1. Genes and Its Expression

- 1.1 Definition, Concept and Function of Gene
- 1.2 **Genetic Code** - Concept of Codon, Properties of genetic code
- 1.3 **Transcription of Gene** - Initiation , Elongation and Termination.
- 1.4 **Translation of Gene** - Initiation , Elongation and Termination.

Genes

- **Definition- Gene** is a particular segment of DNA which is responsible for the expression and inheritance of a character.

Evolution of Gene Concept –

The hereditary units which are transmitted from one generation to the next generation are called genes. A gene is the fundamental biological unit. **Mendel** while explaning the results of his monohybrid and dihybrid crosses, first of all conceived the genes as particulate units and referred them by various names such as **hereditary factors** or **hereditary elements.** The term **Gene** was coined by **Wilhelm Johansen** in**1909** to describe a heritable factor responsible for the transmission and expression of a given biological character but without reference to any particular theory of inheritance. A more precise idea of the physical and functional basis of the gene arose from many sources in the first half of the twentieth century. In 1909 **A. E. Garrod** showed that the human metabolic disorder **Alkaptonuria** resulted from the failure of some specific enzyme and could be transmitted in an autosomal recessive fashion. He called this disorder as an Inborn errors of metabolism. Garrod was unaware about Mendelian inheritance and the significance of his discovery was not appreciated for 30 years, until **George Beadle and Edward Tatum** 1940 found that X-ray induced mutations in **Fungi (Ex-Neurospora)** often caused specific biochemical defects. This led to the One gene one enzyme model of gene function. In 1911 **Thomas Hunt Morgan** showed that **genes were located on chromosomes** and were physically were considered as beads and chromosomes as strings of beads. Mutation was supposed to alter the bead structure and recombination (crossing over) was regarded to involve a breakage between two beds followed by their exchange between paired chromosome .Each bead was thought of to control one character by controlling some biochemical steps. Thus a gene was considered to be a unit of **Mutation, Recombination and Function.**

Concept of Gene

The gene concept was introduced by **Sutton**. The study of **Morgan, Bridges and Muller** etc elaborated it. The essential features of modern concept of genes are the following-

1. Genes determine the physical as well as physiological characteristics. These are transmitted from parents to the offspring's generation after generation.

2. Genes are situated in the **chromosomes**.
3. Since the number of genes in each organism is very large in comparison to the number of chromosomes, several genes are located in each chromosome. In man about **40,000** genes are known to be located on **23** pairs of chromosomes.
4. Each gene occupies a specific position in a specific chromosome. This position is known as **locus**. The chromosomal aberrations(like translocation, inversion) may bring relative changes in the specific position of genes and these help in the origin of chromosomes with new sequences of genes and help in the origin of new species.
5. Genes in the chromosomes are arranged in a single linear order.
6.A single gene may occur in several forms or in several functional states. The forms other than normal are known as **alleles**. Many genes have only two alleles, one of them is normal and other one is its mutant .For example **Red** and **White colour** of flowers in **Pea plant** are controlled by two different alleles of the gene controlling flower colour.
7. The alleles may be related as dominant or recessive but not always.
8. Some genes mutate more than once and have more than two alleles. These are known as **multiple alleles**. Whatever may be the number of alleles in a multiple series only two of them are found in an individual because of the presence of two homologous chromosomes of each type. **Example Blood groups in man.**
9. The gene may undergo sudden change in expression due to change in its composition. The changed gene is known as mutant gene and the phenomenon of change is known as **Mutation**.
10. Genes duplicate themselves very accurately. The phenomenon is known as replication. Self duplication of genes leads to chromosomal duplication.
11. Genes express themselves by producing enzymes which are proteins. It means each gene synthesizes a particular protein which acts as enzyme and brings about an appropriate change.
12. According to the recent information a gene is a segment of DNA which contains the information for one enzyme or one polypeptide chain coded in the language of nitrogen bases or the nucleotides. The sequence of nucleotides in a DNA molecule representing one gene determines the sequence of amino acids in the polypeptide chain(the genetic code).The sequence of three nucleotides reads for one amino acid(codon).

Function of Gene

Genes act mainly by producing enzymes which are involved in different steps of metabolism. Enzymes are proteins formed of a polypeptide chain of amino acids. There are about 20 amino acids. Their arrangement determines different types of proteins . The sequence of amino acids in a polypeptide chain is determined by the genes, which contain the information in the form of genetic code. The genetic code is the triplet sequence of nitrogenous bases in mRNA molecule, which it has copied from the DNA molecule. Two theories have been put forward to explain the mechanism of gene action.

One gene one enzyme theory –

One gene one enzyme theory was proposed by the geneticist Beadle and Tatum 1940 while working on the **Fungi red mold Neurospora crassa**. According to this theory each

gene in an organism controls the production of a specific enzyme. The normal strain of Neurospora is able to grow in the minimal medium containing sucrose, salts and one of the vitamins B-biotin. It can synthesize all other complex substances of the culture medium. These biochemical synthesize reactions are brought about by specific enzymes. Beadle and Tatum exposed some of the asexual spores of Neurospora crassa to mutagen (ultraviolet rays). These mutated ascospores were found unable to grow in the minimal medium, but required the addition of **Thiamine** for the normal growth. When this mutated form was mated with a normal one, the resulted spores were found to contain four mutated and four normal ascospores. These were grown in a minimal medium containing **Thiamine**. After these had multiplied and established their colonies, these were transferred to tubes containing minimal medium (no thiamine). Al transferred spores, which failed to grow in **Thiamine** less medium were separated. These represented the mutant form in which some specific gene has mutated in such a way that it no more produces enzyme necessary for the synthesis of thiamine.

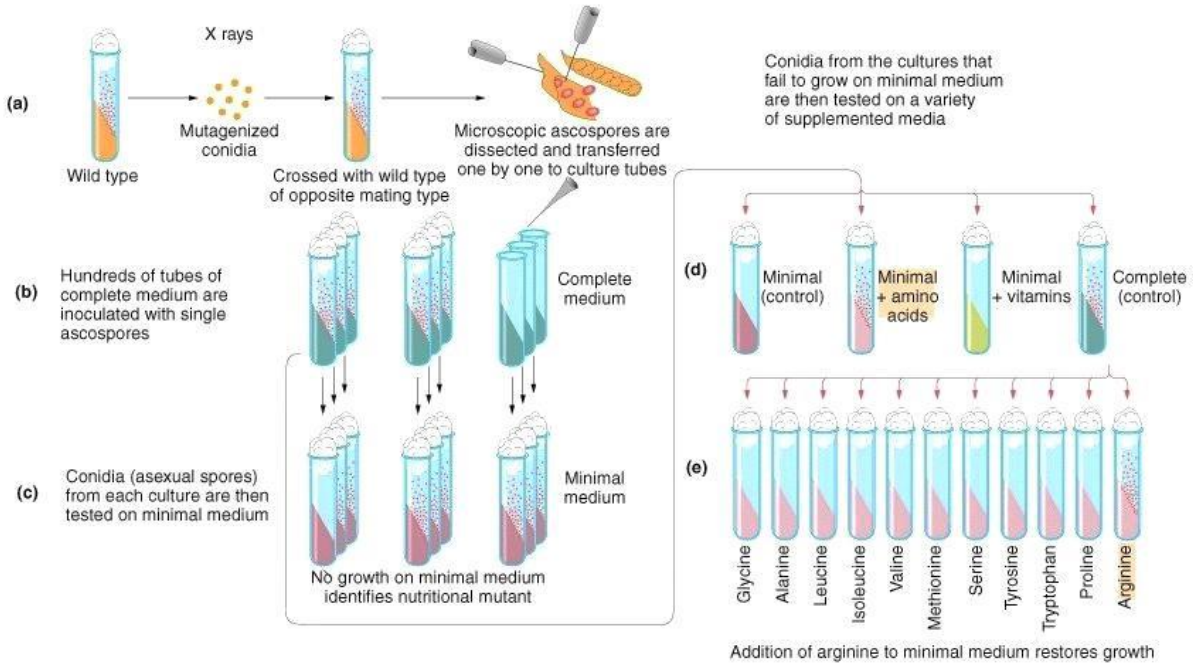
The controlled experiments by Beadle and Tatum have shown that manufacture of Thiamine from simple substance of minimal medium is completed in a number of steps and each step requires the presence of a specific enzyme and each enzyme is produced by a separate gene.

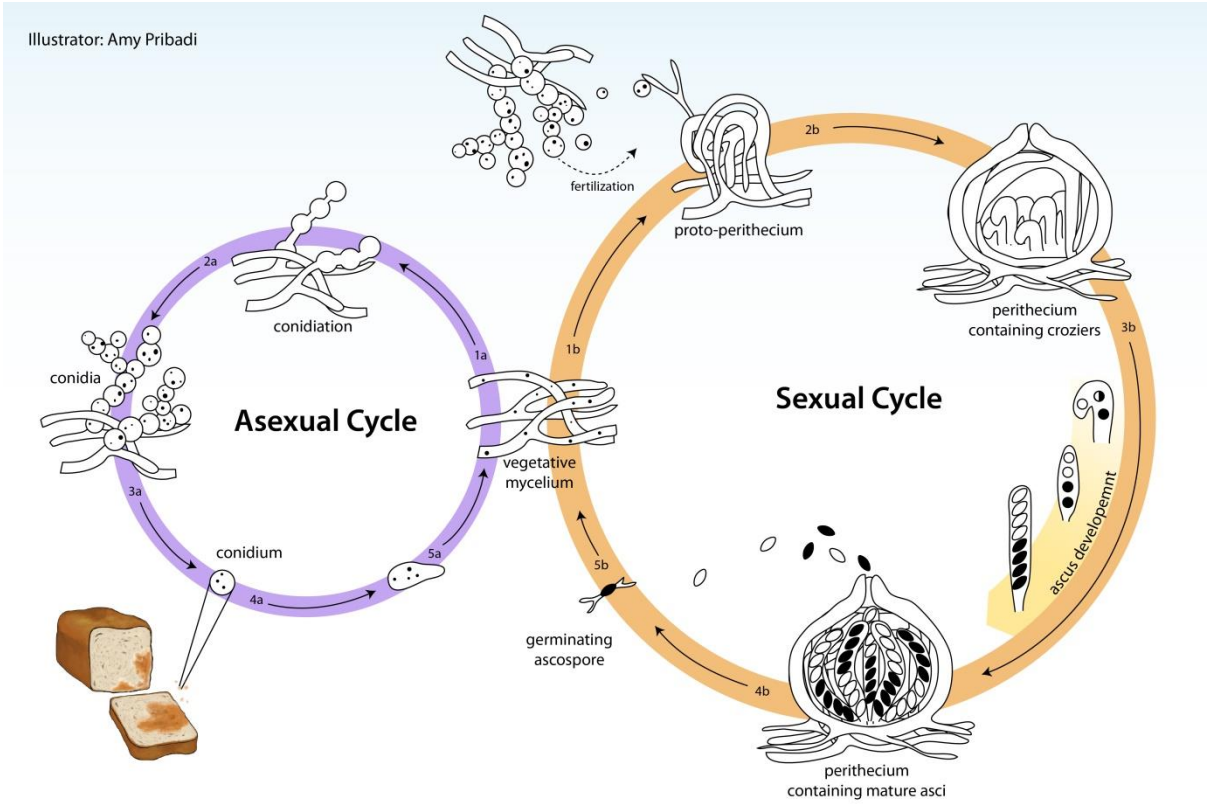
Let us presume that the synthesis of this vitamin involves ten chemical reactions and each one of them is governed by a specific enzyme S1 represents the raw material from the medium, S11 is the final product (Thiamine) and S2..S3…S10 are the intermediate products; the enzymes involved during this process are represented as E1…E2…E10 and genes synthesizing these enzymes G1..G2…G10.These entire process of synthesis of Thiamine can be represented.

By adding intermediate compounds (precursors) to medium, Beadle and Tatum were able to locate that particular step in Thiamine synthesis which was blocked in the mutant strain resulting in the non-production of a specific enzyme. On this basis they presented one gene one enzyme theory.

One gene one enzyme theory of Beadle and Tatum explained some of the human heredity diseases described by Garrod 1908 as inborn errors of metabolism in man. These are Alcaptonuria, Phenylketonuria etc.

Figure – Beadle and Tatum Experiment


(a)
X rays
Wild type
Mutagenized conidia
Crossed with wild type of opposite mating type
Microscopic ascospores are dissected and transferred one by one to culture tubes
(b)
Hundreds of tubes of complete medium are inoculated with single ascospores
Complete medium
(c)
Conidia (asexual spores) from each culture are then tested on minimal medium
Minimal medium
No growth on minimal medium identifies nutritional mutant
Conidia from the cultures that fail to grow on minimal medium are then tested on a variety of supplemented media
(d)
Minimal (control)
Minimal + amino acids
Minimal + vitamins
Complete (control)
(e)
Glycine
Alanine
Leucine
Isoleucine
Valine
Methionine
Serine
Tyrosine
Tryptophan
Proline
Arginine
Addition of arginine to minimal medium restores growth


Illustrator: Amy Pribadi
fertilization
proto-perithecium
2b
perithecium containing croziers
3b
ascus developemnt
perithecium containing mature asci
4b
germinating ascospore
5b
vegetative mycelium
1b
1a
conidiation
2a
conidia
Asexual Cycle
3a
conidium
4a
5a
Sexual Cycle

The One-Gene-One Enzyme Hypothesis

- George Beadle and Edward Tatum were among the first to investigate biosynthetic pathways
- They studied growth variants of the fungus, *Neurospora crassa*
- Their proposal, the **one-gene-one enzyme** hypothesis came out of their experiments

George Wells Beadle (1903 - 1989)

Edward Lawrie Tatum (1909 - 1975)

Red Bread Mold; http://www.biosci.missouri.edu/shiu/

Genetic Code

Concept of genetic code –

Several theories were proposed to explain the mechanism by which a particular sequence of nitrogenous bases in DNA by transcribing complementary bases in mRNA determines the position of specific amino acid in the protein molecule. The theory which widely accepted till now was proposed by F.H.C. Crick 1961.The theory holds the existence of a genetic code and its smallest unit which codes for one amino acid is known as Codon.

A codon is the nucleotide or nucleotide sequence in mRNA which codes for particular amino acid ; whereas the genetic code is the sequence of nitrogenous bases in mRNA molecule, which encloses information for the synthesis of protein molecules.

Triplet Code –

The main problem of genetic code was to determine the exact number of nucleotides in a codon which codes for one amino acid. Since there are only four nitrogenous bases in mRNA for 20 amino acids, combination of only one or two nitrogenous bases cannot provide sufficient code words for 20 amino acids. A single code consisting of only one nucleotide provides just for codons **A, C, G** and **U**. These are insufficient to code for 20 amino acids. Similarly combination of two nitrogenous bases (doublet code) provides 4 x 4 = 16 codons still insufficient for 20 amino acids.

Gamow 1954 pointed out the possibility of three letter code i.e. each codon consists of three nitrogenous bases. This will give 4 x 4 x 4 = 64 code words or codons which are more than enough to code for twenty amino acids. The table given provides the list of codons for each amino acid. It is evident from the table that several of the triplets have the same letters but in different sequences and these code for different amino acids. It means that the sequence of letter in the triplets is most important in determining what amino acid is to be coded. Although information are coded in the form of nitrogenous base sequence in DNA molecule, it is customary to represent the code letters of RNA because the message from DNA is carried out in the cytoplasm by mRNA and the code on mRNA is translated into the sequence of amino acids in polypeptide chain.

The existence of a triplet code was simply an assumption till **Nirenberg** (Noble prize winner) and **Mathaei in 1961** proved its existence by experiments. They were able to synthesize artificial mRNA which contained molecules of only one base Uracil. It was named as Polyuridylic (Poly U) molecule. The synthetic poly U was placed in a cell free system containing protein synthesizing enzymes extracted from Escherichia coli and the twenty amino acids together with necessary ATP after some time a small protein like molecule was produced which was formed by the linking of phenylalanine. It means UUU is the codon for phenylalanine.

First base	Second base: U	Second base: C	Second base: A	Second base: G	Third base
U	UUU, UUC – phenylalanine (phe) UUA, UUG – leucine (leu)	UCU, UCC, UCA, UCG – serine (ser)	UAU, UAC – tyrosine (tyr) UAA stop UAG stop	UGU, UGC – cysteine (cys) UGA stop UGG tryptophan (trp)	U C A G
C	CUU, CUC, CUA, CUG – leucine (leu)	CCU, CCC, CCA, CCG – proline (pro)	CAU, CAC – histidine (his) CAA, CAG – glutamine (gln)	CGU, CGC, CGA, CGG – arginine (arg)	U C A G
A	AUU, AUC, AUA – isoleucine (ile) AUG methionine (met) (start)	ACU, ACC, ACA, ACG – threonine (thr)	AAU, AAC – asparagine (asn) AAA, AAG – lysine (lys)	AGU, AGC – serine (ser) AGA, AGG – arginine (arg)	U C A G
G	GUU, GUC, GUA, GUG – valine (val)	GCU, GCC, GCA, GCG – alanine (ala)	GAU, GAC – aspartic acid (asp) GAA, GAG – glutamic acid (glu)	GGU, GGC, GGA, GGG – glycine (gly)	U C A G

Table – The genetic dictionary –The trinucleotide codons are written in the 5' ⟶ 3' direction.

Har Govind Khorana was born in 1919 in Raipur, Punjab (Now in Pakistan).He made remarkable contribution in artificial synthesis of nucleic acids. He discovered how to synthesize triplet RNA molecules of known sequence thereby assigning the genetic code. He also synthesized artificial genes (long DNA molecule). **Khorana shared 1968 Nobel prize with Marshall Nirenberg and Robert Holley.**

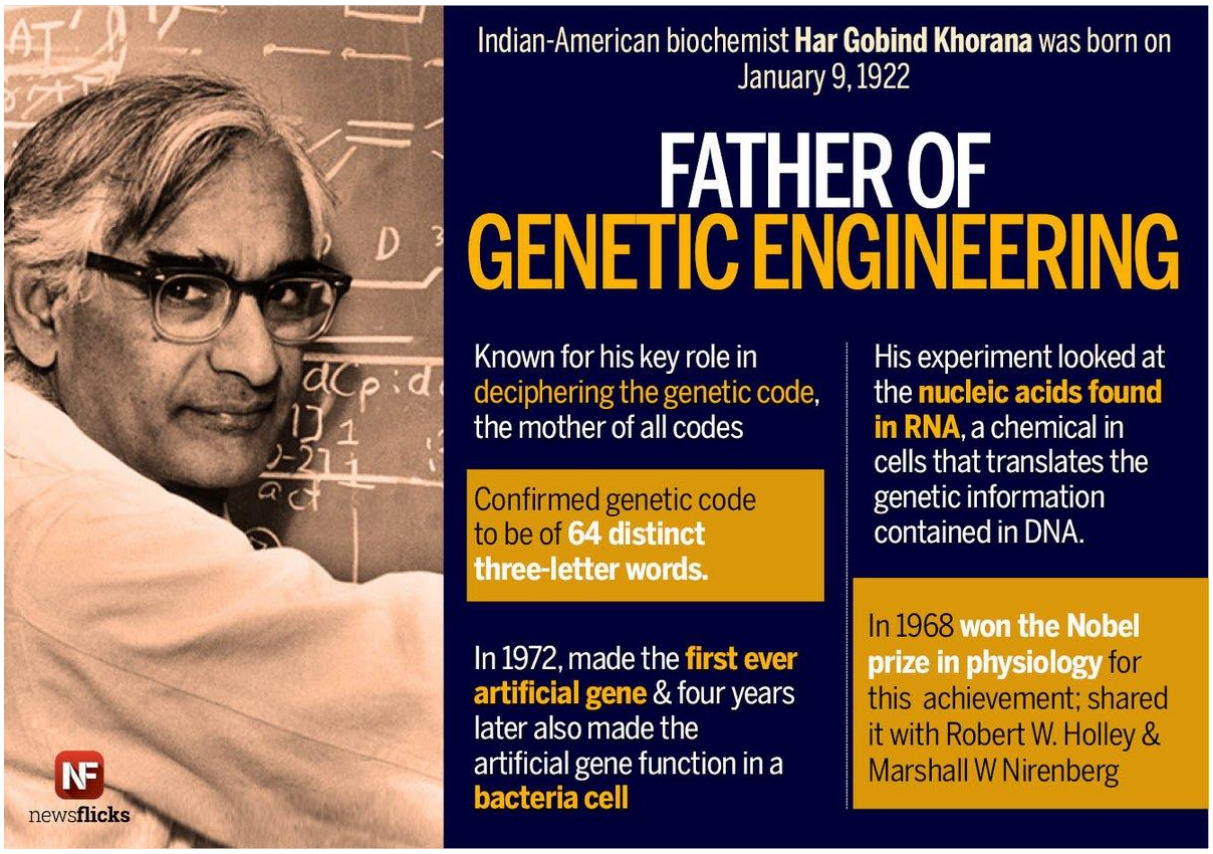
Contribution of Khorana –

Indian born biochemist Dr. H. G. Khorana has devised an ingenious technique for artificially synthesizing mRNA with repeated sequences of known nucleotides. For this valuable contribution he was awarded Noble prize in 1968 . By using synthetic DNA Khorana and his co-workers prepared chains of polyribonucleotide's with known repeating sequences of two or three nucleotides as follows-

i) Poly CUC UCU CUC UCU ……..
Leucine Serine Leucine Serine

ii) Poly CUA CUA CUA CUA ……
Leucine Leucine Leucine Leucine

Polypeptide chains having two amino acids Leucine and Serine


Indian-American biochemist Har Gobind Khorana was born on January 9, 1922
FATHER OF GENETIC ENGINEERING
Known for his key role in deciphering the genetic code, the mother of all codes
Confirmed genetic code to be of 64 distinct three-letter words.
In 1972, made the first ever artificial gene & four years later also made the artificial gene function in a bacteria cell
His experiment looked at the nucleic acids found in RNA, a chemical in cells that translates the genetic information contained in DNA.
In 1968 won the Nobel prize in physiology for this achievement; shared it with Robert W. Holley & Marshall W Nirenberg
newsflicks

ZOOLOGY

Semester III

Paper - X Genetics - II

1.Gnes and its expression :-

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2. Population Genetics :-

Population may be defined as a group of interbreeding individuals of a species, living in a particular area at a particular time. The study of inheritance of phenotypic trait (character) in a given population is called Population genetics. This is founded on a principle proposed independently by Hardy in England and Weinberg in Germany (1908). The basic principal of population genetics can be derived directly from Mendelian inheritance.

Organic evolution and the diversification of plant and animal species are to be understand only through an understanding of the population genetics. A population of a particular species includes many inbreeding groups. The inbreeding groups within particular area are called Mendelian populations or Population Genetics.

2.1 Gene Pool :-

Gene pool is defined as "The sum total of genes present in a Mendelian population". It includes all the genes of all the individuals of a population. A study of the gene pool of a population gives the number of genes, the variety of genes and the type of genes present in a population. Gene pool helps to understand the ratio between the different types of genes.

The gene pool of each population maintains its integrity as long as there is no interbreeding between populations. When there is interbreeding between populations the genes of one gene pool enter another and vice versa. The transfer of genes from one gene pool to another is called gene flow. Gene floe leads to the mixing and reshuffling of gene pools.

The gene pool becomes larger by the addition of genes to the gene pool. This is brought about by immigration and mutation.Immmigration is the inward migration of individuals in to a population from other populations. This brings about crossing or hybridization between the immigrants and the old residents of the population. This leads to an addition of genes to the gene pool.

Mutations reduces new genes. These new genes spread in the gene pool by sexual reproduction.

The gene pool becomes smaller by the removal of genes from the gene pool. This is brought about by emigration and natural selection. Emigration is the outward migration of individuals from a population. This brings about the reduction in the size f the gene pool. Secondly natural selection continuously eliminates the unfavorable genes from the population and builds up adaptive gene complexes.

The gene pool is not static in sexually reproducing and cross fertilizing organisms, the cycle o gametogensis,meiosis and fertilization brings about a constant reshuffling of genes is called genetic recombination.

2.2 Gene Frequency :-

The ratio of one allele to other alleles in a gene pool or a population is called gene frequency. In other words gene frequency is the proportion of one allele in the gene pool to other alleles of the same locus.

Ex- In rat population Black color is dominant and grey color is recessive. Black colour is due to a recessive gene m. It is a simple mendelian character.

There are three types of individuals in the population. They are homozygous dominant MM, heterozygous Mm and homozygous recessive mm. Assume that the population contains 100 individuals of which 40 are MM, 40 are Mm and the remaining 20 are mm. These three types of individuals carry two types of genes namely M and m. As each MM individuals carries two M genes and each Mm individuals carries one M gene, the total number of M genes in the population is 2X 40 + 40 = 120. As each mm individual carries two m genes and each Mm individual carries one m gene the total number of m genes in the population is 2 X 20 + 40 = 80. Thus a population of 100 individuals contains 100 X 2 = 200 M and m alleles in the gene pool.

Now the frequency or proportion of any one allele in the population is calculated by dividing the number of the given allele by the total number of alleles located on the same locus in the population. Thus

The gene frequency of allele M is = 120/200 = 0.6

The gene frequency of allele m is = 80/200 = 0.4

When the gene frequency of one allele is known, the frequency of other allele in the population can be calculated by applying a simple formula.

Let the gene frequency of M allele be p and of m allele be q. Then p + q = 1

When q is known, p can be calculated p = 1- q Similarly, when p is known q can be calculated q = 1- p

For example p=0.6

Then q = 1- p

= 1 – 0.6

= 0.4

q = 0.4

2.3 Hardy Weinberg's Law :-

Hardy Wienberg law was proposed by Hardy Wienberg in 1908.

It states that gene frequencies of various genes of a population remain constant generation after generation when te population is large, mating is at random and in the absence of mutation, selection and migration.

When the gene frequency remains constant generation after generation the population is in genetic equilibrium or Hardy Wienberg equilibrium.

When the population is in genetic equilibrium the rate of evolution is zero. That is when a population obeys Hardy Wienberg law the population will not undergo evolution.

So evolution occurs only when Hardy Wienberg equilibrium is altered. The Hardy Weinberg law is represented by a simple formula.

$$p + q = 1$$

p= Frequency of a dominant gene
q= Frequency of a recessive gene

This formula is used to find out the frequency of dominant gene and recessive gene in a population.

The frequency of heterozygotes and homozygotes in a population can be calculated by another Hardy Weinberg formula

$$(p+q)^2 = p^2 + 2\,pq + q^2$$

p = Frequency of a dominant gene
q = Frequency of a recessive gene
p^2= Frequency of dominant homozygote
2pq = Frequency of heterozygote
q^2 = Frequency of recessive homozygote

Hardy Weinberg law lays the foundation for the study of population genetics. It gives a mathematical approach for Genetics and Evolution.

Applications of Hardy Weinberg law

Hardy Weinberg law has two main applications

1) Calculation of frequencies of recessive and dominant genes in a population.
2) Calculation of heterozygotes in a population.

Ex- Let us assume a Hamster population containing 100 individuals of these 300 individuals are grey with recessive genes mm. the remaining 700 individuals are black.yhe black individuals may be homozygous dominant MM and heterozygotes Mm.The two types of black individuals cannot be identified externally.

By applying Hardy Weinberg formula the frequency of M and m genes can be calculated. Similarly the frequency of heterozygotes individuals can be calculated.

Total population = 1000
Grey(mm) = 300
Black (MM and Mm) = 700

Frequency of Grey = 300/1000 = 0.3

Frequency of Black = 700/1000 = 0.7

Let the frequency of M gene is = p
The frequency of m gene is = q

Apply the Hardy Weinberg formula

$(p+q)^2 = p^2 + 2pq + q^2$
$= 0.7^2 + 2 \times 0.7 \times 0.3 + 0.3^2$
= 0.49+0.42+0.09
= 0.49 MM: 0.42 Mm : 0.09 mm
= 49% MM individuals
= 42% Mm individuals
= 9% mm individuals

So the frequency of heterozygote in the population is 42%.So out of 1000 individuals 420 are Mm individuals and 490 are MM individuals and 90 are mm individuals. The frequency of M and m genes can be calculated as follows

490 MM individuals contain = 980 M genes
420 Mm individuals contain = 420 M genes

Total M genes = 1400

Frequency of M genes (p) =1400/2000 =0.7
90mm individuals contain = 180 m genes
420 Mm individuals contain = 420 m genes

Total m genes = 600

Frequency of m gene (q) =600/2000 = 0.3

p + q = 1
0.7+0.3 = 1

4. Mcrobial Genetics

Genetic Exchange and Recombination in Bacteria

Recombination, however is undoubtedly important in the evolution of bacteria just as it is in the evolution of eukaryotes.Three different processes have evolved that mediate transfer of genetic material from one bacterium to another, making possible the subsequent recombination events.

Lederberg and Tatum (1946) showed that i) bacteria undergo **Conjugation**, a parasexual process in which the genetic information of one bacterium is transferred to and combined with that of another bacterium. It is a process during which DNA from a donor or male cell is transferred to recipient or female cell through a sex pilus of conjugation tube ii) **Transformation** involves the uptake of naked DNA molecules from one bacterium (the donor cell) to another bacterium (recipient cell) iii) **Transduction** occurs when bacterial genes are carried from a donor cell to a recipient cell by a bacteriophage.

4.1. Conjugation :-

Lederberg and Tatum observed recombination of genetic traits in bacteria (E. coli).During conjugation DNA is transferred from a donor cell to a recipient cell through a specialized intracellular connection or conjugation tube. The transfer of genetic information is thus a one way transfer during conjugation. Cells that have the capacity to serve as donors during conjugation are differentiated by the presence of specialized cell surface appendages called F pilli. The synthesis of these F pilli is controlled by several genes that are carried by a small circular molecule of DNA or minichromosome called the F factor (for fertility factor, also called sex factor and F plasmid) cells carrying an F factor (donor cell) from conjugation tubes and DNA transfer after making contact with cells not carrying F factor called F^- cells (recipient cell).

The F factor can exist in two different states :-

i) The Autonomous State in which it replicates independently of the host chromosome and ii) the Integrated State, in which it is inserted in to the host chromosome and replicates along with the host chromosome like any other set of chromosomal genes. The F factor is thus like the chromosomes of specialized transducing phages, an example of a class genetic elements called episomes.

A donor cell containing the F factor in the autonomous state is called an F^+ cell. When F^+

Donor cell conjugates with an F^- recipient cell, only the autonomous F^+ factor is transferred. Both exconjugants become F^+ because the F factor replicates during transfer. Thus mixing a population of F^+ with a population of F^- cells results in all of the cells in the new population becoming F^+.

4.2. **Transformation :-**

In this method the donor bacterium liberates the DNA by the dissolution of the cell and the DNA is absorbed from the medium by the recipient bacterium. Hence the recipient bacterium is transformed into another strain. This is called Transformation. Transformation is described by Griffith (1928) in Pneumococcus pneumonia.

Griffith in 1928 carried out a series of experiments with Pneumococcus.There are two types of Pneumococcus bacteria, namely virulent and avirulent.Virulent strain have smooth carbohydrate capsules and give smooth colonies.Avirulent strains have no capsules and give rough colonies. these two strains also differ in their antigenic properties and virulence for the disease pneumonia. Virulence is determined by genetic factors. When virulent strains are injected into mice, they kill them with pneumococcal infection. When mice are inoculated with avirulent bacteria there was no ill effect. Then mice are injected with virulent bacteria there was no ill effect. Then mice are injected with virulent bacteria after killing with heat. In this case no ill effect is produced and the mice survive. Finally mice are injected with a mixture of avirulent and heat killed virulent bacteria. In this experiment the mice die due to pneumococcal infection. The analysis of dead mice shows that it contains virulent bacteria. The heat killed virulent bacteria are responsible for the transformation of avirulent bacteria into virulent smooth bacteria. Something from the heat killed (dead) virulent bacteria was apparently transferred to the live avirulent bacteria. This phenomenon is known as Griffith effect or Bacterial Transformation.

A series of experiments from M.Foxs laboratory (Fox1966,Fox & Allen 1968,Gurney & Fox 1971,and Hotchkiss and Gabor 1970) have demonstrated the occurrence of recombination during transformation of pnemococcus.These experiments have indicated that during transformation the end product of the recombination event itself is a duplex in which one strand derives from the donor DNA and one from the host chromosome. Further transforming or donor DNA is never found free in the cytoplasm and is capable of producing a phenotypic effect only after integration.

4.3 Transduction :-

Transduction involves the exchange of DNA between bacteria using bacterial viruses (bacteriophage) as an intermediate. There are two types of transduction, generalized transduction and specialized transduction, that differ in their mechanism and in the DNA that gets transferred.

Life Cycle of a Bacteriophage :-

When a phage infects a bacterial cell, it injects its DNA into the cell. The viral DNA is replicated numerous times, and viral genes are expressed, producing the proteins that make up the viral capsid (or protein coat) and nucleases that digest the host genome into fragments. The newly replicated viral DNA molecules are packaged into viral capsids, and the bacterial cell is lysed (burst, and therefore killed), releasing hundreds of viral progeny, which then go on to infect other cells.

A) Generalized Transduction :-

Sometimes, during bacteriophage replication, a mistake is made, and a fragment of the host DNA gets packaged into a viral capsid. The resulting phage would be able to infect another cell, but it would not have any viral genes, so it would not be able to replicate. The cell infected by this phage would survive, and would have an extra piece of bacterial DNA present, which could undergo recombination with the host chromosome, and perhaps cause a gene conversion event. Because it is a random fragment that gets packaged into the viral capsid, any segment of the bacterial DNA can be transferred this way (hence the name 'generalized').

B) Specialized Transduction :-

Specialized transduction occurs only with certain types of bacteriophage, such as phage **lambda**. Lambda has the ability to establish what is called a **lysogenic infection** in a bacterial cell. In a lysogenic infection, the viral DNA becomes incorporated into the host chromosome, much as the F factor did in Hfr (High Frequency of Recombination Cell) cells. In a lysogenic infection by lambda, the DNA integrates into a very specific spot in the host chromosome. The integrated viral DNA can remain integrated for long periods of time, without disturbing the cell. Under the appropriate conditions (the regulation of this is very complex, so don't worry about it), the viral DNA will excise itself from the chromosome, and enter the **lytic phase**, in which the virus replicates just as described above. The cell gets lysed, and new bacteriophage particles are released to infect other cells. As with excision of the F factor (when Hfr cells become F'), sometimes the excision of lambda is sloppy, and some bacteria DNA is excised along with it. When the resulting virus infects another cell, it will pass that bacterial DNA into the cell, along with its own DNA. If the infected cell survives (it can happen; there are bacterial defenses against viral infection), it will contain a new piece of bacterial DNA, which can undergo recombination and possibly cause gene conversion. Because the viral DNA integrates into a specific location, when it excises, the bacterial DNA removed with it will be the same in all cases. Therefore, the DNA transferred

to the second cell will be the same segment of the bacterial chromosome. This is why this process is called 'specialized' transduction.

Bacterial Recombination: Summary of Key Points

- Bacteria can pick up loose DNA in their environment through the process of **transformation**. The newly acquired DNA is rendered single stranded, and can recombine with the host chromosome.
- Bacteria can exchange DNA through the process of **conjugation**. The F factor confers the ability to initiate conjugation. If the F factor alone is transferred, no recombination will occur. Under certain circumstances, chromosomal DNA can be transferred to the recipient cell. In these cases, recombination will occur.
- Bacteria can receive bacterial DNA from viruses through the process of **transduction**. Bacterial viruses can accidentally pick up pieces of bacterial DNA. When they subsequently infect a cell, they transfer the pice of bacterial DNA, which can undergo recombination with the host bacterial chromosome.
- The result of recombination in the above cases may be gene conversion, in which a mutant allele becomes wild-type or vice versa.
- Conjugation involving Hfr (High Frequency of Recombination Cell) bacteria can be used to map genes along the bacterial chromosome. This done by determining in what order genes are transferred during conjugation, what is the time difference between the transfer of genes.

3. Human Genetics

3.1 Human Chromosomes

3.2 Sex linked inheritance – X and Y linked

3.3 Dizygotic and Monozygotic Twins

3.4 Inborn Errors of Metabolism in Man (PKU & Albinism)

3.5 Genetic Disorders - SYNDROMES

3.6 Use of Human Genetics in Medical Sciences - Disease Diagnosis, Gene Therapy and DNA Finger Printing

3. Human Genetics

3.1 Human Chromosomes

In human beings like other organisms, the hereditary material is carried in the chromosomes. Somatic or body cells contain two identical sets of chromosomes which is referred to as diploid or 2n. During mitosis each chromosome is replicated and is represented in each of the two daughter cells. The normal diploid number of chromosomes in man is 46. The chromosomal constitution of an organism is called karyotype.

Painter (1923) while studying the testicular material of man found a heteromorphic pair of sex determination . In 1956 two cytologists J.H.Tijo and A Levan cultured somatic cells of human embryos and established the presence of 46 chromosomes.

As mentioned above the normal diploid number of chromosomes in human being is 46. Of these two chromosomes are related to sex determination and are called sex chromosomes or heterosomes or allosomes. The remaining chromosomes constitute the autosomes. In females the sex chromosomes are morphologically similar and are called XX chromosomes while in male they are dissimilar and are called XY. Thus the human karyotype for female is 44+XX and that for male is 44+XY. The X chromosome of male is similar to the X of the female, while the Y chromosome is much smaller in size than the X. The remaining 22 pairs of autosomes are alike in males and females. Since females are XX all ova carry X chromosomes where as males are XY, therefore produced X bearing and Y bearing sperms. The human females are referred to as homogametic while the males as heterogametic sex.

Structure of a Chromosome -

Each pair of homologus chromosomes shows a common basic structure durng cell division. For details usually the metaphasic chromosomes are studied. Each metaphasic chromosome consists of two parallel identical filaments called chromatids. The two filaments are joined at a narrowed constricted region called primary constriction or centromere. The parts of the chromatids on either side of the centromere constitute the arms. The shorter arm is called (p) i.e. Petit and the next alphabet is given to longer arm. It is called (q). Depending upon the position of the centromere the chromosomes are classified as i) Telocentric ii)Acrocentric iii)Metacentric and iv) Submetacentric.

In addition to the primary constriction certain chromosomes show one more constriction called secondary constriction. Its position and extent is characteristic identifying a particular chromosome in a set.Certain chromosomes posses a rounded body connected to the chromosome by a short or long delicate chromatin.Such rounded bodies are called satellite and the chromosomes with it are called SAT-chromosomes.There are certain chromosomes which bear specialized regions called nucleolar organizers concerned with the formation of the nucleolus. The end points of the chromosomes are called Telomeres. They prevent other chromosomes from joining it. The morphological details of the chromosomes are useful in the analysis of a karyotype of a particular organism.

Table - Characteristics of the Chromosomes in the Human Karyotype.

Sr. No	**Denver Report (1960)**	**London Report (1963)**	**Description**
1	Group 1-3	Group 1-3 (A)	Large chromosomes with approximately median centromeres; 1,2 and 3 can usually be identified morphologically.
2	Group 4-5	Group 4-5 (B)	Large Submetacentric Chromosomes.
3	Group 6-12	Group 6-12, X (C)	Medium sized Submetacentric Chromosomes.
4	Group 13-15	Group 13-15 (D)	Large Acrocentric Chromosomes.
5	Group 16-18	Group 16-18 (E)	No 16 is Metacentric;No 17-18 are small Submetacentric Chromosomes.
6	Group 19-20	Group 19-20 (F)	Small Metacentric Chromosomes.
7	Group 21-22	Group 21-22 +**Y**(G)	Short Acrocentric Chromosomes.
8	**Sex Chromosomes** X Y		The Y Chromosome belongs to this group, but has no Satellites; it is of variable size and can usually be identified morphologically.

Banding Patterns in Human Chromosomes –

Recently special techniques have been developed to identify accurately each chromosome pair in the human karyotype . A banding pattern was observed by using fluorescent

staining with Quinocrine mustards. It was also found that Quinocrine specially stains the Y Chromosome not only at metaphase but also during interphase. Other techniques are based on the treatment of the cells with alkali or acids and on staining with Giemsa. These and other techniques have led to the observation of several types of banding patterns (Quinocrine Q Bands, Centromeric C Bands, Giemsa G Bands, Telomeric T Bands, and Revers R Bands). These patterns were used in the Paris Conference of 1971 to establish a new map of Human Chromosomes.

3.4 Inborn Errors of Metabolism in Man (PKU & Albinism)

Definition - Metabolic diseases due to recessive genes arising from birth are called inborn errors of metabolism. The term inborn errors of metabolism was coined by A.E. Garrod in 1909.

There are Three main types of errors of metabolism out of Five errors of metabolism.They are as follows-

1.Phenylketonuria 2. Alcaptonuria 3. Albinism 4. Tyrosinosis 5. Goitrous Cretinism.

1.Phenylketonuria

Phenylketonuria is a metabolic disease characterized by the accumulation of Phenylalanine in the blood. It is an inborn error in metabolism. It is hereditary disease. It is a recessive character caused by recessive genes represented by pp. It is a simple Mendelian character. It is inherited like a typical monohybrid cross.

When these recessive genes pp are present the enzyme Phenylalanine hydoxylase is not produced. When this enzyme is absent phenylalanine can not be converted in to Tyrosine.

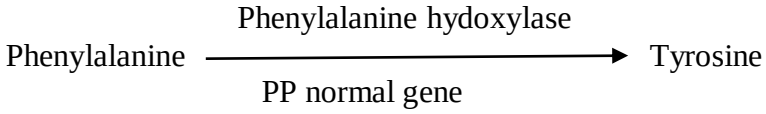

Fig – Reaction in Normal Man

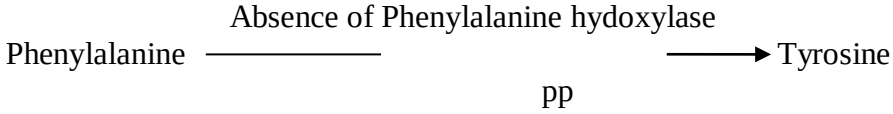

Fig – Reaction in Phenylketonuric Person

Phenylalanine and its derivatives like phenyl pyruvic acid ,phenyl lactic acid etc accumulate in the blood and cerebrospinal fluid(Brain Nerve Cells).The excess of phenylalanine is excreted in the urine. The children suffering from this disease are known as phenylpyruvic idiots. The disese is characterized by mental defect,feble mindedness.This disease can be controlled by phenylalanine free diet.

2.Alcaptonuria

Alcaptonuria is a metabolic disease charaterised by the accumulation of alcapton (Homogentisic Acid) in the blood.It is an inborn error in metabolism. It is hereditary disese. It is a recessive character caused by recessive genes represented by hh. It is a simple Mendelian character. It is inherited like a typical Mendelian monohybrid cross.

When the recessive genes hh are present the enzyme Homogentisic acid oxidase is not produced. When this enzyme is absent Alcapton (Homogentisic Acid) a derivative of tyrosine can not be converted in to acetoacetic acid. As a result Alcapton (Homogentisic Acid) accumulates in the blood. The urine of such persons turns black when exposed to air. The excess of Alcapton (Homogentisic Acid) is attached to the collagen of cartilage and other connective tissues due to which the ear and sclera are stained black.

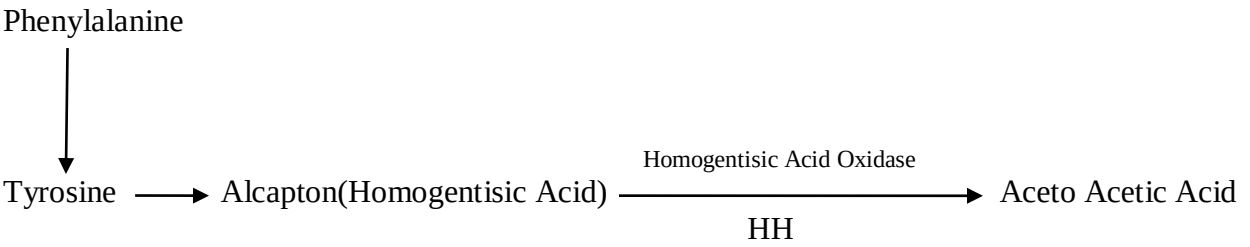

Fig – Reaction in Normal Man

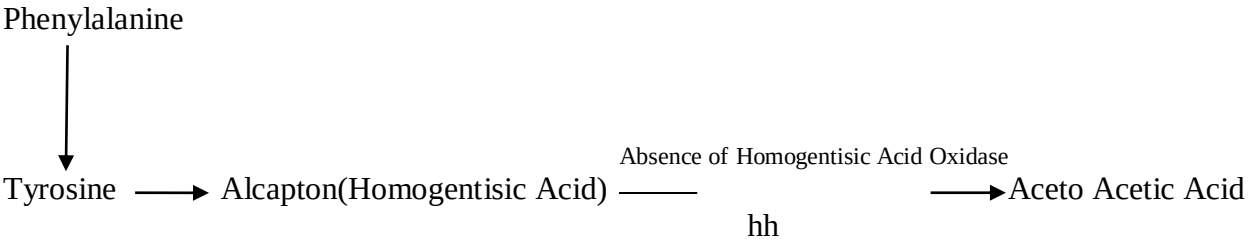

Fig – Reaction in Alcaptonuric Person

3. Albinism

It is hereditary defect where the melanin pigments are absent from the skin,hair,eye etc.Hence affected persons are pale in colour and their hairs and eyes are brown in colour. Such persons are called Albinos. The albinos are highly sensitive to light.

It is an inborn error in metabolism. It is a recessive hereditary disease caused by recessive genes represented by aa. It follows simple Mendelian inheritance.

When these recessive genes are present,the enzyme Tyrosinase cannot be produced. Hence Tyrosine cannot be converted in to Melanin Pigments.

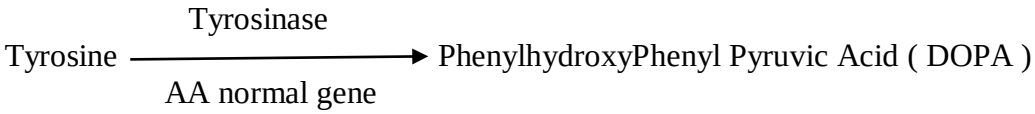

Fig – Reaction in Normal Man

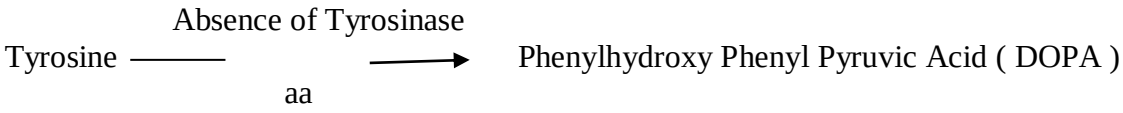

Fig – Reaction in Albinism Man

4.Tyrosinosis

The recessive gene t in its homozygous condition tt blocks the conversion of P-Hydroxypyruvic acid in to 2,5 dihydroxyphenylpyruvic acid. This leads to the accumulation of Tyrosine . Its excess is excreted with the urine.

5.Goitrous Cretinism (Goiter)

Such persons lack enzyme which is required for the conversion of Tyrosine in to thyroxine and triiodothyronin hormones. Deficiency of thyroid hormones causes goitrous cretinism characterized by physical and mental retardation and hypertrophy of thyroid gland.

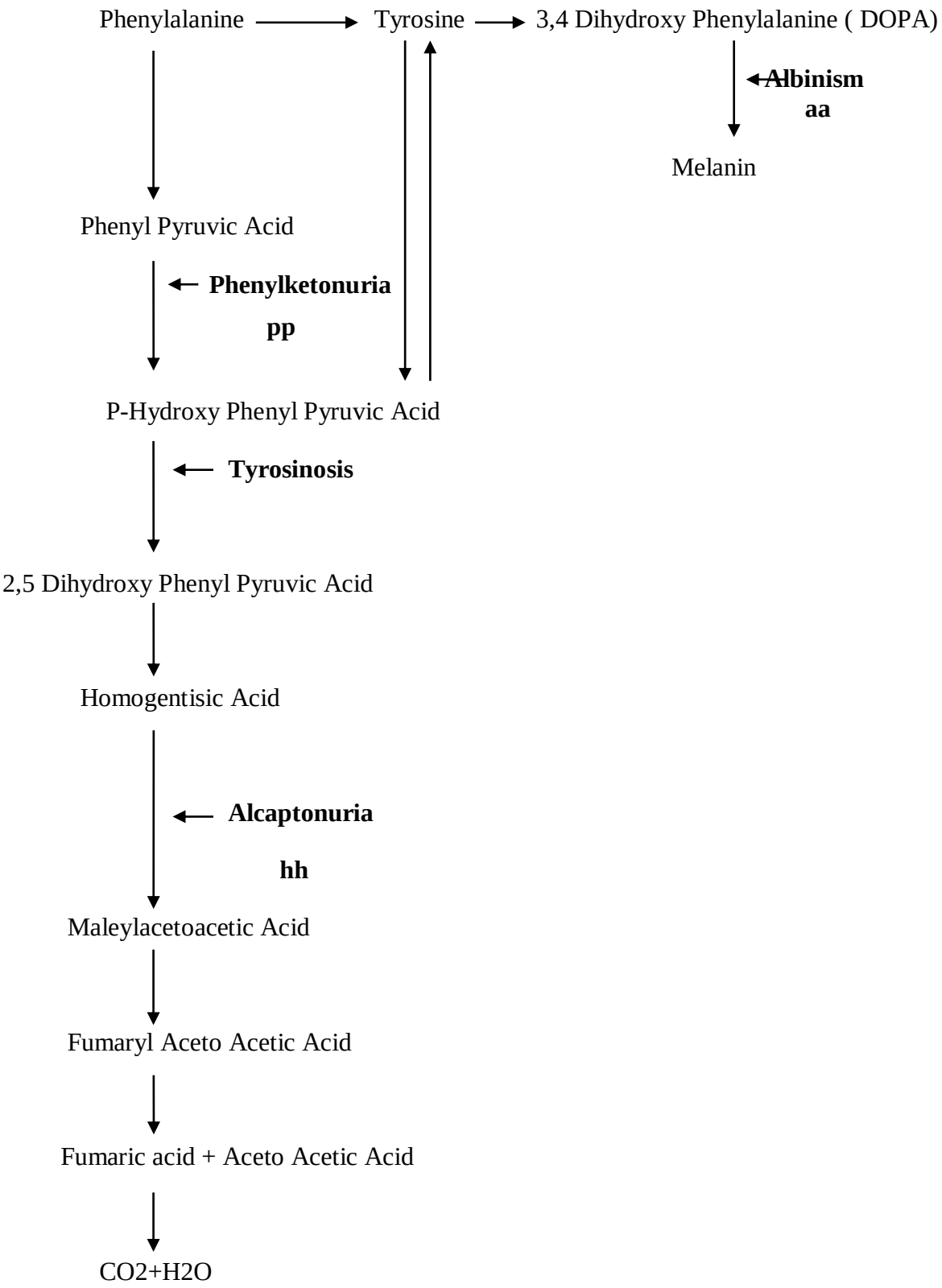

Fig – Biochemical Pathway of Phenylalanine Tyrosine Metabolism in Normal Man.

3.5 Genetic Disorders

SYNDROMES

A syndrome is a group of signs and symptoms that occur together and characterize a particular abnormality. In humans following syndromes are related with various chromosomal aberrations.

Sex – Chromosome abnormalities in Man

Two cytologists J. H. Tijo and A. Levan (1956) of Sweden showed that the normal chromosome number of man is 46. Later several investigators recorded that some individuals with chromosome below or above 46. Such individuals show physical, mental and reproductive abnormalities.

An increase or reduction in the number of sex chromosomes in the normal complement of Female XX or Male XY results into a chromosomal abnormalities in man and this condition is called the syndrome. The two abnormalities commonly observed in human population are the Turners Syndrome and Klinefelters Syndrome.

1. Turner's Syndrome (XO Females)

This is first described by Turner H. H. in 1938 these individuals phenotypically females with 45 (44+XO) somatic number of chromosomes. This is the monosomic condition due to missing of Y chromosome. In human beings it appears as if a single X chromosome can bring about the development of a female body, but that two X chromosomes are required for the development of normal ovarian tissue and the secretion of the hormones required for maturity of the female organs and reproduction. Non disjunction during meiosis of either the male or the female can produce gametes with odd sex chromosome complements and can result in these sexual abnormalities.

About one out of every 3000 female births results in a child with this abnormality. These are phenotypic Females but the adult characteristics do not develop normally and they never

reach functional maturity. Persons affected with Turners syndrome are also dwarfed physically when adult and often show mental retardation. Many of them have a characteristic short neck with webbed skin and wide spaced nipples of the mammary glands, although these glands do not enlarge as in normal women. Persons with this syndrome show that their ovaries are composed mainly of masses of connective tissue, so that there is little or no secretion of Estrogenic hormones.

2. Klinefelter's Syndrome (XXY)

In 1942 H.F. Klinefelter reported this abnormality in males and demonstrated that these individuals have 47 chromosomes i.e. Trisomy to XXY chromosome number in their somatic cells. They posses two X chromosomes and Y chromosome. It is caused by non disjunction of XX chromosomes. About one male child out of every 5000 who are born expresses the symptoms characterizing this syndrome. The affected individuals appear normal in childhood but the abnormalities become visible only in adult males. This syndrome is characterized by enlargement of the breasts, small testes and absence of spermatogenesis. Amount of Androgen (male hormones) is low, Genitalia are poorly developed, sterile. They are tall and feminine fat deposits and female distribution of abdominal and facial hair. In a population the persons of this syndrome can be detected by mentally retarded with infertility and develop a verity of psychiatric problems.

3. Down's Syndrome (Trisomy- 21)

Down's syndrome is named after the physician J. Langdon Down who first described this genetic defect in 1866 and it was formally called Mongolism or Mongolian Idiocy. It is usually associated with a trisomic condition for one of the smallest human autosomes i.e. chromosome number 21. It is the most common chromosomal abnormality in live births one in 650 births. These are mild or moderate mental retardation, eyes that slant up and out with internal epicanthal folds, a tongue that is large swollen and protruding, small and underdeveloped ears . Short stature, stubby fingers an enlarged liver and spleen. Women over 45 years of age are about twenty times more likely to give birth to a child with Down's syndrome than women aged 20. Non disjunction of chromosome pair 21 during oogenesis is the main cause of occurrence of trisomy 21.

3.6 Use of Human Genetics in Medical Sciences

If knowledge about genetics of human disease is available it can be used in a variety of ways to avoid or reduce the incidence of some of these diseases. This can be achieved in a variety of ways and we will describe four of them, namely 1. Genetic Counseling 2. Antenatal Diagnosis 3. Gene Therapy and 4. DNA Finger Printing.

1. Genetic Counseling

Genetic counseling for couples who believed that there may be a risk of producing a defective child, has now become routine aspect of medical practice, particularly in the developed countries. These parents may either voluntarily away from having any child or may undergo selective abortions on doubtful or after finding out it through antenatal diagnosis.

A genetic counselor should first be able to identify the disease and therefore should be first a clinician and then a geneticist. The simplest cases asking for genetic counseling will be those having a family history of disease and the parents may like to know the chances of having a child free of that disease. A couple may have one defective child and would like to know the chances of having a normal child and on the next pregnancy. In such a case if the defect is known to be single gene recessive and both parents are normal the chance is three in four of having a normal child, although even a normal child will have a two third chance of being a carrier. The parents may like to give birth to such a child who may be a carrier, because the chance of his or her spouse also being a carrier will be remote. However in such cases even the possibility of having the defective grand child can be worked out if the frequency of heterozygotes in the population available for marriage of the child is known. For instance in case of Fibrocystic Disease (Cystic Fibrosis) of the pancreas, the frequency of general population (perhaps in UK) is 1/22. The chance of the normal child to be a carrier being 2/3 and the chance of spouse to be a carrier being 1/22 the chance of grand child to be defective would be 2/22 X 1/22 X ¼ = 1/132. Since ¼ is the chance that a child born to both heterozygote parents will be defective. A risk of one in 132 may or may not be worth taking depending upon temperament and circumstances. More detailed calculation can be done in these simple cases and also in cases of polygenic nature and variable penetrance.

In some cases detection of heterozygote may also be useful and possible. Following are the situations where it is possible i.)When heterozygote though phenotypically normal produces a particular enzyme activity intermediate to those found in two homozygote's its presence can be detected using electrophoresis. In such cases if biochemistry laboratory is available deficiency like HGPRT (Lesch-Nyhan Syndrome) in heterozygous condition can be detected from a blood sample or some skin cells. ii.) When the mutant produced an altered form of gene product the heterozygote may produce two different forms of protein that can be separated by electrophoresis to enable the identification of heterozygote. iii.) If the defect is associated with chromosome structure then with the availability of cytogenetics laboratory such an abnormality in heterozygous condition can be identified.

2. Gene Therapy

Gene therapy is an experimental treatment that involves introducing genetic material into a person's cells to fight or prevent disease. Researchers are studying gene therapy for a number of diseases, such as severe combined immuno-deficiencies, Hemophilia, Parkinson's disease, Cancer and even HIV, through a number of different approaches. A gene can be delivered to a cell using a carrier known as a "vector." The most common types of vectors used in gene therapy are viruses. The viruses used in gene therapy are altered to make them safe, although some risks still exist with gene therapy. The technology is still in its infancy, but it has been used with some success.

If a child is diagnosed to carry a defective gene leading to disability one may like to get this gene replaced by a normal functional gene. This is gene therapy in theory. One would like to ask that if there is need and demand for gene therapy can it be done ? The answer to this question has changed from no to yes in recent years. Gene therapy can be ued at two different levels 1.Patient Therapy and 2.Embryo Therapy. It is belived that in future gene therapy of both types will possible.

1.Patient Therapy

Patient therapy in which cells with healthy gene may be introduced in the affected tissue.So that the healthy ene overcomes the defect without affecting the inheritance of the patient. Patient therapy will involve the following steps i.) The deective gene should be identified

ii) normal healthy gene should either be isolated or synthesized iii) Isolation of cells of the tissue where the normal healthy gene will need to function iv) The normal gene should be placed in to a cell where it can function. The gene will have to be placed into the correct site on the host chromosome so that the gene may function or even one may have to delete the defective gene. There are three main problems in this connection. First the introduced gene may not function,second that when corrected cells are reintroduced, these may be outnumbered by the non cured resident cells and third there are only few diseases affecting only a single tissue.

Utilizing the above approach first clinical gene transfer approved in USA was achived in 1989. It ws a marker gene (Neomycin resistance = NeoR) introduced in to tumor infiltaring lymphocytes (TIL). These NeoR/TIL were transferred in to patient with advanced cancer, to ensure that the approved protocol really works. The first approved gene therapy protocol for correction of adenosine deaminase (ADA) deficiency, however began in 1900 and by the end of 1992, two dozen active clinical protocols on three continents became available for trails. However the technology is still very expensive and specialized to be used extensively. Other less expensive techniques (involving delivery of gene through Vectors) are being developed.

2. Embryo Therapy

This involve the following steps which have been tried in case of mouse or rabbit only i) In vitro fertilization of the egg ii) Insertion of normal gene into embryo at post zygotic level, either with viruses or directly by microinjections iii) Integration of inserted gene in host DNA where it may or may not function. The inserted genes have been found to be inactive generally but in few animals genes have been switched on in a tissue specific way but their activity is at a very low level. However it is not yet possible that the therapeutic newly inserted genes function under normal control in the animal in time space or quantity.

3.6 DNA Fingerprinting in Forensic Science
(The Ultimate Identification Test)

The Technique of DNA fingerprinting was developed for the first time in 1985 by Alec Jeffreys and his collegues at Lecister University in UK. Inthis field establishment of the identify of a person with the help of Blood Stains, Semen (sperms) stains or Hair will be possible with almost absolute certainty.

The technique of DNA fingerprinting relies on developments from recombinant DNA technology and allows an examination of each individuals unique genetic blueprint DNA. The technique is based on the fact that the DNA of each individual is interrupted by a series of identical DNA sequences called repetitive DNA. The pattern length and number of these repeats are unique for each individual. Jeffreys developed a series of DNA probes which are short pieces of DNA that seek out any specific sequence they match and base pair with that sequence. Such molecular probes are used to detect the unique repetitive DNA patterns characteristic of each individual. The procedure of DNA fingerprinting has the following steps-

i) DNA is purified from a small sample of blood semen or other DNA bearing cells and digested into smaller fragments with restriction endonucleases.

ii) The fragments are separated by agarose gel electrophoresis.

iii) The separated fragments are transferred to a nylon membrane by the technique of Southern Blotting.

iv) The DNA probes labeled with radioactive material are added to a solution containing the nylon membrane.

v) Wherever the probes fit a band containing repetitive DNA sequences they attach.

vi) The X ray film is pressed against the nylon filter and exposed at bands carrying the radioactive probes attached to the fragments.

vii) The pattern of bands obtained on the film is 100 percent unique for each person, except for identical twins who would have the same pattern.

The forensic application of the DNA fingerprinting technique involves a comparison between the DNA fingerprinting obtained from cells at a crime scene with a DNA fingerprint

from cells provided by the suspect. If the DNA pattern matches exactly, certain identification is made. For paternity determination, DNA fingerprint of the mother, child and alleged father are compared. In this case one half of the bands in the child comes from the mother and the other half from the father. All the paternal bands in Childs DNA fingerprinting must match with the alleged father for positive paternity identification.

In India DNA fingerprinting tests are carried out at the Centre for Cellular and Molecular Biology (CCMB) Hyderabad. For this purpose a test with the BKM- DNA probe (Banded Krait minor satellite DNA) earlier used for identification of sex chromosomes has been found to cost one tenth of the cost of tests used in Europe and USA. Paternity dispute cases are much more common in India and most of them are referred to CCMB for DNA evidence. The first test on DNA fingerprinting was used in June 1989 to settle a drawn out paternity case in Madras.