of a fine needle.
- One end of the segment is tied securely to the aeration tube & transferred to organ bath (already filled with Tyrode's solution & bubbled with gas). The other end of the ileum is attached to the transducer; the tension of the thread is adjusted.
- The baseline record of contraction on the physiograph and the sensitivity are adjusted before the addition of drugs.
- The normal tone of ileum is recorded for 1 min. then different volumes of St. histamine are started to be added. The contact time for the each histamine volume is 30 seconds. At the end of each cycle, the tissue is washed two times with the Tyrode's solution each time the tissue is allowed in contact with the solution for 30 seconds.(Cycle time= 2.5min, contact time for agonist(30 sec.) + normal record(1min.)+ washing twice(30 sec.))
- Different doses of St. histamine solution are added (0.05ml, 0.1ml, 0.2ml, 0.3ml &0.4ml) to chose two proper doses and bracketing the test in between them. The amount of standard which produce responses matching those of the dose of test is tried and attempt is made to reduce the limits as far as possible.
- Changing in St. histamine doses, followed by the dose of test, is continued till the test dose was bracketed between 2 doses of St. histamine given nearly the same response of the test.

BIOASSAY OF VASOPRESSIN

Definition:

Bioassay is defined as estimation of the concentration or potency of a substance by measuring its biological response in a living system.

Principle:

Potency of vasopressin injection is determined by comparing test activity with that of standard preparation of vasopressin.

Standard Preparation:

It is a dried acetone extract of posterior lobes of pituitary gland of oxen or any other suitable preparation.

Standard unit: Specific pressor activity corresponding to that yielded by 0.0005gm of standard preparation (20units/ml).

Procedure:

- **Animal:** Albino rat of 300g weight.
- Anaesthetize it by S.C. injection of Ethyl carbamate.
- After 40-60 min., cannulate the trachea with polyethylene tube of 2.5 mm external diameter.
- Dissect carotid artery for cannulation.
- Cannulate femoral vein close to inguinal ligament by the following process
- Retract abdominal muscles to expose the inguinal ligament and superficial prudential vein to one side.
- Dissect femoral vein towards inguinal ligament from corresponding artery.
- Tie a short polyethylene cannula (1 mm external diameter) into femoral vein by two ligatures, joined by short piece of rubber tubing to 1 ml burette with an attached thistle funnel containing saline solution.
- Fix a wet cotton swab & tie to cover the incision and cannula .
- Inject 200U heparin in saline solution/100 g body weight.
- Connect carotid artery cannula with mercury manometer (2-3mm internal diameter).
- Inject all solutions through venous cannula by 1 ml syringe.
- A suitable hypotensive agent is given into tail vein to produce a constant basal pressure of 50 torr.
- Dilute standard & test preparations such that volume to be injected is between 0.1- 0.5 ml.

- Choose 2 doses of standard so that lower dose produces 30 torr B.P. & higher produces 50 torr B.P.
- i.e., ratio of doses should be 3:5.
- Select test doses according to standard doses.
- Doses are added at intervals of 3-5 min. in a random order.
- Record rise in B.P. in response to each dose.

Method 2:

- Anaesthetize healthy cat with volatile anaesthetic agent.
- Insert a tracheal tube for artificial respiration.
- Expose spinal cord from behind by removing second cervical vertebrae.
- Destroy brain by passing suitable instrument through foramen magnum.
- Start artificial respiration through tracheal tube & leave animal for an hour to remove anaesthetic effect.
- Cannulate carotid artery for B.P. measurement & femoral vein for injection of drug solutions.
- Maintain normal B.P. at 50-100 torr.
- Select 2 doses of test & standard; inject 0.05-0.1 units at 30 min. interval.
- Record maximum rise in B.P. in response to each dose.

BIOASSAY OF ACTH

Official preparation

- Corticotropin injection: is a sterile solution, in a suitable diluents, of the polypeptide from the pituitary glands of mammals. Potency range should be 80.0 – 120.0% of cartiotropin units.

Purpose and Rationale:

- This is a historical assay method.
- Administration of pituitary gland ACTH decrease the ascorbic acid present in the adrenals.
- The depletion of adrenal ascorbic acid is a function of the dose of ACTH administered.
- This relationship has been used for a quantitative assay of ACTH.

Solution:

- Five units of test or standard dissolved in 0.25 ml of 0.5% phenol solution and diluted with 8.1 ml of 15% gelatin solution (Now 0.5 ml contains 300 mU ACTH).
- (Solution A)
- Three ml of solution A diluted with 6 ml of gelatin solution. Now concentration reduced to 100 mU ACTH/0.5 ml) (Solution B).
- Again 3 ml of solution B diluted with 6 ml of gelatin solution, the resulting solution contains 33 mUACTH/0.5 ml.

Procedure:

- Male wistar rat (100-200 g) are hypophysectomized (pituitary gland removed by surgery) one day prior to the test.
- For one test with 3 dose of test preparation and standard.
- Number of hypophysectomized rats required: at leat 36 (preferably 60).
- The hypophysectomized rats are randomly distributed in to six groups. Each rat receives subcutaneous 0.5 ml of the various concentration of test or standard.
- Three hours after injection, the animals are anesthetized and both adrenal and removed, freed from extraneous tissue and weighed. The rats are sacrificed and the skull opened to verify completeness of hypophysectomy.
- The adrenal are homogenized in glass tubes contains 200 mg pure sand and 8.0 ml of 4% trichloroacetic acid and the ascorbic acid determined.
- The potency ratio including confidence limits is calculated with the 3+3 point assay.

Estimation of Ascorbic Acid:

- Preparation of 1 mg/ml conc. Of ascorbic acid in 4% TCA (stock) solution A.
- Use solution A to prepare 0.2% of ascorbic acid in 4% TCA (solution B).
- Use solution B to prepare 0.02% of ascorbic acid in 4% TCA (solution B).
- The calibration curve is established at a wave length of 540 mm using the solutions without ascorbic acid as blank.

BIOASSAY OF 5HT

Objective: To record the concentration response curve of 5 HT using isolated rat fundus strip preparation.

Principle:

- The basic principle of bioassay is to compare the test substance with the Standard preparation of the same and to find out how much test substance is required to produce the same biological effect, as produced by the standard.
- Rat fundus is a very sensitive tissue for the study of the action of several naturally occurring substances like 5HT, Histamine, Acetyl Choline and Bradykinin.
- Unlike the intestinal smooth muscle this preparation is slow contracting and slow relaxing serotonin.
- Rat fundus preparation is generally employed for the bioassay of serotonin.
- The fundus is grey in colour and therefore, easily identified from pyloric part.
- A zig-zag preparation of the fundal strip is prepared so as to expose maximum portion of the tissue to drug.
- The tissue is sensitive to 1 ng/ml of serotonin.

Procedure:

1. Sacrifice the rat by a blow on the head and carotid bleeding.
2. Cut open the abdomen and expose the stomach.
3. Identify the fundus of the stomach, incise it from the junction of pyloric part and put it in the dish containing Krebs solution.
4. Incise the fundus from the lesser curvature and open it longitudinally. Give alternate Zig-Zag cuts to make a fundal strip preparation, tie both the ends with thread and mount in the organ bath containing Krebs solution at 37˚C. Aerate the tissue.
5. Apply 1g load and allow the preparation to equilibrate for 30 min. using frontal writing lever with 10-12 magnification record the contraction due to increasing concentration of serotonin. Since the muscle contracts slowly and relaxes slowly, a contact time of 90sec and 5 min time cycle is followed for proper recording of the concentration response curve.
6. Label and fix the tracing. Plot the concentration response curve.

IMPORTANT QUESTIONS

Very Short Answer Type Questions

1. What are contraceptives?
2. What are the uterine stimulants?
3. What are uterine relaxants?
4. What are androgens?
5. Define bioassay.
6. Classify bioassay.
7. Define quintal bioassay.
8. Describe the graded bioassay
9. What is potency?
10. What is efficacy?
11. Write the use of bioassay.
12. What is radioimmuno assay?
13. Explain the principle of oxytocin bioassay.
14. Explain the principle of vasopressin bioassay.
15. Explain the principle of D-tubocuranie bioassay.

Short Answer Type Questions

1. Discuss corticosteroids; write the mechanism of action of prednisolone.
2. Describe androgens and its functions.
3. Write a note on oral contraceptive.
4. Describe the pharmacology of oxytocin.
5. Define bioassay and its types in details.
6. Explain the principle of bioassay with example.
7. Discuss the bioassay of digitalis.
8. Describe the method of biological assay of oxytocin.
9. Explain the principle of vasopressin bioassay.
10. Explain the principle of insulin bioassay.