GENETICS & ANIMAL PHYSIOLOGY

PRACTICAL

M.Sc., ZOOLOGY First Year

Semester –II, Paper-V

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M.Sc., ZOOLOGY - GENETICS & ANIMAL PHYSIOLOGY

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A^{+}' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the doorstep of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
M.Tech.,Ph.D.,
Vice-Chancellor I/c
Acharya Nagarjuna University

M.Sc.–Zoology

SEMESTER-II

GENETICS & ANIMAL PHYSIOLOGY (205ZO24)

Syllabus

GENETICS

1. Genetic disorders – photographs
    a. Klinefelter syndrome
    b. Turner syndrome
    c. Cri-du-chat syndrome
    d. Patau syndrome
    e. Down syndrome
    f. Edwards Syndrome
2. Genetic exercise/problems based on:

a Dihybrid cross

b Law of independent assortment

c Multiple alleles

d Interaction of genes

ANIMAL PHYSIOLOGY

1. Estimation of glycogen
2. Estimation of proteins
3. Estimation of lipids
4. Estimation of haemoglobin
5. Qualitative identification and estimation of ammonia
6. Qualitative identification and estimation of urea

CODE: 205ZO24

M.Sc DEGREE EXAMINATION
Second Semester
Zoology:: Paper V – GENETICS & ANIMAL PHYSIOLOGY

MODEL QUESTION PAPER

Time : Three hours **Maximum : 70 marks**

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1. Estimation of glycogen in the given sample **(20 M)**

2. Qualitative identification and estimation of ammonia in the given sample **(10 M)**

3. Write detail note on dihybrid cross with example **(15 M)**

4. Identify the given spotters **(5x3=15)**

- **a.** Klinefelter syndrome
- **b.** Down syndrome
- **c.** Patav syndrome
- **d.** Turner syndrome
- **e.** Cri-du-chat syndrome

5. Record & viva **(10M)**

GENETIC DISORDERS

A typical human being has 46 chromosomes: 28 paras of autosomes & one pair of sex chromosome that determines the child sex. Children normally inherit 23 chromosomes from each parent. Sometimes child may end up with either sometimes more (or) less than normal number of chromosomes because of Problems with the father's sperm (or) the mother's egg due to non- disjunction.

Non- disjunction (not coming part)

The failure of chromosome pairs to separate properly during cell division is known as nondisjunction. This can occur due to the failure of homologous chromosomes to separate during meiosis I, or the failure of sister chromatids to separate during meiosis II. As a result of this error, cells may have an imbalance in chromosome number. A cell that loses a single chromosome is said to have monosomy **(2n − 1),** while a cell that has one extra chromosome in addition to a pair is said to have trisomy **(2n + 1).**

Genetic disorders due to non-disjunction of chromosomes

a. KLINEFELTER SYNDROME (47, XXY)

It is a genetic disorder caused by the nondisjunction of allosomes (sex chromosomes). Normally, the disjunction or proper separation of chromosomes occurs during meiosis. However, when homologous chromosomes fail to separate during meiosis, the process is referred to as nondisjunction. When nondisjunction involves the allosomes, it results in allosomal aneuploidies of various types. These aneuploid karyotypes include:

Monosomy: single chromosome in a homologous pair

Trisomy: three chromosomes in place of a homologous pair

Tetrasomy: four chromosomes in a homologous pair

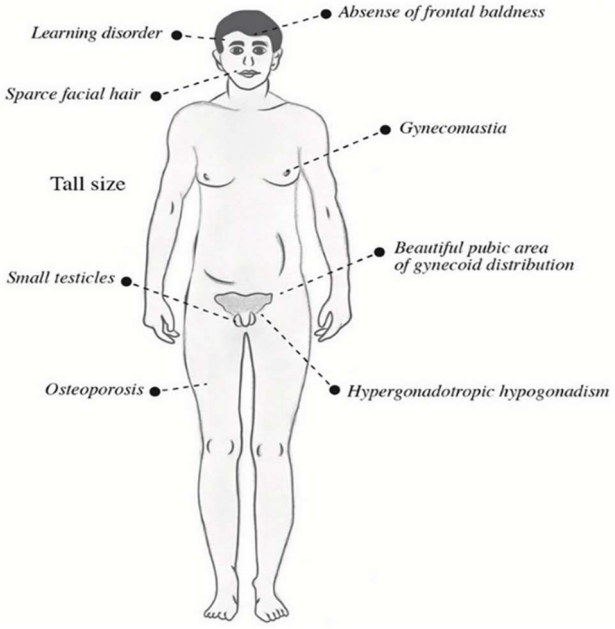


Figure: Klinefelter syndrome

Klinefelter male:

[Karyotype – 47] [44XXY]

Klinefelter syndrome is a genetic disorder that affects males who have an extra X chromosome, giving them a 47, XXY karyotype instead of the normal 46, XY. It was first described in 1942 by Dr. Harry Klinefelter, who worked with Fuller Albright at Massachusetts General Hospital in Boston. The condition occurs approximately in 1 out of every 2000 male births. The extra X chromosome affects the development of the testes, leading to reduced testosterone levels and causing a variety of physical, hormonal, and cognitive changes.

Klinefelter males usually appear physically normal until puberty, when symptoms begin to become noticeable. Due to low testosterone levels and increased FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone), affected males may show female secondary sexual characteristics, such as breast enlargement. Variations of the syndrome also exist, such as 48, XXXY (two Barr bodies) and 49, XXXXY (three Barr bodies).

Symptoms:

1. Tall stature with disproportionately long legs and arms.

2. Abnormal body proportions (long legs, short trunk).

3. Small testes (hypogonadism) and small penis.

4. Gynecomastia (enlarged breasts).

5. Sparse facial and body hair.

6. Low muscle tone and reduced body strength.

7. Low testosterone with high FSH and LH levels.

8. Infertility and diminished sexual drive.

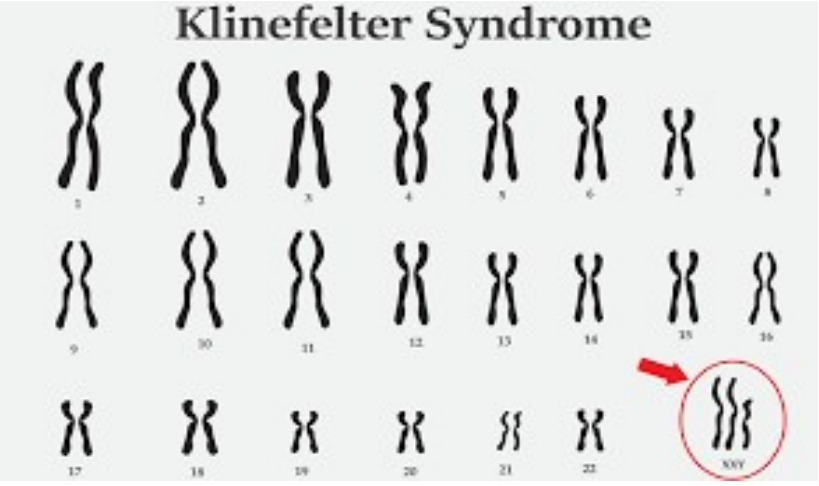
9. Speech and language delays often learn to speak later than peers.

10. Mild learning disabilities and difficulty in reading and writing.

11. Attention Deficit Hyperactivity Disorder (ADHD) or social immaturity.

12. Personality changes shyness, low confidence, and emotional sensitivity.

13. Normal to borderline intelligence.



b. TURNER FEMALE (45, X)

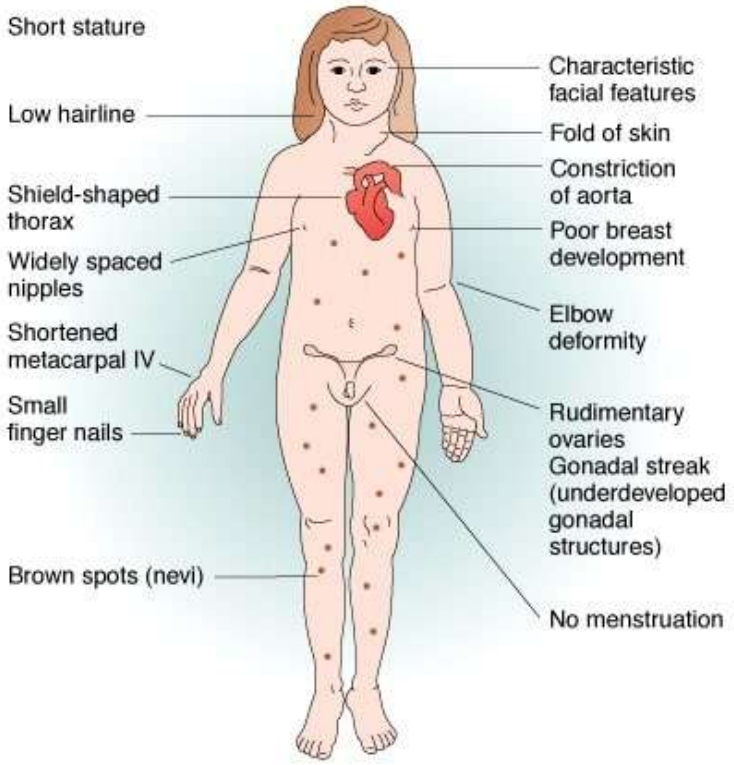


Figure: Turner syndrome

Turner syndrome is a genetic disorder that affects females, caused by the absence of one X chromosome. The general karyotype is 45, X (instead of the normal 46, XX). This condition results from nondisjunction during meiosis, where one of the sex chromosomes fails to separate properly. It occurs approximately in 1 out of every 5000 female births.

The affected individual has a female phenotype, but with several developmental abnormalities. Since there is only one X chromosome, no Barr body is present in the cells. The ovaries are usually rudimentary (underdeveloped), leading to infertility and absence of menstruation (amenorrhea). Turner females are usually shorter than average and may show distinct physical features such as a webbed neck and broad chest with widely spaced nipples.

Symptoms:

1. Short stature (below normal height for age).
2. Webbed neck (skin folds on the sides of the neck).
3. Lymphedema (swelling) of hands and feet at birth.
4. Broad, shield-shaped chest with widely spaced nipples.
5. Low hairline at the back of the neck.

6. Low-set ears.

7. Rudimentary or streak ovaries, causing infertility.

8. Amenorrhea (absence of menstrual periods).

9. Underdeveloped secondary sexual characteristics (e.g., lack of breast development).

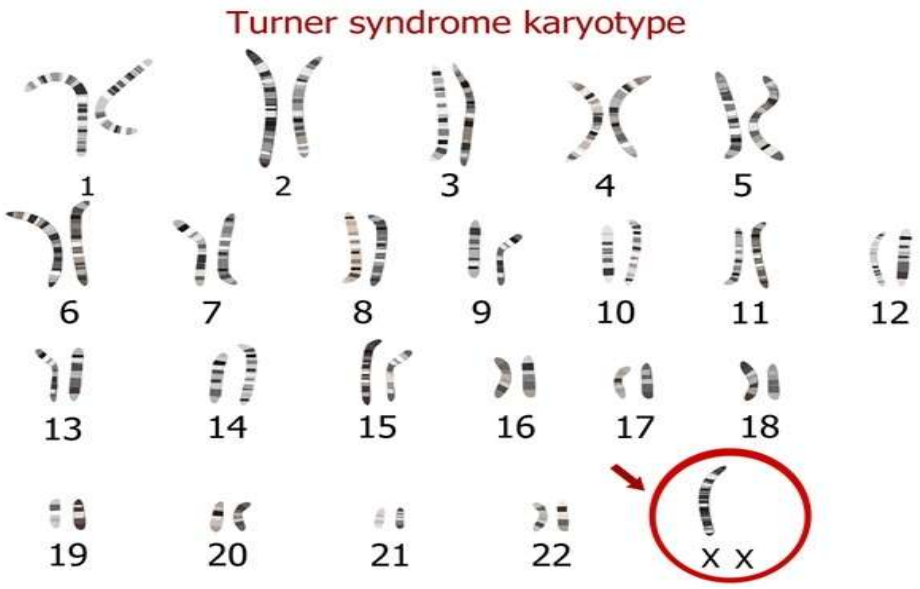
10. Increased body weight or obesity.

11. Shortened metacarpals (short fingers).

12. Small fingernails and small finger joints.

13. Possible heart, kidney, or thyroid disorders.

14. Normal intelligence, but may have difficulty with spatial or mathematical tasks.



c. CRI-DU-CHAT SYNDROME (Cat Cry Syndrome / 5p– Syndrome):

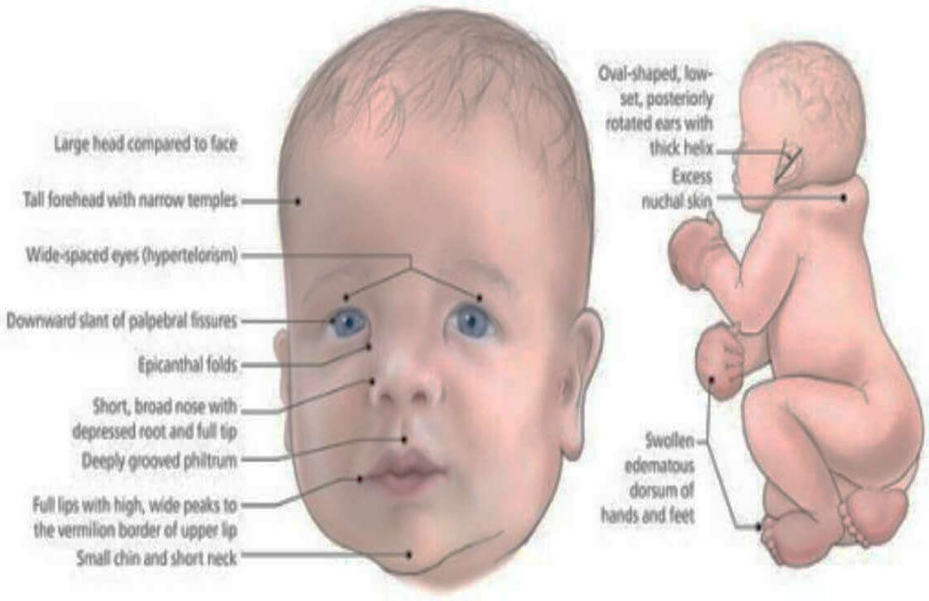


Figure: Cri-du-chat syndrome

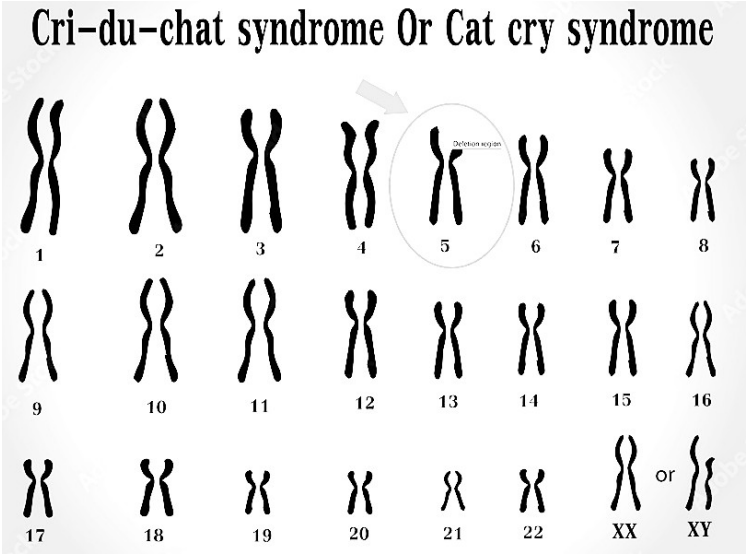
Cri-du-chat syndrome (French for "cry of the cat") is a rare genetic disorder caused by a partial deletion (loss) of the short arm of chromosome 5 (5p-). The condition was first described by Jerome Lejeune in 1963, and it is also known as Lejeune's Syndrome or Chromosome 5p Deletion Syndrome.

The characteristic name "Cri-du-chat" comes from the distinctive, high-pitched, cat-like cry of affected infants, caused by abnormalities in the larynx and nervous system. The general karyotype is 46, XX, 5p- for females and 46, XY, 5p- for males. The syndrome occurs in approximately 1 in 50,000 live births.

Symptoms:

1. High-pitched, cat-like cry in infancy.
2. Feeding difficulties due to problems with sucking and swallowing.
3. Poor growth and low birth weight.
4. Severe developmental delay, including speech and motor delays.
5. Behavioural problems such as hyperactivity, aggression, tantrums, and repetitive movements.

6. Distinct facial features, which may include small head (microcephaly), round face, wide-set eyes, and low-set ears.
7. Excessive drooling.
8. Constipation and digestive problems.
9. Intellectual disability, ranging from moderate to severe.



d. PATAU SYNDROME (TRISOMY 13)

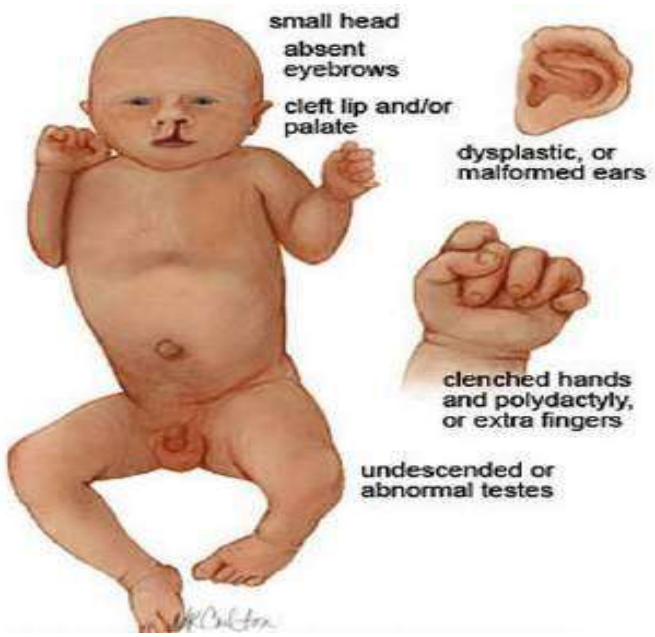


Figure: Patau syndrome

Patau syndrome, also known as Trisomy 13, is a congenital genetic disorder caused by the presence of an extra copy of chromosome 13. The karyotype is 47, XX, +13 for females and 47, XY, +13 for males. It was first described in 1960 by Dr. Klaus Patau, after whom the syndrome is named. The condition occurs in approximately 1 in 10,000 live births.

The extra chromosome disrupts normal development, resulting in severe physical and mental abnormalities. Most affected infants have multiple organ defects and a very short life expectancy. The risk increases with advanced maternal age (above 30 years).

Symptoms:

Nervous System Abnormalities

1. Severe mental and motor retardation.
2. Microcephaly (small head).
3. Holoprosencephaly (failure of the forebrain to divide properly).
4. Structural eye defects such as:

- Microphthalmia (small eyes)

- Coloboma (cleft or gap in structures of the eye)
- Cataract or retinal dysplasia
- Nystagmus and optic nerve defects

5. Cleft palate and cleft lip.

6. Meningomyelocele (spinal defect).

Musculoskeletal and Skin Abnormalities

7. Polydactyly (extra fingers or toes).

8. Low-set ears.

9. Prominent heel, known as rocker-bottom feet (deformed feet).

10. Omphalocele (abdominal wall defect where intestines protrude).

11. Clenched hands with overlapping fingers.

12. Cutis aplasia (absence of patches of skin or scalp hair).

Urogenital and Other Defects

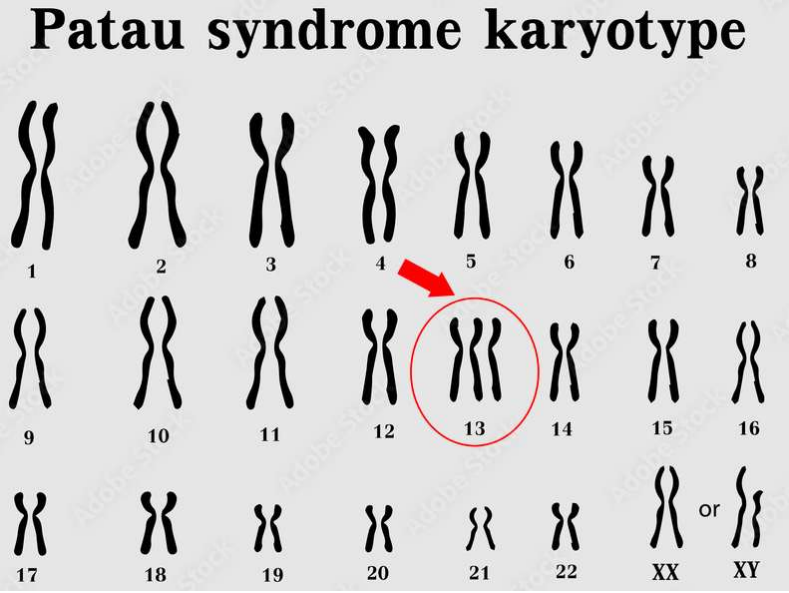
13. Abnormal genitalia.

14. Kidney defects.

15. Heart defects, especially ventricular septal defect (VSD).

16. Single umbilical artery instead of two.

17. Abnormal lung development.



e. DOWN SYNDROME (TRISOMY 21)

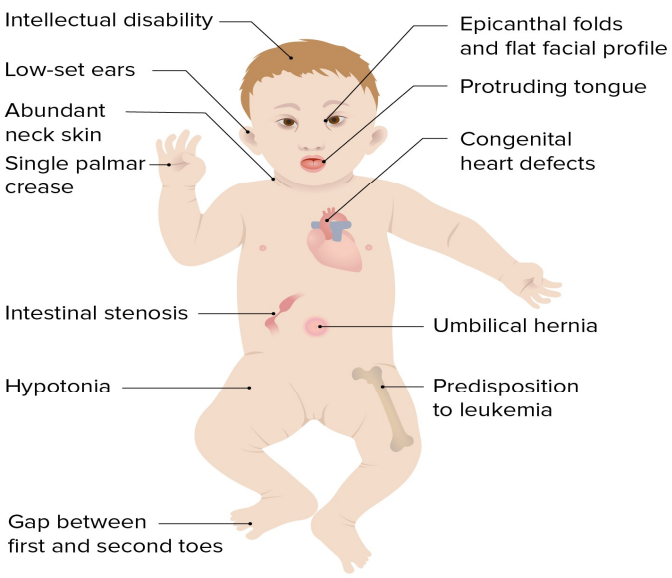


Figure: Down syndrome

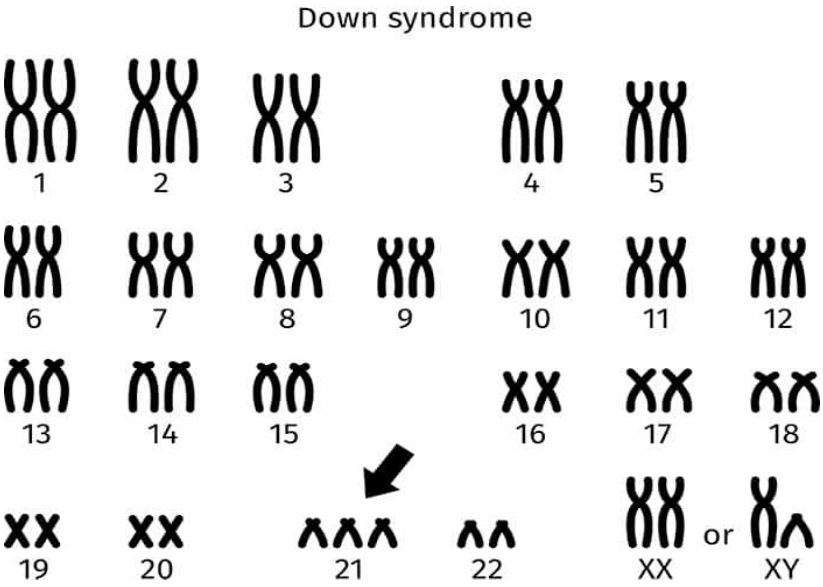
Down syndrome is an aneuploid genetic disorder caused by the presence of an extra copy of chromosome 21. The karyotype is 47, XX, +21 for females and 47, XY, +21 for males. It is also called Trisomy 21 because there are three copies of chromosome 21 instead of the normal two.

The syndrome was first described by the British physician Dr. John Langdon Down in 1866, after whom the condition is named. The incidence is approximately 1 in every 800 to 1000 live births, and the risk increases with maternal age, especially after 35 years. The extra chromosome arises due to nondisjunction during meiosis, which occurs when chromosomes fail to separate properly during cell division.

Symptoms:

1. Short stature and stocky build.
2. Small, round head with a flat facial profile.
3. Partially open mouth with a protruding, furrowed tongue.
4. Epicanthic folds (skin folds at the inner corners of the eyes giving an almond-shaped appearance).
5. Short, broad hands with a single transverse palm crease (simian crease).

6. Short fingers with characteristic palm and finger patterns.
7. Poor muscle tone (hypotonia) and delayed motor development.
8. Physical, psychomotor, and mental retardation.
9. IQ usually below 70.
10. Congenital heart defects are common.
11. Life expectancy is reduced (30), but many individuals can live into adulthood with proper care.



f. EDWARDS SYNDROME (TRISOMY 18)

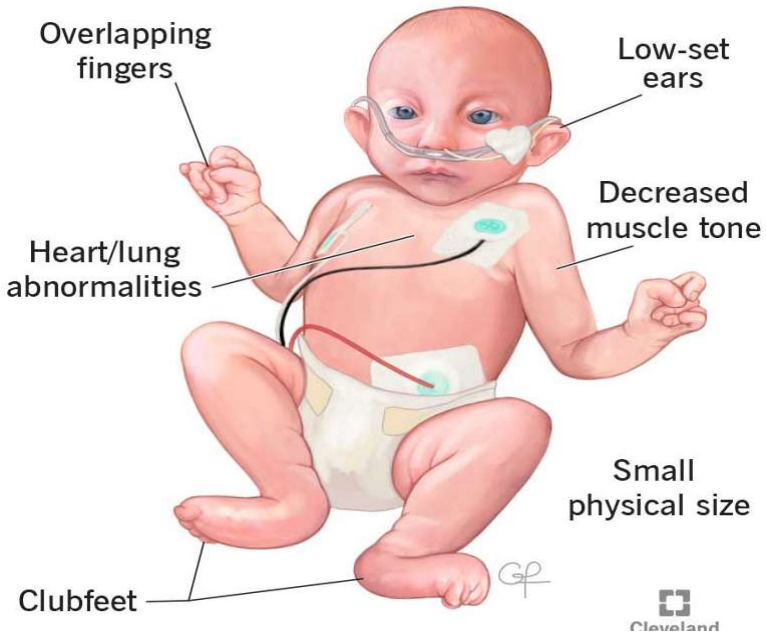


Figure: Edwards Syndrome

Edwards Syndrome, also known as Trisomy 18, is a genetic disorder caused by the presence of an extra copy of chromosome 18. The karyotype is 47, XX, +18 for females and 47, XY, +18 for males. It was first described by John H. Edwards in 1960, and the syndrome was named after him.

The incidence of Edwards Syndrome is approximately 1 in 3,000 to 10,000 live births. The condition is more common with increasing maternal age, especially after 30 years. The presence of the extra chromosome affects nearly every organ system, causing multiple congenital abnormalities and severe developmental delays. Most affected infants are stillborn or die within the first few weeks of life, although a small number may survive for a few months.

Symptoms:

1. Microcephaly-small head with a prominent back of the skull (occiput).
2. Low-set, malformed ears.
3. Small jaw (micrognathia) and cleft lip or palate.
4. Upturned nose and narrow eyelid openings (palpebral fissures).
5. Widely spaced eyes (ocular hypertelorism).

6. Drooping upper eyelids (ptosis).

7. Short breastbone (sternum).

8. Clenched hands with overlapping fingers and choroid plexus cysts (in brain).

9. Underdeveloped or absent thumbs and nails.

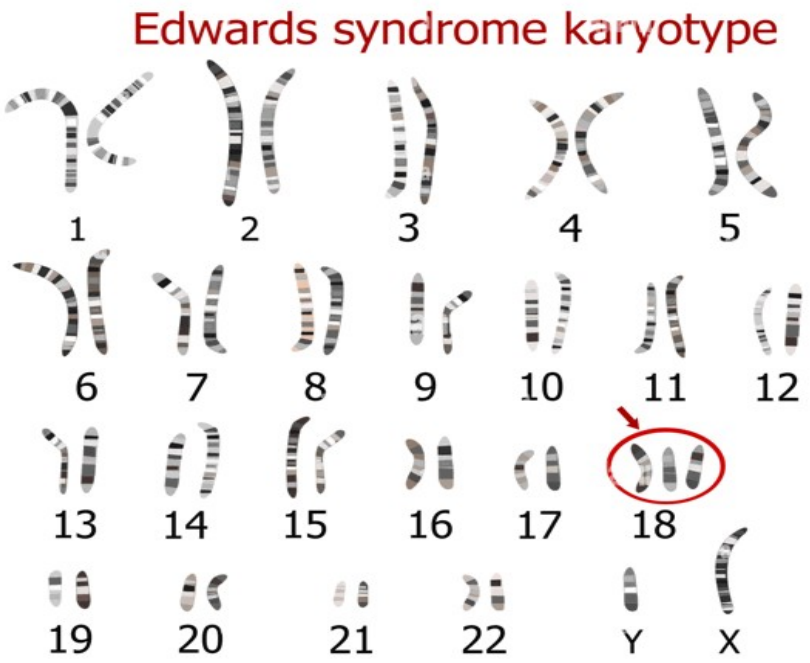
10. Webbing between the second and third toes.

11. Clubfoot (talipes) or rocker-bottom feet.

12. Undescended testicles (cryptorchidism) in males.

13. Congenital heart defects and kidney abnormalities.

14. Severe growth retardation and mental disability.



GENETIC PROBLEMS

a. DIHYBRID CROSS

A dihybrid cross is a cross between two individuals that differ in two traits. The term "di" indicates that two different characters (or pairs of alleles) are involved. A dihybrid cross usually occurs between two parents (a male and a female) that are heterozygous for both traits. The rules of meiosis explain the results of a dihybrid cross through Mendel's two laws - the Law of Segregation and the Law of Independent Assortment. These laws describe how alleles separate and assort independently during gamete formation.

When black feathered long beaked female is crossed with white feathered short beaked male what will be the F_1 hybrid and what are the phenotypic and genotypic ratios in the F_2 generation?

	Female ♀	**Male ♂**
Parent generation:	Black feathered long beak	white feathered short beak
Genotype:	BBLL	bbll
Gamets:	BL , BL	bl , bl

F_1 generation: BbLl

Selfing of F_1 generation: BbLl X BbLl

Gamets: BL, Bl, BL, bl BL, Bl, BL, bl

F_2 generation:

♂ / ♀	**BL**	**Bl**	**bL**	**Bl**
BL	BBLL	BBLl	BbLL	BbLl
Bl	BBLl	BBll	BbLl	Bbll
bL	BbLL	BbLl	bbLL	bbLl
Bl	BbLl	Bbll	bbLl	Bbll

Phenotypic ratio: 9:3:3:1

Black feathered long beak: **9**

Black feathered short beak: **3**

White feathered long beak: **3**

White feathered short beak: **1**

Genotypic ratio: 1:2:1: 2:4:2: 1:2:1

b. LAW OF INDEPENDENT ASSARTMENT

The Law of Independent Assortment, proposed by Gregor Mendel, states that the inheritance of one pair of factors (genes) is independent of the inheritance of another pair.
This law applies only to genes located on different chromosomes or on the same chromosome but far apart (so they do not show linkage). It explains how different traits are transmitted independently from parents to offspring.

When a black, short-haired guinea pig is crossed with a brown, long-haired guinea pig, all F_1 offspring are black and short-haired, because black color (B) and short hair (S) are dominant traits over brown color (b) and long hair (s).

Parent generation: Black short hair Brown long hair

Genotype: BBSS bbss

Gametes: BS , BS bs , bs

F_1 generation: BbSs

Selfing of F_1 generation: BbSs X BbSs

Gametes: BS, Bs, bS, bs BS, Bs, bS, bs

F_2 generation:

♂ / ♀	**BS**	**Bs**	**bS**	**Bs**
BS	BBSS	BBSs	BbSS	BbSs
Bs	BBSs	BBss	BbSs	Bbss
bS	BbSS	BbSs	bbSS	bbSs
Bs	BbSs	Bbss	bbSs	bbss

Phenotypic ratio: 9:3:3:1

Black short hair: **9**

Black long hair: **3**

brown short hair: **3**

brown long hair: **1**

Genotypic ratio: 1:2:1: 2:4:2: 1:2:1

c. MULTIPLE ALLELES

A gene that exists in more than two alternative forms is said to have multiple alleles. Generally, a gene has two alleles, but in some cases, there are more than two alternative forms of the same gene in a population.

The number of possible genotypes for multiple alleles is given by the formula:

$$\text{Number of genotypes} = \frac{n(n+1)}{2}$$

where n = number of alleles.

If a female with blood group A and a male with blood group B are involved,

1. Types of blood groups appearing in the offspring:
2. Genotypes of the offspring:

Parent generation: Group A (female) ♀ Group B (male) ♂

Genotype: i^Ai^O i^Bi^O

Gametes: i^A , i^O i^B , i^O

Fertilization:

♀ \ ♂	i^B	i^O
i^A	i^A i^B	i^Ai^O
i^O	i^Bi^O	i^O i^O

Phenotypic ratio: i^A i^B , i^Ai^O , i^Bi^O , i^O i^O

Genotypic ratio: group AB , groupA , group B , groupO

d. INTERACTION OF GENES

Usually, a single gene controls one character, and its alleles follow simple dominant–recessive relationships, as described in Mendel's classical genetics.
However, in some cases, a character may be influenced by more than one gene. These genes can modify, enhance, or suppress each other's effects.

This phenomenon is called gene interaction or factor interaction. Gene interaction can lead to variations in the expected Mendelian ratios in both monohybrid and dihybrid crosses, producing modified ratios such as 9:3:4, 9:7, or 13:3, instead of the classical 3:1 or 9:3:3:1

The white plumage of White Leghorn fowls is dominant over coloured plumage, such as black. When a White Leghorn is crossed with a White Wyandotte (which may have patterned or coloured feathers), the F_2 generation typically shows white plumage.

Parent generation: white (leghorn) White (Wyandotte)

Genotype: IICC iicc

Gametes: IC , IC ic , ic

F_1 generation: IiCc

Selfing of F_1 generation: IiCc X IiCc

Gametes: IC, Ic, iC, ic IC, Ic, iC, ic

F_2 generation:

generation:

♂ / ♀	**IC**	**Ic**	**iC**	**ic**
IC	IICC **white**	IICc **white**	IiCC **white**	IiCc **white**
Ic	IICc **white**	IIcc **white**	IiCc **white**	Iicc **white**
Ic	IiCC **white**	IiCc **white**	iiCC **coloured**	iiCc **coloure**
Ic	IiCc **white**	Iicc **white**	iiCc **coloured**	iicc **white**

Phenotypic Ratio (F_2):

White : Coloured = 13 : 3

ANIMAL PHYSIOLOGY

1. ESTIMATION OF GLYCOGEN

INTRODUCTION:

They are chief energy giving compounds of the body. The daily carbohydrate requirement is 450-550gm

They are hydrolysed to monosaccharides such as glucose, galactose and fructose by enzymes of the digestive track

Often, they are absorbed into the body, they carried out to liver where galactose and fructose are converted to glucose

AIM:

To estimate the amount of glucose in the given tissue

REQUIREMENTS:

Pipette, test tube, hot air oven, water bath, calorimeter or spectrophotometer, tissue and required chemicals.

METHOD:

Calorimeter method

REAGENTS:

1) 10% TCA- take 10ml of TCA dissolved in 100ml od distilled water
2) 80% ethanol- take 5% of ethanol dissolved in 20% of distilled water
3) Concentric H_2SO_4

PRINCIPLE:

when the tissue is homogenised with 80% ethanol reduced sugar will be repeated treatment with H_2SO_4 sugar will be reacted with H_2SO_4 to form Hydroxy methyl perfectly a brown colour complex. The brown colour complex is proportional to the presence of carbohydrates.

PROCEDURE:

Take 30-50gm of fresh tissue from the freshly killed animal and homogenate it with 5ml of 80% ethanol then centrifuge for 10min at 3000rpm. supernatant is dissolved in 5ml of 5%

TCA mixed well boil the content for 15min at 100%. Then cool the content under the running tap water and the solution is make up to 5ml of TCA because same amount of TCA will be evaporated now centrifuge the contents for 10min at 3000rpm. 2ml of the supplement supernatant is taken and 6ml of through the walls of the Conc H_2SO_4 is added through the walls of the test tube. Boil the contents to 10min and cool them in room temperature finally the Coloured hydroxymethyl is formed. The optical density of the above solution is measured at 520nm through spectrophotometer

PREPARATION OF STANDARD GLUCOSE SOLUTION:

200mg glucose is dissolved in 100ml of distilled water in a standard volumetric flask to get a stock solution of 10mg for 100ml conc H_2SO_4 take 1ml of above glucose standard solution and make up to 2ml of 5% TCA and added 5ml of concH_2SO_4 boil the contents for 10min and cooled at room temperature at then the brown colour Hydroxy methyl is formed

PREPARATION OF BLANK:

Take 2ml of distilled water and add 6ml of conc H_2SO_4 then set 'o' with the blank.

CALCULATION:

Amount of glycogen present in sample :

$$\frac{\text{O.D of sample x amount present in standared}}{O.D\ of\ standard\ x\ volume\ of\ sample}\ x\ 1000$$

O.D of sample = 0.03

O.D of standard = 0.01

Amount in the sample = 0.05

Weight of tissue = 20

$$\frac{0.3\ x\ 0.5}{0.01\ x\ 2.0}\ \text{x}\ 1000$$

= 750mg

RESULT:

Amount of glycogen present in given sample is **750mg/ml**

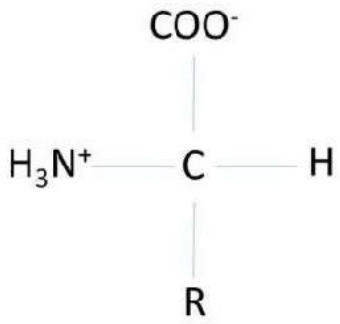
2. ESTIMATION OF PROTEINS

INTRODUCTION:

Proteins are the important receiver for the living organisms' proteins are available through different food resources like Beans, meat, egg etc, almost everything that occurs in the cell involved one or more proteins are among the most abundant in their function.

proteins carry out cellular respiration they are linear polymers of high mole weight. All proteins contain carbon, hydrogen, nitrogen, the presence of nitrogen distinguish from them. carbohydrates and the molecular weight of proteins varies from about 12,000 dalton's to several million Daltons.

Amino acids are building blocks of proteins. The structure of Amino acid as fructose.



Structure of Amino acid

AIM:

To estimate the amount of proteins quantitatively present in the given sample.

METHOD:

Lowry-etal method (1951)

REQUIREMENTS:

Teat tube, centrifuge machine, centrifuge tubes, homogenizer, spectrophotometer and test tube, stand, Pipette, Beaker, weighing Machine

PREPRATION OF REAGENTS:

Take 4ml of NaOH dissolved in 100ml. of distilled water to prepare 0.1N NaOH solution

0.1N NaOH is now dissolved in 100ml of distilled water.

Reagent A:

Dissolve 2gm of Na_2CO_3 in 100ml of NaOH

Reagent B:

Dissolve 500mg of cupric sulphate and add 1mg of sodium potassium tartarated in 100ml of distilled water.

Reagent C:

Take 500ml of Reagent-A and added 1ml of reagent-B

Reagent D:

Prepare 1:1 ratio of Folin-phenol reagent and add 1ml of distilled water

STANDARD BOVIENE AMALGOMIN SOLUTION

Take 5mg of bovine serum albumin and dissolved in 2.5ml of In NaOH that is 0.2mg 1ml concentration.

PRINCIPLE:

This method is about 10 times more sensitive than the first method where the tissue is homogenized with 5% of TCA The tissue constituents are precipitated when the precipitation is dissolved in the measure of IN of NaOH

The carbonyl groups of the protein molecules react with the copper and potassium of reagent-c, and gives copper potassium billet complex

the reagent-D called Folin reagent is quite complex and contains phosphomolybdic acid. The protein constitutes aromatic amino acids like tyrosine and tryptophan. The aromatic amino acids, tyrosine and phenolic compounds present in the proteins, reduce the Folin reagent to phosphomolybdate, producing a dark blue colour.

PROCEDURE:

Take 50mg of fresh tissue and homogenize it with 5ml of 5% TCA (trichloroacetic acid) and centrifuge the contents for about 10-15 minutes at 3000 rpm. then discard the supernatant dissolve the precipitate by adding 1ml of 1N NaOH. From this take 0.2 ml of solution and then add 5ml of reagent -D keep it aside for 30min and read the colour intensity ay 520nm of spectrophotometer (or) calorimeter.

The colour intensity is proportional to the amount present in the sample.

Preparation of standard:

Take 0.2ml of bovine serum albumin standard solution add 0.5ml Folin phenol reagent keep for 10min and read the colour

Preparation of blank:

Take 0.2ml of 1N NaOH and add 5ml of reagent-A then add 25ml of reagent-B and set to blank.

Example:

- 5ml of sample solution contains 25mg of protein
- 0.2ml of sample solution contains 1mg of protein
- 0.4ml of sample solution contains 2mg of protein
- 0.6ml of sample solution contains 5mg of protein
- 0.8ml of sample solution contains 4mg of protein
- 1ml of sample solution contains 5mg of protein

CALCULATION:

To estimate amount of protein present in sample :

$$\frac{\text{O.D of sample} \quad \text{x amount present in standared}}{O.D\ of\ standard\ x\ volume\ of\ sample}\ x\ 1000$$

O.D of sample = 1.33

O.D of standard = 0.7

Amount present in standard = 0.2mg/ml

Amount in the sample = 30

$$\frac{1.33\ x\ 0.2}{0.7\ x\ 30} \text{ x } 1000$$

= 0.01266 x 1000

12.66mg/ml

RESULT:

Amount of proteins present in sample is **12.66mg/ml.**

3. ESTIMATION OF LIPIDS

INTRODUCTION:

Lipids are a large and diverse group of naturally occurring organic compounds that are insoluble in water and soluble in non-polar solvents such as chloroform. It is found as a structural component of cell membrane

Functions of Lipids:

- Storage form of energy
- Structural components of bio membranes
- Non-polar lipids act as electrical insulators
- Provide shape to the body
- Act as metabolic regulators

AIM:

To estimate the amount of lipids, present in the sample.

METHOD:

Folch et al. (1951)

REAGENTS:

- Chloroform-methanol solution
- Calcium chloride solution
- Sodium chloride solution
- Lipid standard solution (e.g., palmitic acid)
- Potassium dichromate reagent

PROCEDURE:

Homogenize the tissue sample with a known volume of cold double-distilled water. Transfer 5 mL of chloroform-methanol solution (2:1, v/v) into a glass-stoppered centrifuge tube and add 0.3 mL of the tissue homogenate. Gradually add chloroform drop by drop while mixing and shaking gently to form a finely clumped precipitate. Allow the mixture to stand at room temperature for 1 hour. After this incubation, add 5 mL of calcium chloride solution (0.1 M) and shake the tube gently for 5 minutes to ensure thorough mixing of the two phases.

For the standard, prepare a stock lipid solution (e.g., palmitic acid dissolved in chloroform-methanol) at a concentration of 10 mg/mL. Dilute this stock solution to 1 mg/mL to create a working standard solution, and pipette 0.1 mL of the working solution into a test tube. For both the test samples and the standard, add 2 mL of potassium dichromate reagent and mix well. Place the test tubes in a heating block set at 85°C and incubate for 30 minutes to ensure the reaction is complete.

After incubation, mix the solutions thoroughly and measure their absorbance at 430 nm using a spectrophotometer. Use water as the blank for calibration. The lipid concentration in the tissue samples is calculated using the standard curve and expressed as milligrams of lipid per milligram of tissue weight.

CALCULATION:

To estimate amount of lipids present in sample :

$$\frac{\text{O.D of sample} \quad \text{x amount present in standared}}{O.D\ of\ standard\ x\ volume\ of\ sample}\ x\ 1000$$

O.D of sample = 0.72

O.D of standard = 1.5

Amount present in standard = 0.2mg/ml

Volume of sample = 4ml

Amount of lipid present in the sample= $\frac{0.72\ x\ 0.2}{1.5\ x\ 4}$ x 1000

$$\frac{0.144}{6}\ x\ 1000\text{mg/ml}$$

= 0.024 x 1000

= 24mg/ml

RESULT:

The amount of lipids present in given sample is **24mg/ml.**

4. ESTIMATION OF HEMOGLOBIN

AIM:

To estimate the percentage of haemoglobin, present in the give blood sample

METHOD:

Salts haemoglobinometer method

REQUIREMENTS:

0.1N Hcl, distilled water

PRINCIPLE:

When the blood is treated with 0.1N Hcl the haemoglobin break to form Heam and globin (or) globulin molecules and to exhibit peculiar colour. This colour will be matched with the colour of the standard haemometer rods, indicating % of haemoglobin.

PROCEDURE:

Take the dilute standard Hcl of 1% dilution into haemometer tube up to the lowest dilution mark with the colour of the standard haemometer rods with the help of dropper Sterilize the pricking needle and finger with a small pad of cotton dipped in spirit. Prick the finger gently with the help of the needle and discard the first drop of blood. Later, suck the blood through the Haemoglobin pipette up to the mark ($20mm^3$ or 0.02ml). Care should be taken that no air bubbles enter into the capillary pipette. Immediately transfer the blood from the pipette into the haemoglobin tube containing HCl. Rinse the pipette 2-3 times. Then, in the haemoglobinometer tube, add distilled water drop by drop until the colour of the content matches with the colour of the permanent rods of the Haemometer. Find out the rod point where the colour of the solution becomes light by the addition of the next drop. The experiment should be repeated twice to avoid errors. Readings should be taken in g/dl of haemoglobin/100ml of blood.

Precautions:

- The entire apparatus should be cleaned before proceeding with the experiment.
- The finger should be cleaned before taking blood.
- There should be a clear column of blood in the pipette without air bubbles.

- While transferring blood, the tip of the pipette should not touch the walls of the test tube.
- The stirrer should not be removed until the experiment is finished.
- Stirring should be done thoroughly and gently.
- The colour should be compared preferably in daylight.
- 0.1N HCl should be taken up to the mark in the calculation tube.

RESULT:

In the given sample, **16g/dl** of blood haemoglobin is present.

Normal haemoglobin concentration:

Males = 14-16 g/dl

Females = 12-15 g/dl

At birth = 2 g/dl

Macrocytic anaemia = increased level of Hb

Microcytic anaemia = decreased level of Hb

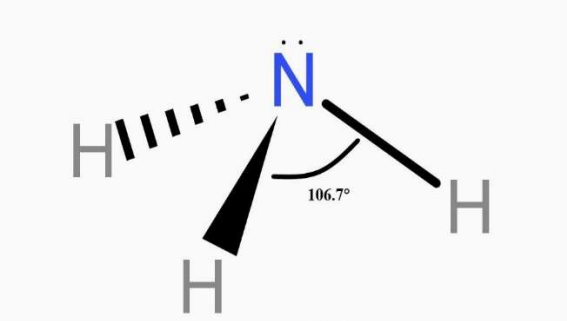
5. QUALITATIVE IDENTIFICATION AND ESTIMATION OF AMMONIA

INTRODUCTION:

In every living organism nitrogen balance is crucial. The excreted nitrogenous wastes constitute several waste products, which differ significantly among species. Ammonia is commonly produced by the oxidation (or) hydrolytic determination of amino acids it is one of the important nitrogenous waste compounds and is highly toxic to the body. So, it is present in low amount toxic to the body. So, it is present in the low concentration excess of ammonia is retain in the body causes toxicity.

The animals that eliminate ammonia is the major excretory product are generally ammonotelic. Fishes are ammonotelic in nature as ammonotelic is associated with the availability of water because of the toxic nature of ammonia it is not allowed to accumulate in the cells.

Ammonia is highly soluble in water and is excreted by aquatic animals.



Chemical structure of ammonia

AIM:

To estimate the amount of ammonia, present in the given sample.

MEATHOD:

Nesslerization by Berg & Mayer (1965).

REQUIREMENTS:

Test tubes, pipettes, Nessler's reagent, spectrophotometer or calorimeter.

PRINCIPLE:

The ammonia that is present in the sample will react with Nessler's reagent to forms a product called Di mercuric ammonium iodide

The intensity of the colour is directly proportional to the ammonia that is present in the sample.

PROCEDURE:

About 5ml of sample was taken into a clean test tube and add 0.5ml of Nessler's reagent the colour intensity of the solution is reacts at the 945nm. Against reagent blank in spectrophotometer.

PREPARATION OF BLANK:

To 5ml of distilled water 0.5ml of Nessler's reagent were added.

CALCULATION:

NH_3+ sample solution = $\frac{\text{O.D of sample x amount present in standared}}{O.D\ of\ standard\ x\ volume\ of\ sample}\ x\ 1000$

O.D of sample = 0.42

O.D of standard = 1.2

Amount present in the sample = 0.1

Volume of sample taken = 5ml

NH_3+ sample solution = $\frac{0.4\ x\ 0.1}{1.2\ x\ 5}$ x 1000

1.2 x 5

$\frac{42}{6}$= 7mg/ml

RESULT:

The amount present in the sample solution is expressed in **7mg/l.**

6. ESTIMATION OF UREA

INTRODUCTION:

Urea is the second major nitrogenous waste produced by animals. It is derived from the breakdown of organic compounds like purines, amino acids, and ammonia. The liver is the organ capable of making urea.

In adult animals, reabsorption of water in the tubules allows them to excrete urea in a concentrated form, which is less toxic than ammonia and less soluble than ammonia. It has been reported that a high protein diet leads to a marked increase in urea output.

Chemically, urea consists of two amino groups (-NH2) and one carbonyl group (CO). The cyclic chain of chemical reactions is known as the "ornithine cycle." Specific enzymes are responsible for catalysing the formation of urea.

AIM:

To identify and estimate the amounts of Urea present in the given sample.

METHOD:

Nessler's method by Bergmeyer (1968).

REQUIREMENTS:

Test tubes, pipette, Nessler's reagent, and urease enzyme.

PRINCIPLE:

Urea present in the sample will be hydrolyzed with urease enzyme, and the ammonia liberated will be estimated using Nessler's reagent.

PROCEDURE:

Take 5mL of sample in a clean test tube and add 0.5mL of urease enzyme solution. After a few minutes, add 0.5mL of Nessler's reagent to the sample, which gives a brown-coloured product, "Dimercuric Ammonium Iodide."

The intensity of the colour is measured spectrophotometrically at 495 nm

Against a reagent blank prepared with 5mL of distilled water, 0.5mL of urease solution, and 0.5mL of Nessler's reagent.

CALCULATION:

To estimate amount of urea present in sample :

$$\frac{\text{O.D of sample} \quad \text{x amount present in standared}}{O.D\ of\ standard\ \ x\ \ volume\ of\ sample}\ x\ 1000$$

O.D of sample = 0.48

O.D of standard = 1.2

Amount present in standard = 0.1

Volume of sample sample = 5ml

Amount of urea present in the sample= $\frac{0.48\ x\ 0.1}{1.2\ x\ 5}$ x 1000

$\frac{48}{6}$mg/ml

= 8mg/ml

RESULT:

The amount of urea present in the given sample is **8mg/ml.**

Prof V. Venkat Ratnamma