TEACHERS' HANDBOOK OF TRANSACTION OF NEW BIOLOGY TEXTBOOKS OF KERALA AT +2 LEVEL

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2004

PREFACE

New Biology Text Books for +2 level of Kerala State were introduced recently. The text books are designed on NCERT text book plan. New areas in Biology like Biotechnology, Tissue culture, environmental studies find place in these books. These topics not only demand for more understating about subject but new approaches or strategies to teach them. The +2 teachers felt that some amount of input in new areas could equip them for transacting subject in effectively in classrooms. In view of this Kerala State Government proposed to organize training programme and requested Regional Institute of Education, Mysore to provide required training to Key Resource Persons, since the number of +2 teachers is very large. The KRPs in turn train +2 teachers in teaching Biology. State Government deputed 20 key resource persons but only ten persons attended the programme.

Prior to this an inhouse meeting was organized to chalk out modalities of the programme. The programme was designed keeping the following objectives in mind.

1) Analyse of the Biology content of new books of 11^{th} and 12^{th} standards.
2) Identifying the new areas where teacher felt difficulty in classroom transaction.
3) Designing new strategies or approaches for teaching these areas.
4) Supplementing the content areas with hands on experiences in the laboratories.

The whole programme was planned to provide adequate training and information through

1. Lecture cum discussions by the resource persons of RIE.
2. Hands on experience through relevant laboratories activities
3. Visits to institutions where more information about new areas could be obtained
4. Arranging special lectures by the experts in respective areas form the University departments etc.,

It was also planned to develop a hand book to provide relevant and additional information which could come handy to teachers while transacting Biology in classes. The handbook can further be multiplied by state Government for the use of teachers.

This hand book contains sufficient information about the difficult areas and also relevant and sufficient inputs in conducting laboratory activities, with evaluatory procedures. Latest practices in tissue cultures, procedure for tissue culture are also included in this book. Improvements by addition or deletions are always possible once we receive feed back from teachers.

I am grateful to NCERT for providing funds to carry out this programme. I am thankful to Dr. G. Ravindra, Principal, RIE, Mysore for the eternal support and encouragements. I take this opportunity to thank all resource persons of this institution and University departments whose help was very much essential in making this programme meaningful and successful one.

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RESOURCE PERSONS

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Human genome Project

The Human Genome Project (HGP) is an international 13-year effort formally begun in October 1990. The project was planned to last 15 years, but rapid technological advances have accelerated the expected completion date to 2003. Project goals are to determine the complete sequence of the 3 billion DNA subunits (bases), identify all human genes, and make them accessible for further biological study. The Department of Energy's Human Genome Program and the National Institutes of Health's National Human Genome Research Institute (NHGRI) together make up the U.S. Human Genome Project.

Project goals are to

- identify all the approximate 30,000 genes in human DNA,
- determine the sequences of the 3 billion chemical base pairs that make up human DNA,
- store this information in databases,
- improve tools for data analysis,
- transfer related technologies to the private sector, and
- address the ethical, legal, and social issues (ELSI) that may arise from the project.

To help achieve these goals, researchers also are studying the genetic makeup of several nonhuman organisms. These include the common human gut bacterium Escherichia coli, the fruit fly, and the laboratory mouse.

Human Genome Project research has been funded at many laboratories around the U.S. by the Department of Energy (DOE), the National Institutes of Health (NIH), or both. Other researchers at numerous colleges, universities, and laboratories throughout the United States have also received DOE and NIH funding for human genome research. In addition, many large and small private U.S. companies are conducting genome research. At least 18 other countries have participated in the Human Genome Project. At least 18 countries have established human genome research programs. Some of the larger programs are in Australia, Brazil, Canada, China, Denmark, European Union, France, Germany, Israel, Italy, Japan, Korea, Mexico, Netherlands, Russia, Sweden, United Kingdom, and the United States. Some developing countries are participating through studies of molecular biology techniques for genome research and studies of organisms that are particularly interesting to

their geographical regions. The Human Genome Organisation (HUGO) has helped to coordinate international collaboration in the genome project.

Steps used in genome sequencing:

- Chromosomes, which range in size from 50 million to 250 million bases, must first be broken into much shorter pieces (*subcloning step*).
- Each short piece is used as a template to generate a set of fragments that differ in length from each other by a single base that will be identified in a later step (*template preparation and sequencing reaction steps*).
- The fragments in a set are separated by gel electrophoresis (*separation step*).
- New fluorescent dyes allow separation of all four fragments in a single lane on the gel.
- The final base at the end of each fragment is identified (*base-calling step*). This process recreates the original sequence of As, Ts, Cs, and Gs for each short piece generated in the first step.
- Automated sequencers analyze the resulting electropherograms, and the output is a four-color chromatogram showing peaks that represent each of the four DNA bases.
- After the bases are "read," computers are used to assemble the short sequences (in blocks of about 500 bases each, called the read length) into long continuous stretches that are analyzed for errors, gene-coding regions, and other characteristics.
- Finished sequence is submitted to major public sequence databases, such as GenBank. Human Genome Project sequence data are thus freely available to anyone around the world.

Meeting Human Genome Project sequencing goals by 2003 has required continual improvements in sequencing speed, reliability, and costs. Previously, standard methods were based on separating DNA fragments by gel electrophoresis, which was extremely labor intensive and expensive. Total sequencing output in the community was about 200 Mb for 1998. In January 2003, the DOE Joint Genome Institute alone sequenced 1.5 billion bases for the month. Gel-based sequencers use multiple tiny (capillary) tubes to run standard electrophoretic separations. These separations are much faster because the tubes dissipate heat well and allow the use of much higher electric fields to complete sequencing in shorter times.

The human reference sequence does not represent an exact match for any one person's genome. In the Human Genome Project (HGP), researchers collected blood (female) or sperm (male) samples from a large number of donors. Only a few samples were processed as DNA resources, and

the source names were protected so neither donors nor scientists knew whose DNA was being sequenced. The knowledge obtained is applicable to everyone because all humans share the same basic set of genes and genomic regulatory regions that control the development and maintenance of their biological structures and processes. In addition to generating a reference sequence, another important goal of the HGP was to identify many of the small regions of DNA that vary among individuals. Scientists believe these variations underlie disease susceptibility and drug responsiveness, particularly the most common variations called SNPs (single nucleotide polymorphisms). The DNA resources used for these studies came from anonymous donors of European, African, American (north, central, south), and Asian ancestry.

Craig Venter (former head of Celera Genomics), Ari Patrinos (director of DOE Human Genome Program and Biological and Environmental Research Program), and Francis Collins (director, NIH National Human Genome Research Institute).

A working draft of the entire human genome sequence was announced in June 2000, with analyses published in February 2001, in Nature and Science. In generating the draft sequence, scientists determined the order of base pairs in each chromosomal area at least 4 to 5 times (4x to 5x) to ensure data accuracy and to help with reassembling DNA fragments in their original order. This repeated sequencing is known as genome "depth of coverage." Draft sequence data are mostly in the form of 10,000 base pair-sized fragments whose approximate chromosomal locations are known. To generate high-quality reference sequence, additional sequencing was done to close gaps, reduce ambiguities, and allow for only a single error every 10,000 bases, the agreed-upon standard for the HGP.

Investigators believe that a high-quality sequence is critical for recognizing regulatory components of genes that are very important in understanding human biology and such disorders as heart disease, cancer, and diabetes. The finished version provides an estimated 8x to 9x coverage of each chromosome. "Final" version of the complete human genome project includes 99% of gene-containing DNA was announced on April 14th 2003 by Francis Collins, who heads the US National Human Genome Research Institute (NHGRI). **The sequence is not completely complete, and perhaps never will be.**

Although the completion of the Human Genome Project was celebrated in April 2003 and sequencing of the human chromosomes (1–22, X, and Y) is essentially "finished," the exact number of

genes encoded by the genome is still unknown. Latest estimates from gene-prediction programs suggest that there might be 24,500 or fewer protein-coding genes. When analysis of the draft human genome sequence was published by the International Human Genome Sequencing Consortium on February 15, 2001, the paper estimated only about 30,000 to 40,000 protein-coding genes, much lower than previous estimates of around 100,000. This lower estimate came as a shock to many scientists because counting genes was viewed as a way of quantifying genetic complexity. With around 30,000, the human gene count would be only one-third greater than that of the simple roundworm *C. elegans* at about 20,000 genes.

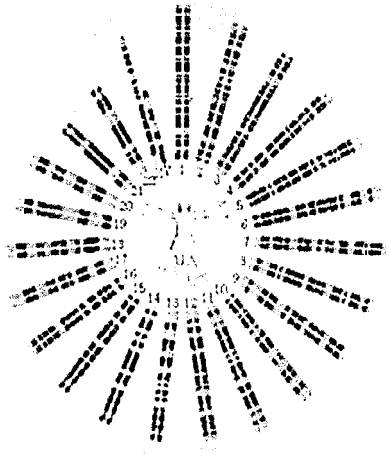
In the human genome, about 3 billion bases are arranged along the chromosomes in a particular order for each unique individual. To get an idea of the size of the human genome present in each of our cells, consider the following analogy: If the DNA sequence of the human genome were compiled in books, the equivalent of 200 volumes the size of a Manhattan telephone book (at 1000 pages each) would be needed to hold it all. It would take about 9.5 years to read out loud (without stopping) the 3 billion bases in a person's genome sequence. This is calculated on a reading rate of 10 bases per second, equaling 600 bases/minute, 36,000 bases/hour, 864,000 bases/day, 315,360,000 bases/year. Storing all this information is a great challenge to computer experts known as bioinformatics specialists. One million bases (called a megabase and abbreviated Mb) of DNA sequence data are roughly equivalent to 1 megabyte of computer data storage space. Since the human genome is 3 billion base pairs long, 3 gigabytes of computer data storage space are needed to store the entire genome. This includes nucleotide sequence data only and does not include data annotations and other information that can be associated with sequence data. Morey Parang and Richard Mural of Oak Ridge National Laboratory; and Mark Adams of The Institute of Genome Research made contributions to this answer.

3.4.1 Human Chromosome Launchpad

Links to Research Resources for Each Chromosome

This site is provided as a single-source launchpad to information about each human chromosome. Choose databases and other information resources by "pointing and clicking" on one of the pictured chromosomes or by using the list of links below. Each chromosome page provides links to gene maps, sequences, associated genetic disorders, nonhuman genetic models, identified genes, research efforts and laboratories, and other information as available.

Broader applications reaching into many areas of the economy include the following:



- **Clinical medicine.** Many more prognostics, individualized diagnostics and drugs, and other therapies.
- **Agriculture and livestock.** Hardier, more nutritious, and healthier crops and animals.
- **Industrial processes.** Cleaner and more efficient manufacturing in such sectors as chemicals, pulp and paper, textiles, food, fuels, metals, and minerals.
- **Environmental biotechnology.** Biodegradable products, new energy resources, environmental diagnostics, and less hazardous cleanup of mixed toxic-waste sites.
- **DNA fingerprinting.** Identification of humans and other animals, plants, and microbes; evolutionary and human anthropological studies; and detection of and resistance to harmful agents that might be used in biological warfare.

The comparative genome sizes of humans and other organisms being studied

organism	estimated size	estimated gene number	average gene density	chromosome number
Homo sapiens (human)	3000 million bases	~30,000	1 gene per 100,000 bases	46
Mus musculus (mouse)	3000 million bases	~30,000	1 gene per 100,000 bases	40
Drosophila melanogaster (fruit fly)	180 million bases	13,600	1 gene per 9,000 bases	8

Arabidopsis thaliana (plant)	125 million bases	25,500	1 gene per 4000 bases	5
Caenorhabditis elegans (roundworm)	97 million bases	19,100	1 gene per 5000 bases	6
Saccharomyces cerevisiae (yeast)	12 million bases	6300	1 gene per 2000 bases	16
Escherichia coli (bacteria)	4.7 million bases	3200	1 gene per 1400 bases	1
H. influenzae (bacteria)	1.8 million bases	1700	1 gene per 1000 bases	1

**Information extracted from genome publication papers below.*

Deriving meaningful knowledge from DNA sequence will define biological research through the coming decades and require the expertise and creativity of teams of biologists, chemists, engineers, and computational scientists, among others.

PHOTOSYNTHESIS

INTRODUCTION

Life on earth finally depends on energy derived from the sun. This is achieved by the process of Photosynthesis in green plants. The literal meaning of the word Photo-synthesis is "synthesis using light". The primary function of this process is to transform and harvest the radiant energy of the sun into useful form of chemical energy that inturn is used by cells in many ways.

This is the only large scale process of biological importance which utilizes the cheapest raw materials like CO_2 from the air, water from the soil, sunlight from the sun, in the presence chlorophyll libertes oxygen and produces carbohydrates. Each year the plants of the earth combine about 150 billion tons of carbon with 25 billion tons of hydrogen and set free 400 billion tons of oxygen.

KEY CONCEPTS

(1) Photosynthesis in green plants occur in a highly specialized well organized membrane system of subcellular organelles known as chloroplasts.

(2) The primary events of photosynthesis occur in organized membrane system of thylakoids and inter-granal membranes. Photosynthetic Carbon Reduction (PCR) cycle occur in the stroma

(3) Chlorophyll pigments are the photo receptor molecules.

(4) Light of two wave lengths activate two photochemical systems. They provide electrons, protons and energy rich molecules required to convert CO_2 and water into carbohydrates.

(5) The antenna (accessory pigments) Chlorophyll molecules transfer their excitation energy to a specialised Chlorophylla at the reaction centers namely P_{680} and P_{700}.

(6) The Oxygen evolved during Photosynthesis comes from water.

(7) Photosynthesis requires the interaction of two light reactions namely Photosystem II and Photosystem I which are spatially separated and structurally distinct.

(8) Photosystem II produces a strong oxidant that splits water and proton gradient is formed as electrons move from Photosystem II to Photosystem I during which ATP synthesis occurs through non cyclic Photophosphorylation.

(9) Photosystem I produces - a strong reductant that reduces NADP to NADPH. If NADPH is not utilized during PCR cycle or when NADP is not regenerated electrons fall back from Ferrodoxin to P_{700} via cytochrome f-b complex during which also ATP synthesis occurs through cyclic Photophosphorylation.

CONTENT ENRICHMENT

In majority of algae and in higher plants Photosynthesis takes place in the intricate membrane filled structure known as the chloroplast. The Chloroplasts act as chemical pumps and obtain necessary power from the absorption of light.

The most striking aspect of structure of Chloroplast is the extensive system of internal membranes known as Thylakoids. All the chlorophyll is contained within this membrane system, which is the site of light reaction of Photosynthesis. The Carbon reductions or dark reactions which are catalyzed by water soluble enzymes takes place in the stroma region of the chloroplast outside the thylakoids. Thylakoid membranes are stacked one above the other to form grana. A single Chloroplast may contain many grana. Each granum made up of 10 to 60 thylakoid membranes. The grana in a chloroplast are connected by intergranal membranes or stroma lamellae.

The chloroplast also contain its own RNA and ribosomes. A wide variety of proteins essential to photosynthesis are embedded in the thylakoid membrane and extend into portions of intergranal or stroma lamellae also.

The reaction centers P_{680} and P_{700}, the light gathering antenna pigments-protein complexes and the most of the electron transport enzymes are all integral membrane proteins.

During the photochemical reaction sun's radiant energy is harvested and transformed into Chemically useful energy and this is one of the most important event during photosynthesis.

The majority of chlorophyll pigments, serve as antenna collating and transferring (funnelling) the energy to the photochemical reaction centers where the chemical reaction leading to long term energy storage take place. The chlorophyll pigments harvest solar energy by catalysing an oxidation-reduction process by which Hydrogen atoms (H^+) are boosted from water to organic matter.

The Photosynthetic pigments like chlorophyll a,b,c,d,e etc. Carotenoids, Phylobillins and Xanthophylls are grouped in such a way that they act as light gathering antenna collating light and transferring the energy to the specialized chlorophyll protein complex known as the reaction center or Photochemical center.

The experiments conducted with regard to the rate and Quantum yield of Photosynthesis at different wave lengths, led to the establishment of operation of two photosystems in the photochemical reactions of photo-synthesis. Robert Emerson and his colleagues found that although Photosynthesis is not efficient at wavelengths more than 680 nm known as Red drop. That is the Quantum yield of Photosynthesis falls of drastically for "far red" light of wavelength > 680 nm. This is referred to as Red drop.

They observed that the yield can be enhanced by adding light of shorter wavelength for example at 650 nm. The rate of Photosynthesis in the presence of both wave lengths is greater than the sum of the rates obtained when two wavelengths are supplied separately. This is known as Emerson enhance-ment effect. This can be easily explained if photosynthesis is assumed to require two light activated Photosystems both of which can be driven by light of less than 680nm but only one by light of longer wavelength.

In these two photosystems the light energy is transferred from one chlorophyll to another until it reaches a chlorophyll with special properties at a site called reaction center namely Photosystems-I activated by Chlorophyll$_a$ P_{700} and Photosystem-II which is activated by chlorophyll$_a$ P_{680}.

These two photosystems are spatially separated structurally different and are connected in tandem. In recent years it has become well established that the photosystem-II is a larger unit reaction center with it, unique chlorophyll$_a$ of absorption peak at 680nm with its associated light gathering antenna chlorophylls like chlorophyll$_a$, chlorophyll$_b$, carotenoids, light harvesting enzymes Mn, Z Protein complex, plasto quinone, and plastocy anin electron transporting proteins are located predominantly in the stacked region of grana lamellae (Anderson and Anderson, 1988).

The Photosystem I is a smaller unit reaction center with its special chlorophyll$_a$ of absorption peak at 700nm P_{700} is associated with its chlorophyll$_a$, chlorophyll$_b$, caroteroids, light harvesting protein as well as the coupling factor enzymes that catalyses the formation of ATP are found almostl exclusively in the stroma lamellae (non stacked stroma) and at the edger of the grana lamellae.

The cytochrome b_6-f complex is a large multi-subunit protein factor connect the two photosystems is evenly distributed in both grana and intergranallar lamellae.

The chlorophyll$_a$ and $_b$ have major and distinctive absorption bands in the blue and red regions of the spectrum. When chlorophyll$_a$ or one of the other pigments absorb photons. The pigment passes from its lower energy state (ground electronic state) to a higher energy state (excited electron state). That is the electrons in outer orbit of these pigments are energized and move to the excited state.

The energy of excitation reaches a Photosynthetic reaction center where it is finally transferred to a special long wavelength form of chlorophyll$_a$ and it is considered a kind of energy sink.

The transfer of excitation energy from an excited molecule and normal chlorophyll$_a$ to such a special chlorophyll$_a$ P_{680} (PII) or P_{700} (PSI) probably taken place

within 10-12 seconds which is 1000 times faster than the time for the "waste" energy and Chlorophylla to emerge as fluorescence. Thus there is sufficient time for excited chlorophyll molecule to disperse its energy in a chemically useful way.

The absorbed energy when once relayed to reaction center initiates a complex series of chemical oxidation and reduction reactions that capture energy and lead to the formation of an oxidant and a reductunt. The oxidant must be capable of splitting water into free oxygen, protons and electrons.

Robert Hill and Fay Bendall Proposed a scheme showing how electrons could be transported along a biochemical chain in which two separate reactions are driven by light of less than 680 nm but only one by light of longer wavelength.

The coordination and cooperation of two light reactions are required for the effective operation of photosynthesis.

The Z scheme of photosynthesis reveals that red light absorbed by Photosystem II (P. 680) Sensitizes a reaction that produces a strong oxidant and a weak reductant. The strong oxidant oxidizes water (Photolysis and water) releasing free oxygen, electron and proton

$$2H_2O \text{ -------> } O_2 + 4e^- + 4H^-$$

The electrons and two protons reduce NADP to NADPH.

A proton gradient across the thylakoid membrane is generated when O_2 is evolved and electron flow through an electron transport chain linking the photosystems.

Far red light absorbed by photosystem I (PSI) produces a weak oxidant and a strong reductant. The strong reductant reduces NADP to $NADPH^+$. The two photochemical reaction systems are linked in series by electron carriers and acceptors.

The weak reductant produced in PSII is oxidized by Pheopytin which transfers its electron to Quinone A and then Quinone B. Quinone B transfers its electrons to cytochrome-b_6-f complex. This inturn transfer its electron to plastocyanin. Then plastocyanin transfer its electrons to chlorophyll$_a$ P_{700} of PSI.

Duysens and his co-workers have given evidences to show that the two photosystems act in series. The synthesis of ATP is driven by this proton gradient as in oxidative phosphorylation. The weak reductant produced by PSII is oxidized by the longer wavelength light absorbed by PSI. This type of antogonistic effect indicate that the plasto quinone A, plasto quinone B, cytochrome b_6-f complex and plastocyanins lie in the path of electron flow between two systems.

Van Niel proposed a new hypothesis that light splits water to make H and OH radicals. The (H) supplies the

Hydrogen to convert CO_2 to carbohydrate. In plants the (OH) reacts to form oxygen. Van Niel has proved beyond doubt that in green plants, oxygen is liberated comes from water and not from CO_2.

THE C_3 PHOTOSYNTHETIC CARBON REDUCTION CYCLE (PCR CYCLE)

The reactions associated with the reduction of CO_2 to carbohydrate are coupled to the consumption of NADPH and ATP. These reactions are referred to as the dark reaction of Photosynthesis, since light in not directly involved.

The only requirement for the synthetic Carbon reduction cycle is that it is coupled to the generation of reduced pyridine nucleotide (NADPH) and ATP. In Photosynthetic organisms the capacity is provided by light. But in chemosynthetic organisms (such as the Hydrozen Bacteria) ATP and NADPH are generated by the oxidation of some inorganic substrate.

All Photosynthetic eukaryotes, from the most primitive algae to the most advanced Angiosperms reduce CO_2 to carbohydrate through the basic mechanism, the C_3 Photosynthetic Carbon Reduction (PCR) cycle. The PCR cycle is some time referred to as the calvin cycle in honour of its discoverer Melvin Calvin. Other metabolic pathways associated with the Photosynthetic fixation CO_2 such as the C_4 Photosynthetic Carbon Assimilation (PCA) cycle and the C_2 Photorespiratory Carbon oxidation (PCO) cycle, are either auxiliary or dependent on the basic PCR cycle.

[C_3] PCR cycle

In C_3 PCR cycle CO_2 from the atmosphere and water in the soil are enzymatically combine with a 5C carbon acceptor molecule to generate two molecules of a three carbon intermediate. These intermediates are reduced to carbohydrate using the photo-chemically generated ATP and NADPH in the light reaction.

The cycle is completed by regeneration of the five carbon acceptor. The C_3 PCR cycle proceeds in three stages.

1) Carboxylation of the CO_2 acceptor, ribulose 1,5 biphosphate, to form two molecules of 3-phosphoglycerate, the first stable intermediate of the PCR cycle.
2) Reduction of this carboxylic and to a carbohydrate in the form of glyceraldehyde-3-Phosphate
3) Regeneration of the CO_2 acceptor ribulose 1,5 biphosphate from glyceraldehyde-3-phosphate.

SUMMARY

Photosynthesis is a food-making process that occurs in green plants. It is the chief function of leaves. The word photosynthesis means putting together with light. Green plants use energy from light to combine Co_2 and water to make sugar and other chemical compounds. Sugar and other chemical compounds. All our food comes from this important energy converting activity of green plants. Light energy is converted to chemical energy and is stored in the food made by green plants.

The light used in photosynthesis in absorbed by a green pigment called chlorophyll. Each food-making cell in a plant leaf contain chlorophyll in small bodies called chloroplasts. In Chloroplasts, light energy causes water drawn from the soil to split into Hydrogen and oxygen. In a series of complicated steps, the H_2 combines with CO_2 from the air, forming a simple sugar. Oxygen from the water molecules is given off in the process. From sugar together with N_2, sulphur and phosphorus from soil - green plants can make starch, fat, protein, vitamins, and other complex compounds essential for - life photosynthesis provides the chemical energy that is needed to produce these compounds.

Certain bacteria and algae can also capture light energy and use it to make food. Photosynthetic bacteria contain chlorophyll in tiny bodies called chromatophores. In these compounds other than water are combined with CO_2 to form sugar. No oxygen is released.

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AIM: Experiment to demonstrate the evolution of oxygen during photosynthesis.

MATERIALS REQUIRED: Test tube, short-stem funnel, 250 or 500ml beaker, fresh Hydrilla plants, Sodium-bi-carbonate, pyrogallol, water.

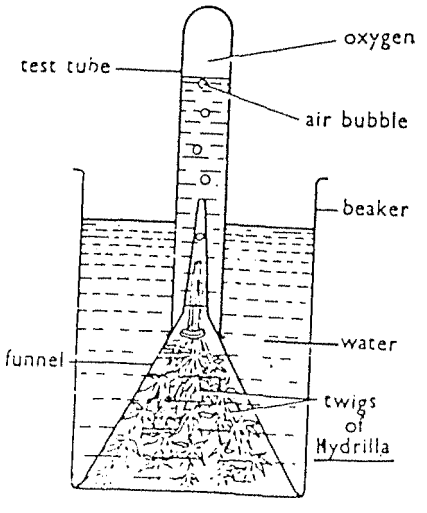
THEORETICAL BACKGROUND

Photosynthesis in an important metabolic process in plants which helps in the synthesis of simple sugars, by utilizing CO_2, H_2O, light energy and Chlorophyll. Photosynthesis involves 2 major stages - the light reaction and the dark reaction. In light reaction, the water molecule dissociates into H^+, e^- and O_2 is released as a by product.

PROCEDURE

1. Fill a beaker with pond water.
2. Place several (8-10) Hydrilla plants in the mouth of funnel in such a way that cut ends of plants are directed towards the stem of funnel.
3. Invert the funnel with plants and place it inside the beaker with water.
4. Fill a test tube completely with water and invert it over the stem of funnel in the beaker. When the apparatus is set-up there should be a continuous column of water in test-tube without any air-gaps.
5. Keep the set up in bright sunlight and put a pinch of Sodium-bi-carbonate into the beaker.

6. Observe the evolution of bubbles of a gas from the plants.
7. After 1 to 1 1/2 hours observe the set up.



OBSERVATION

A gas is collected at the top of the inverted test tube by downward displacement of water. When tested, the gas is found to be oxygen.

Testing for oxygen is rather difficult. The traditional test of introducing a burning splinter will not be possible as the collected O_2 is inaccessible. A substance, pyrogallol, is most suitable to test for oxygen. Introduce a few mg of the powder into the test tube. This has the ability of absorbing oxygen and the level of water rises in the test tube.

INFERENCE

Bubbles of gas that emerge from the plant and get collected at the top of inverted test-tube is oxygen. It is evolved during light reaction of photosynthesis by photolysis of water molecules.

PRECAUTIONS

1. Select fresh, healthy Hydrilla plants.
2. Apparatus can be successfully set up only if the entire funnel (including tip of its stem) is under water.
3. Place the apparatus in bright sunlight as it enhances the rate of photosynthesis.

APPROXIMATE DURATION: 1 1/2 to 2 hours.

LINK WITH THEORY: Photosynthesis - light reaction.

SAMPLE EVALUATION QUESTIONS

1. Why should we select only fresh, healthy Hydrilla plants?
2. Will the evolution of O_2 be faster inside the room/lab? Give reasons.
3. Why aquatic plants like Hydrilla are ideally suited for this experiment?
4. Why the burning splinter test cannot be done?

GENETIC ENGINEERING

A. Introduction

Genetic engineering is a new methodology which enables the previously impossible experiments to be planned and carried out with success. It is the *in vitro* modification and recombination of genetic material from different organisms to create new gene combinations. It is a very powerful investigative tool that has helped to revolutionize our concept of the gene. It can also be said as any deliberate manipulation of genes within or between species for the purpose of genetic analysis or strain improvement.

One of the first usages of the terms genetic engineering was by Hanscho et al. who reported in October 1971 issue of Diamond Walnut News that "our accumulated knowledge of the mechanisms of hereditary and the demands for efficiency have turned varietal improvement into a problem of biological engineering and the plant breeder into a genetic engineer".

Genetic engineering is the "formations of new combination of heritable material by the insertion of nucleic acid molecules, produced by whatever means outside the cell, into any virus, bacterial plasmid or other vectors, so as to allow their incorporation into a host systems in which they do not naturally occur but they are capable of continued propagations". This is also called as

recombinant DNA technology. Genetic engineering promises great benefits for the advancement of science and society.

B. Key Concepts

* Genetic engineering offers the possibility of bypassing all biological restraints to genetic exchange and mixing from widely differing species.

* Restriction enzymes cleave DNA at target sequences, facilitating the joining of non-homologous DNA, from different sources.

* The cloning vector allows the recombinant DNA molecule to multiply within the host cells.

* It is now possible to rapidly determine the nucleotide sequence of larger and smaller genomes.

* Gene cloning can provide a pure sample of an individual gene, separated from all the other genes that is normally shares the cell with.

* It permits the production of human proteins in bacteria; the generation of transgenic animals and plants; and detection of genetic diseases at an early stage.

C. Content enrichment including activities/experiments

Genetic engineering permits isolation and propagation of individual genes, the study of their structure and functions, their transfer to various species, and efficient

expression of their products. Genetic engineering can be studied under the concepts of gene cloning, gene isolation and gene sequencing.

I. Gene cloning

There are five basic steps in a gene cloning experiment (Fig. 1).

1. A fragment of DNA containing the gene to be cloned is inserted into a circular DNA molecule called a vector, to produce a recombinant DNA molecule.

2. The vector acts as a vehicle that transports the gene into a host.

3. Within the host cell the vector multiplies, producing numerous identical copies not only of itself but also of the gene that it carries.

4. When the host cell divides, copies of the recombinant DNA molecule are passed to the progeny and further vector replication takes place.

5. After a large number of cell divisions, a colony or clone of identical Host cells is produced. Each cell in the clone contains one or more copies of the recombinant DNA molecule and the gene carried by the recombinant molecule is now said to be cloned.

The recombinant DNA process consists of several biological and biochemical manipulations that are made

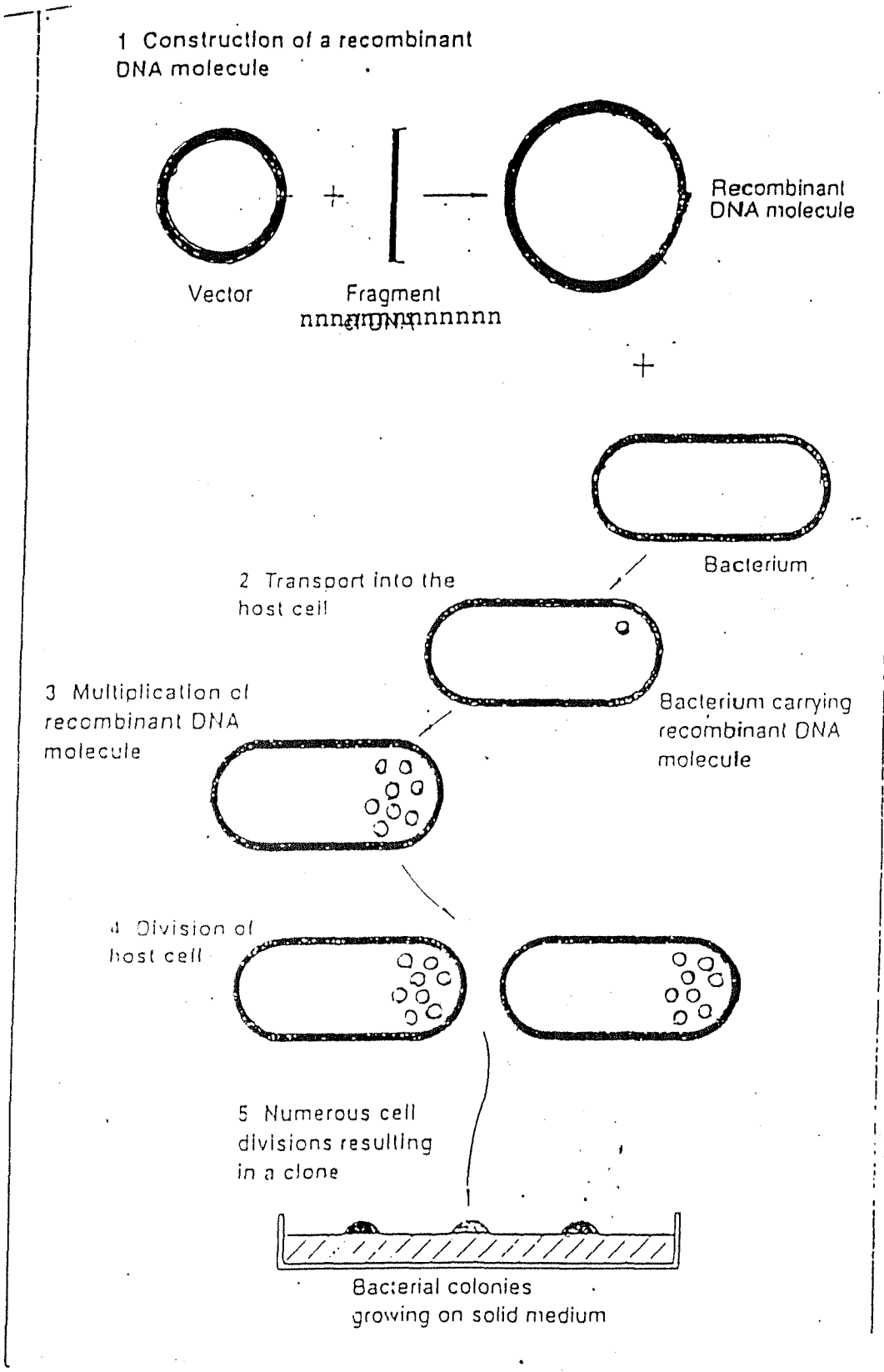


Fig. 1: The basic steps in gene cloning

possible by a series of independent discoveries made after 1950. Some of the important investigations are the following:

a. DNA

The elucidation of the structure of DNA in 1953 by James Watson and Francis Crick was one of the most exciting discoveries in the history of genetics and molecular biology. Now DNA is the darling of the media. After understanding of the functional properties of DNA, viz. replication, transcription, mutation, recombination, repair and transposition, it became possible to manipulate DNA.

One of the first steps in the in vitro manipulation of DNA involves the isolation of both the vector DNA and the DNA to be cloned. DNA does not exist as a free molecule in the cell, but rather as a complex association of DNA, RNA and proteins. The basic steps in the purification of DNA are (1) the release of soluble, high molecular weight DNA from disrupted cell wall and membranes; (2) dissociation of DNA-protein complexes by denaturation or proteolysis; and (3) the separation of DNA from the other macromolecules.

b. Restriction enzymes

Restriction is a limitation to the efficiency of propagation of foreign DNA in a bacterium. Restriction enzymes are sequence specific nucleases that are named from their role in the microbiological processes of restriction and modification. These enzymes have been found in nearly

every microorganisms examined and are known to catalyze double-strand breaks in DNA, to yield restriction fragments. Whole genomes can be subdivided into smaller, discrete pieces so that genes can be isolated, cloned and characterised by nucleotide sequence analysis.

The first observations suggesting the existence of restriction enzymes were made in the early 1960s by Werner Arber and his associates while studying the efficiency of plating of the bacteriophage on different strains of Escherichia coli. The next step in understanding restriction phenomenon did not come until 1970, when Hamilton Smith and his colleagues (1970) isolated the restriction enzyme from Haemophilus influenzae typed. They showed that the enzyme recognised and cut the viral DNA at specific sites. Daniel Nathans (1971) showed that the enzyme discovered by Smith cuts the DNA of the monkey virus SV40 into eleven well defined pieces. This technique was later used to map the DNA of other organisms. Arber, Nathans and Smith were awarded the Nobel prize in physiology and medicine for these discoveries.

Restriction endonucleases are endo-deoxyribonucleases that digest dSDNA after recognising specific nucleotide sequences by cleaving two phosphodiester bonds, one within each strand of the duplex DNA. Evolution endowed different bacterial species with unique endonucleases that allow them to distinguish their own from foreign DNA. They provide

protection against invasion of the cell by foreign DNA. Protection against self-digestion is achieved by the presence of specific DNA methyltransferases which transfer methyl groups to adenine or cytosine residues. Over 900 specific endonucleases have been discovered which recognise more than 90 different specific sequences. These enzymes were isolated from more than 200 bacterial strains. Restrictions enzymes have not been reported from mammalian cells. Not all enzymes cleave DNA at specific sites but all recognise specific nucleotide sequences.

Nomenclature

In order to simplify the naming of these enzymes, a nomenclature has been developed that is based on an abbreviations of the name of the organism from which the enzyme was isolated. The first initial of the genus and the first two initials of the species form the basic name. When the enzyme is present in a specific train, these three italicized letters may be followed by a strain designation. The third portion of the name is reserved for a Roman numerical indicating the order of discovery of the enzyme in the particular strain. For example, ECORI is the first enzyme purified from Escherichia coli strain R. Hind III - is the third enzyme purified from Hasmophilus influenzae strained.

Types of restriction enzymes

All restriction enzymes have been classified into three types based on their gene and protein structure,

cofactor dependence and specificity of binding and cleavage. Type I and Type III restrictions enzymes are multimeric proteins that are capable of both cleaving and modifying DNA and also make cleavages at variable distance from the recognition site. Therefore, these are not useful tools in constructing recombinant DNA molecules. Type II endonucleases are smaller monomeric proteins that require only Mg^{2+} for activity. These enzymes recognise and cleave specific short nucleotide sequences in DNA. Type II enzymes have become one of the primary tools in the analysis and restructuring of DNA. The recognition sites for type II restriction endonucleases are generally 4, 5, 6 or 8 base pairs in length and most of these share the feature of two fold rotational symmetry (Table 1). Single strand complementary tails which are produced by ECORI endonuclease are referred to as sticky or cohesive ends, because they can form base pairs with one another.

Production of sticky ends

```
5'-G-A-A-T-T-C-3     ECORI     5'-G-              -A-A-T-T-C-3'
   | | | | | | |    ---------->   |.       +          |
3'-C-T-T-A-A-G-S'                 3'-C-T-T-A-A-          -G-5'
```

Tangetisite Sticky ends

Production of blunt ends

```
5'-A-G-C-T-3'          AluI        5'-A-G-        -C-T-3'
   | | | |          ---------->                +
3'-T-C-G-A-5'                      3'-T-G         -G-A-5'
```

Target site Blunt ends

AluI = Arthrobacter luteus

Table 1. Recognition sequences of some restriction enzymes

Microbial origin	Enzyme	Recognition site (two fold symmetry)
1. *Escherichia coli*	*Eco*RI	5'GAA TTC 3' 3'CTT AAG 5'
2. *Hemophilus influenzae*	*Hin*dII	5' GTPy PuAC 3' 3' CAPu PyTG 5'
	*Hin*dIII	5' AAG CTT 3' 3' TTC GAA 5'
3. *Hemophilus parainfluenzae*	*Hpa*I	5' GTT AAC 3' 3' CAA TTG 5'
	*Hpa*II	5' CC GG 3' 3' GG CC 5'
4. *Hemophilus aegyptius*	*Hae*III	5' GG CC 3' 3' CC GG 5'
5. *Moraxella* spp.	*Msp*I	5' CC GG 3' 3' GG CC 5'
6. *Proteus vulgaris*	*Pvu*I	5' CGA TCG 3' 3' GCT AGC 5'
7. *Bacillus amyloliquefaciens*	*Bam*HI	5' GGA TCC 3' 3' CCT AGG 5'
8. *Streptomyces albus*	*Sal*I	5' GTC GAC 3' 3' CAG CTG 5'
9. *Providencia stuartii*	*Pst*I	5' CTG CAG 3' 3' GAC GTC 5'

c. Ligases

Ligases are involved in the establishment of covalent linkage between 3'OH group and 5' phosphate group of adjacent nucleotide. These are called as sealing enzymes. Two types of ligases namely E. coli DNA ligase and T_4 DNA ligase are extensively used in cloning experiments.

d. Cloning vectors

The cloning vector is the central component of a gene cloning experiment. It provides the replicative function that allows the recombinant DNA molecule to multiply within the host cell. The modified plasmids, bacteriophages, plant and animal viruses as well as hybrid vectors and artificial chromosomes are being used as cloning vectors in recombinant DNA technology.

1. Plasmids are duplex, supercoiled DNA molecules, which enjoy an autonomous, self replicating status without significantly lowering host viability, exist in bacteria and some eukaryotes in an extrachromosomal state. Plasmids always carry one or more genes which are not found on the main chromosome. These genes are responsible for a useful characteristic such as self transmissibility, resistance to drugs or toxic metal ions, catabolism of certain carbon sources, production of antibodies and restriction - modification system. Some of the naturally occurring plasmids are R100 F factor COLE1 and Ti plasmids. Table 2 provides various properties of these plasmids. Most of the plasmids

Table 2 Some Plasmid Vectors Used in *E. coli*

Plasmid Vector	Replication Mode	Size (bp)	Selective Markers and Some Unique Restriction Endonuclease Sites*
pSC101	stringent	8700	Tetracycline^{res}(*HindIII, BamH1, SalI*); *EcoRI*
pUC7	relaxed	2700	Ampicillin^{res} *lacZα*** (*EcoRI, BamH1, HincII, PstI*)
pMB9	relaxed	5500	Tetracycline^{res}(*HindIII, BamH1, SalI*) Colicin immunity^{I}; *EcoRI; HpaI; SmaI*
pBR322	relaxed	4362	Tetracycline^{res}(*HindIII; BamH1, SalI*); Ampicillin^{res}(*PstI, PvuI*); *EcoRI; AvaI; PvuII; ClaI*
pDF41	stringent	12,800	*TrpE* gene; *BamH1, EcoRI, HindIII, SalI*
pRK2501	stringent	11,100	Tetracycline^{res}(*SalI*) Kanamycin^{res}(*HindIII, XhoI*)

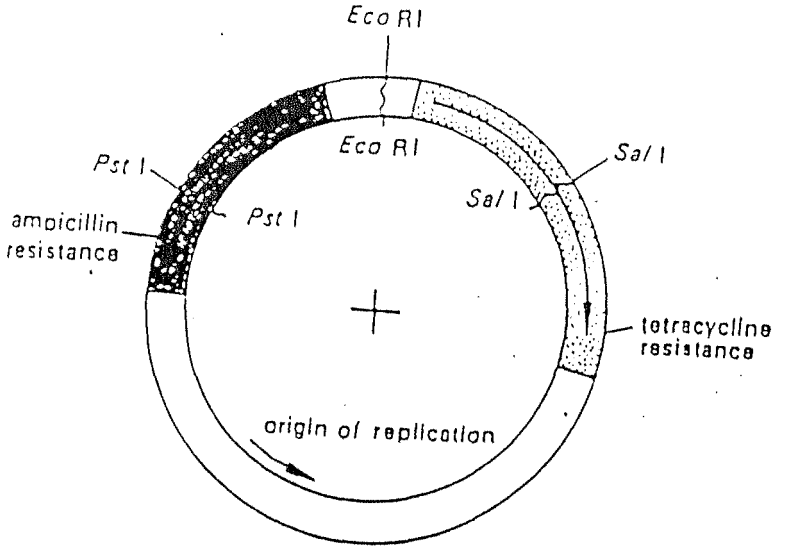


Fig. 2. Plasmid pBR322

used as cloning vectors are derived from natural plasmids, but most have been modified in various ways so that each one is suited for a particular type of cloning experiment.

Literally hundreds of different cloning vectors are now available for use with different types of host cells. The largest number exists for E. coli and the best known of these is the plasmid vector pBR322 given in Fig. 2. The essential features of a cloning vector are as follows:

1. It should have origin of replication that allows the plasmid to replicate as the bacteria divide and multiply.
2. It should have unique restriction endonuclease sites into which foreign DNA may be inserted without disabling the plasmid.
3. It should have a gene for resistance to the antibiotic (for example - amphicillin) that permit the selection of bacteria which have taken up the plasmid.
4. Additional markers are inserted to distinguish between recombinant and non-recombinants.
5. It should be the multiple copy number plasmid.
6. It should be smaller in size for efficient transfer to host cells.
7. The cloning vector should not have transfer and mobility genes for biological safety.

The newly created/engineered plasmids receive a name consisting of a small 'p' (for plasmid) followed by two capital letters (for the person or laboratory of origin) and

a number. For example, PBR322 - plasmid - Boliver and Rodriguez (who prepared this);pSC101 - plasmid discovered in Shigella dyssentriae by Cohen and pUC 18 - plasmid created by (Messing) University of California.

e. Cloning host

When foreign DNA is ligated to the vector, the recombinant vector is to be transferred to a suitable host. This cloning host should be deficient in the major pathway of DNA recombination. This will induce the possibility of undesirable recombinant. The cloning organism should preferably lack restriction enzymes that can degrade foreign DNA.

E. coli is the best cloning host. Many other bacteria, fungi, plant and animal cells have been used as host systems for recombinant DNA experiments.

Basic skills needed to carry out a simple gene cloning experiments are:

a. Preparation of pure sample of DNA.

b. Cutting of DNA molecules.

c. Analysis of DNA fragment sizes.

d. Joining of DNA molecules together.

e. Introduction of DNA into host cells.

f. Identification of cells that contain recombinant DNA molecules.

II. Isolation of Genes

During the last two decades, significant progress has been made in the techniques for isolation of a variety of

genes. In order to isolate genes from a genome, a mixture of clones each carrying DNA derived either from the genomic DNA or from cDNA (which is derived from mRNA) will be created. This mixture may contain thousands of clones, which when derived directly from the genomic DNA are collectively called a genomic library. A genomic library can be constructed by restriction digestion of genomic DNA and cloning of these fragments into a cloning vector. Similarly, complementary DNA (cDNA) libraries can also be prepared by isolating mRNAs from tissues and copying it into cDNA through the use of reverse transcriptase. After converting into double stranded cDNA, it will be cloned into cloning vector. These clones are screened to isolate an interested gene by applying molecular biological processes like, colony screening, molecular probing, southern blotting and hybridization.

III. Sequencing of Gene

Once a gene or a DNA fragment has been cloned and isolated, its further study involves the determinations of order and arrangement of nucleotides. From the nucleotide sequence the amino acid sequence and molecular weight of protein can be understood. From the last two decades, significant progress has been made, in developing the stable DNA sequencing technology and in building the computational tools required for the analysis of sequence data. so far the genomes of E. coli, yeast, C. elegans, B. subtilis, many

microbes and many mitochondrial and chloroplast DNA have been completely sequenced. Those of the fruit fly, mouse and human have been partially sequenced. These sequences have dramatically altered the practice of genetics, molecular biology, developmental biology, immunology and microbiology.

Applications

Recombinant DNA technology offers enormous benefits for the basic and applied science including:

1. better understanding of the biology of the lower and higher organisms,
2. revolutionary improvements in various industrial processes,
3. cleaning up the environment,
4. advances in food production, and
5. advances in medicine.

Genome analysis

The genetic engineering techniques are powerful tools for genetical research as they can help to gain insight into the structure, regulation of genes and to prepare physical maps of the lower and higher genomes. At present, the most exciting facet of research is the 'Human genome project'. This is a coordinated major international research project with the ultimate goal by the year 2005, to sequence the entire human genome and identify the estimated 100,000 genes. An international organisation located in USA, called Human Genome Organisation (HUGO) is coordinating the

research activities of various laboratories of eight countries in this regard.

Industry

The use of recombinant DNA is revolutionizing chemical industry in the developed countries by providing cheaper, better and less pollutive ways of producing both known and new, simple and complex, useful chemical substances. Some of them are L-amino acids, organic acids, vitamins, enzymes, B-carotene, antibiotics and carbohydrates. A new strain of Methylophilus methylotrophus bacterium which is a rich source of a protein has been developed by recombinant DNA technology which is more efficient in producing the required protein. This technology is employed to improve the fermentation capabilities of microbes.

Environment Management

The superbug is a newly constructed bacterium which can degrade most of the major hydrocarbon components of petroleum. The different strains of Pseudomonas pulida contain a plasmid which has genes coding for enzymes that digest a single family of hydrocarbons. The multiplasmid bacterium is able to grow on a diet of crude oil and can degrade more families of hydrocarbons which no single plasmid strain can do by itself. The superbug has potential for cleaning up oil spills.

Agriculture

DNA can now be introduced from other species of plants, animals, or even bacteria. An agriculturally

important example of inserting foreign DNA via T-DNA is a bacterial gene for resistance to the herbicide glyphosate. This gene confers resistance to the transgenic plant, enabling it to withstand the field application of glyphosate as a weed killer. Genetic engineering also promises notable advantages for crop improvement, crop protection and propagation of tissue culture plants. This technique is particularly useful for introducing nitrogen fixing genes into cereal crop plants. Recently, it has been possible to assemble a bacterial plasmid which carries all the 17 ni genes from the nitrogen fixing bacterium Klebsiella pneumoniae. Developments for producing crops with better nutritional status, resistance to frost damage and resistance to viral and fungal diseases have shown great promise.

In animal husbandry, gene transfer, embryo transfer, animal birth control, are some of the methods now being used to improve the milk, meat, eggs, fish and marine products.

Human Health

The recombinant DNA technology has revolutionised the practice of medicine in the following five areas, namely

a. isolation of rare biological molecules,

b. preparation of vaccines,

c. development of highly specific diagnostic laboratory tests,

d. prenatal diagnosis of human genetic diseases, and

e. future prospects of gene therapy.

The commercial use of recombinant DNA technology has already begun to make significant contributions. The products already available on the market include: somatostatin, Human insulin, Growth hormones, Human interferons, Tissue plasminogen activator, Human renin, Interleukin-2, and Vaccines, etc. Numerous other products are at various stages of testing for clearance before commercial production. This technology is also being employed in the production of monoclonal antibodies for developing disease diagnostic kits and for the production of biomedical engineering products such as artificial skin and artificial kidney machines.

Gene Therapy

This is defined as the delivery of a functional gene for expression in somatic tissues with the intent to cure a disease. The prerequisites for effective gene therapy strategies for single gene defects are the identification of the defective gene, functional complementation of the gene defect in vitro with a wild type copy, and in vivo animal models of the disease and its correction. A defective gene can be isolated by the following techniques namely, reverse genetics, restriction fragment length polymorphism and chromosome walking and jumping. In general, two major types of strategies for gene transfer are used. (1) In vivo gene transfer: Direct transfer of genes to cells residing in the body. Ex. Correction of a growth hormone deficiency in dwarf

mice. (2) Ex vivo gene transfer: Cells are removed from body, cultured in vitro for gene transfer and subsequently returned to the body.

The necessary criteria for gene therapy includes high efficiency of gene transfer, stable replication of the introduced gene as either an integrated transgene or as an extrachromosomal element, effective expression of the gene product in the target tissue and adequate safety during the gene transfer period and throughout the life of the patient undergoing gene replacement therapy.

Although the site of insertion of the introduced DNA in higher eukaryotes can be highly variable, and the DNA is generally not found at the homologous locus, human gene therapy has progressed from a distant prospect to reality in a very short space of time. Only five years ago, the first protocol for the treatment of adenosine deaminase (ADA) deficiency in humans was approved in the USA, and in 1990, gene therapy for ADA was first performed. Now there are at least 20 approved gene therapy in the USA and Europe. Some of them are Duchenne Muscular Dystrophy (DMD), Cystic fibrosis (CF), and Familial hypercholesterolaemia (FH). Now, various targeting strategies like targeted drug delivery, specific cell targeting of Retroviral vectors, and mammaliam artificial chromosomes (MACs) are being planned and used to deliver DNA to cells.

After 20 Years

1. Many products of everyday use would be cheaper and better to an extent that might even change our life styles.
2. Production of alcohol by the enzymatic hydrolysis of cellulose could make alcohol so cheap from alcohol based industry.
3. Availability of biodegradable plastics and home diagnostic kits would make life different from what it is today.
4. Availability of techniques that would allow preservation of food for long periods without refrigeration.
5. The control of those few diseases that cannot be fully cured today such as cancer, leprosy, heart disease, brain disorders, and viral diseases like AIDS would be possible.
6. Unlock the record of 3.5 billion years of evolutionary experimentation.
7. Reveal secrets about the fine-tuning of genes and gene networks.
8. Find out all the susceptible genes and create a mutant for every gene.
9. Brings the next question - Second Human Genome Project.
10. These advances would increase our life span to such an extent that the entire social security system would need to be revised.

Therefore, now the processes and products of genetic engineering have become realities and are not line drawings on paper any more. The understanding and applications of genetic engineering is very essential. Our real concern are the common man, the poor and the down trodden. Our priorities should be to help him by this scientific potentiality in many areas but not in one or two spectacular successes.

D. Suggestions for Assessment

1. What is genetic engineering ?
2. What are the essential feature of a cloning vector ?
3. What is gene cloning ?
4. How does the host DNA is protected by its own restriction enzymes ?
5. Name the steps involved in gene isolation.
6. What are DNA libraries ?
7. How do you isolate DNA ?
8. Name important applications of genetic engineering.

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IMMUNITY

KEY CONCEPTS

1) **Hematopoesis:** It includes generation of blood cells. All blood cells originate from a common stem cell present in the bone marrow.
2) **Immunity:** It is the state of protection from infectious diseases. It has both specific and Non-specific components.
3) **Non-specific or innate immunity:** It refers to basic resistance to disease that a given organism has.
4) **Specific immunity:** It refers to the immunity that is acquired during the life time of the organism which results in the selective elimination of a specific foreign body or a foreign molecule.
5) **Primary & Secondary immune response:** Differences occur in the time required for antibody production and the serum antibody concentration, depending on the entry of an antigen in to an individual for the first time or subsequently. This is based on immunological memory.
6) **Cell mediated and Humoral immunity:** Physical involvement of cells in the elimination of antigens is called cell mediated immunity e.g, Ingestion of foreign material by the phagocytes. Humoral immunity includes neutralization

of antigens (leading to its elimination) by the production of antibodies.

ENRICHMENT OF CONTENT

The immune system is a defensive system. It consists of diverse organs and tissues that are widely dispersed throughout the body. It is able to generate an enormous variety of cells and molecules. These cells and molecules orchestrate the complex process of identification, neutralization/destruction and elimination of the foreign invader. The immune system is commonly compared to an army and its various cells to soldiers. It is a remarkably adaptive defense system evolved in vertebrates to protect them from invading pathogenic microorganisms.

Immunity is the state of protection from infectious disease, has both nonspecific and specific components. The nonspecific or innate immunity refers to the basic resistance to diseases/pathogens that invade a species. Defensive barriers such as anatomic, physiologic, endocytic, phagocytic and inflammation accomplish this type of immunity, which are present in the organism right from the time of birth of the organism.

The acquired immunity is specific and reflects the presence of a functional immune system that is capable of specifically recognizing and selectively eliminating foreign bodies including microbes. Unlike innate immunity, acquired

immunity is characterized by specificity, diversity, self recognition and memory.

HISTORICAL PERSPECTIVE

Around the turn of 18th Century, the scrouge of small pox was on rampage throughout Europe, which usually was fatal. Edward Jenner was a country physician at taht time, in Gloucestershire of England. He was intrigued by the fact that milkmaids who contracted (suffered from) cowpox were resistant to smallpox infection. Jenner reasoned that people could be protected from smallpox by inoculating them with the fluid from a cowpox pustule. He tested his on May 14, 1796, by inoculating an eight year old boy with fluid from a cowpox pustule. Later on July 1, 1796, Jenner intentionally infected that boy with live human smallpox, taken directly from a festering pustule. The boy did not develop smallpox.

CELLS OF IMMUNE SYSTEM

Human blood contains a remarkable variety of cells, each precisely performing a specific function in the immune response. They are Monocyte, Neutrophil, Eosinophil, Basophil, Platelets, T-Lymphocyte and B-Lymphocyte. The amazing reality is that, all these cells including Red Blood Corpuscles (RBC) originate from a kind of master cell the blood forming (Hematopoetic) Stem cell,. residing in the bone marrow (Fig. 1).

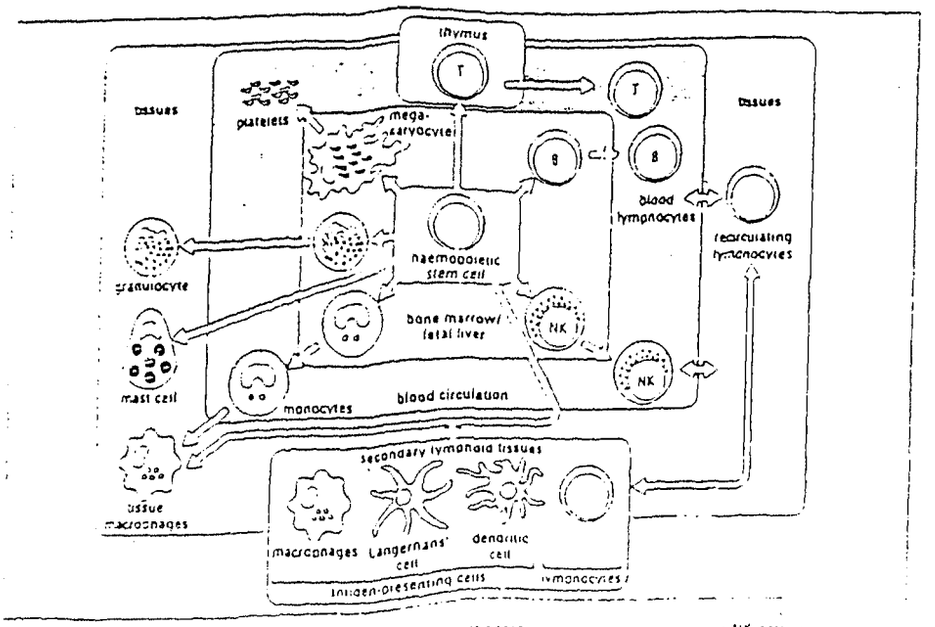

Fig. 1.1. All of these cells arise from the haemopoietic stem cell. Platelets produced by megakaryocytes are released into the circulation; granulocytes pass from the circulation into the tissues. Mast cells are identifiable in all tissues. B cells mature in the fetal liver and bone marrow in mammals, while T cells mature in the thymus. The origin of the large granular lymphocytes with NK activity is uncertain, but is probably the bone marrow. Both lymphocytes and monocytes (which develop into macrophages) can recirculate through secondary lymphoid tissue. Langerhans' cells and dendritic cells act as antigen-presenting cells in secondary lymphoid tissues.

(From Immunology by Roitt, I., Brostoff, J. and Male, D. 1993 Fifth Edition, Mosby, London)

All these cells are short lived and are constantly replenished by the activity of stem cells (See Table 1)

Table 1: Different blood cells and their functions

Blood Cell	Life span in blood	Functions
Erythrocyte	120 days	Oxygen transport
Monocyte	3 days	Host defense Immune Surveillance (Precursor of tissue Phagocyte)
Neutrophil	7 days	Host defense (Phagocytosis)
Eosionophil	Not known	Host defense against Parasites and allergies
Basophil	Not known	Inflammation and allergy
Platelets	7-8 days	Blood clotting
T-Lymphocytes	Not known	Cellular immunity
B-Lymphocytes	Not Known	Antibody defenses (Precursor of plasma cell)
NK Cell	Not known	Target cell lysis

The immune responses, whose ultimate out come is to get rid of the unwanted microbe/molecule, could be of two types:

1) Cell mediated immunity - includes the physical involvement of the cells in the removal of antigens eg., Phagocytosis by Macrophages (Blood monocytes) and Microphages (Neutrophils).
2) Humoral immunity - involves the production of antibodies by plasma cells, which differentiate from B-Lymphocytes upon antigenic stimulation.

INFLAMMATION

Tissue damage due to an injury or invasion by a pathogenic microorganism induces a complex sequence of events, collectively known as the inflammatory response or inflammation. Three major events occur at the site infection/damage. They are

1) Increased blood flow- resulting in engorgement of the capillary network.
2) Increased capillary permeability - facilitating an influx of fluid from the engorged capillaries.
3) Influx of phagocytic cells

Such a concentration of different cells of the immune system and their products at the damaged/infected region is necessary for the lysis of the pathogen or for increased phagocytosis.

VACCINES: An individual can be made resistant (immunized) to an infectious agent by artificially stimulating antibody producing mechanisms. This is accomplished by introduction of the pathogen or some of their component products that cause disease, in a modified form. For this purpose, sub-lethal doses of weakened organisms are employed. The preferred method is to employ killed microorganisms. These are called vaccines. If the disease is caused by toxins, the antibody producing mechanisms are stimulated by administration of non toxic "toxoids" which can act as antigens.

PRIMARY & SECONDARY IMMUNE RESPONSE

The phenomenon can be discussed with an example. Treatment of tetanus bacterial toxin with formaldehyde destroys its toxicity; but it retains antigenic property. Administration of this "toxoid" in to an individual activites the immune system to cause the production of antibodies. Exposure to toxin in a subsequent natural infection stimulates the memory cells, leading to the production of high levels of neutralizing antibody which are protective.

In primary response, after the introduction of the antigen, there follows a lag or latent period of several hours or days. Antibody begins to appear in the serum slowly at first; but within several days, its concentration rises exponentially and then the level tapers off to a plateau subsequently declines.

In a secondary response (when the same antigen subsequently enters), the latent period is shorter. The serum antibody concentrations will be much higher and will remain in circulation for a longer period. This property of the immune system is explited in protecting the individuals (inducing resistance to infection) by means of vaccination followed by administration of booster dose.

ANTIGENS

Antigens are the molecules which when they enter the individual stimulates its immune system and causes the production of antibodies. Chemically an antigen may be a protein lipid polysaccharide glycoprotein lipopolysaccharide etc.

Antibodies when produced will bind to antigens to result in the formation of antigen-antibody complexes. Antibodies do not bind to the whole molecule (antigen). Instead they bind to a particular region of the antigen called "antigenic determinant" or "epitope". A given antigen can have epitopes whose number may vary. These epitopes may be similar or different. Each antibody recognizes an epitope and binds to it.

ANTIBODIES

They are globular glycoproteins. Hence they are also called immunoglobulins (Ig). Human beings have 5 types of Ig molecules. They are IgA, IgD, IgE, IgG and IgM.

The most important of the five is IgG and constitutes 80% of total blood immunoglobulins. It has a molecular weight of 1,60,000. The molecule is made up of 4 polypeptides of two different sizes. These are a pair of identical high molecular weight chains called heavy chains (H-chains) and a pair of identical low molecular weight chains called light chains (L-chains). These 4 polypeptides are held together by disulfide bonds.

IgG has 2 disulfide bonds within the L-chain while there are 4 disulfide bonds within the H-chain. Each of these disulfide bonds encloses a peptide loop of 60-70 amino acids (Fig 2).

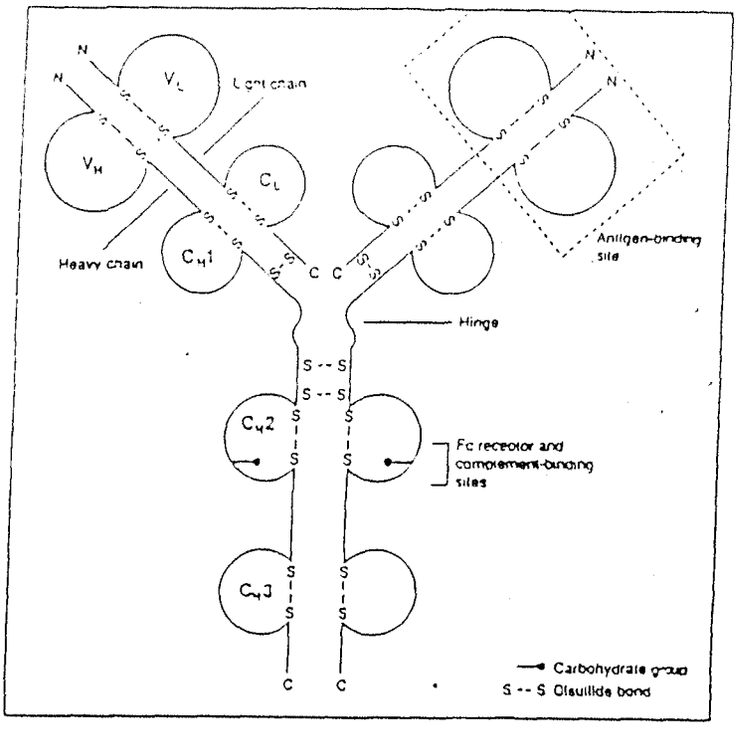

Fig. 2: From Cellular and Molecular Immunology by Abbas, A.K., Lichtmann, A.H. and Pober, J.S., 1994, W.B. Saunders, USA).

The amino acid sequences in these peptide loops are strikingly similar. However, one peptide loop in L-chain and one in H-chain situated at the N-terminal region of the chains will have variable amino acid sequences and hence are called variable domains. Suppose we take 2 IgG molecules produced against different antigenic determinants and compare the amino acid sequences in the peptide loop present at the N-terminal regions of L nd H-chains of one with the other, we find that the sequences differ, while the sequences in rest of the domains will be identical. This accounts for antibody diversity and their ability to ability to combine different antigenic determinants.

The recognition of self/nonself molecules (antigens) is accomplished due to antigen processing and its presentation by the Antigen Presenting Cells, to the T-Lymphocytes. Further, involvement of various cell surface receptors, for e. MHC (Major Histocompatibility Complex) Class I & II molecules, TCR (T-Cell Receptor), CD (Cluster of Differentiation) molecules and secretion of a variety of cytokines by some blood cells are necessary for the destruction of virally infected cells, recognition of non self molecule, proliferation and differentiation of antibody producing cells.

When the self/non self recognition ability of the immune system is lost, it leads to the developments of autoimmune diseases. Due to some reasons, when the immune

system fails to respond to the invading pathogens, it leads to the development of immunodeficiency syndromes as in case of AIDS, which makes way for bizarre opportunistic infections resulting in the untimely death of the individual sooner or later.

FOR FURTHER READING

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CONDUCTION OF NERVE IMPULSE

Structure of Neuron

- Nerve Signals are transmitted from one neuron to another through inter neuronal junctions called "Synapses".

There are two types of synapses.
a) Electrical synapse
b) Chemical Synapse

a) Electrical synapses have narrow gaps in between. They can conduct electricity from one to another neuron. This brings about action potential in neurons. Only few such synapses are found in central nervous system.

b) Chemical synapses are slightly wider gaps and neurons communicate with each other through chemical substances called "neurotransmitters".

Each motor neuron has a) Soma-cell body, b) an axon and c) dendrites

- Ends of other neurons in the form of nerve fibrils with their knob like structures lie on the surface of dendrites and soma. These knobs produce the neurotransmitters.
- Each terminal knob is called presynaptic knob or bulb or end feet.
- Each knob has synaptic vesicles containing nemotransmitters either excitatory or inhibitory and mitochondria that provide energy to synthesise vesicles.
- Each vesicle contains about 2000 to 50,000 molecules of neurotransmitters and each knob may contain nearly hundreds to 10,000 vesicles. This is enough for hundreds of action potentials.
- Role of calcium in release of neurotransmitters.
- Large number of calcium ions are found around the knob and very few inside the knob.
- An action potential reaching presynaptic knob membrane open up *voltage –gated calcium channels.*
- Calcium ions bind with specific proteins and in turn attach with vesicles make them to migrate to surface and open up (exocytosis) to release neurotransmitters.

Post synaptic Neuron

- There are specific protein receptors that bind to neurotransmitters and migrate inside. These in turn make membrane permeable by opening up sodium channels, potassium channels and chloride channels.

Conduction of Impulse

Resting potential

- This stage occurs before action potential.
- The membrane is said to be polarized.
- The exterior of membrane is *positively charged* because of large number of Na^+ ions and interior is *negatively charged* because of negatively charged *large protein molecules* and less number of K^+ ions. (Compared to Na^+ outside).

When nerve fibre receives a chemical, physical or electrical stimulus following changes occur.

Action Potential

- Membrane becomes suddenly permeable to sodium ions.
- Na^+ K^+ pump start working.
- Voltage-gates open up
- There is tremendous flow of sodium ions to the interior of the axon (5000 times).
- The normal polarized state of –90 mV is lost, with potential rising rapidly to positive direction.
- This state is called *Depolarisation.*
- The membrane potential overshoots beyond zero level and becomes positive. Some time in large fibres goes up to +35 mV.
- All these events happen in fraction of seconds.

Repolarisation

- Once the maximum movement of sodium ions takes place Na^+ gates are closed.
- K^+ gates open up and rapid diffusion of potassium ions to exterior takes place.
- By this positive charges are lost slowly from interior.
- This goes on till normal negative membrane potential inside is re-established.
- This is called *repolarisation.*

Reestablishment of membrane potential

- Reestablishment of sodium potassium ions around the membrane becomes necessary after every *action potential.*
- This is done through Na^+ K^+ pump.

- Since the pump requires *energy* for operation, the process is called recharging.
- The energy is derived from ATP.
- Lot of heat is produced during this process.

Excitation

It is brought about by opening up sodium channels to maximum extend and suppressing potassium and chloride channels.

Inhibition

When more potassium and chloride channels open up suppressing sodium channels, the inhibition occurs.

Active Transport Mechanism

a) **Sodium Potassium Pump**

- This pump has two separate globular proteins one large and another small protein molecules.
- Large protein has 3 receptor sites for binding sodium and small protein has 2 receptor sites for 2 potassium ions.
- Inside there is a site for ATPase activity.
- High energy produced brings about confrontational change in protein molecule and interchange of ions take place.
- This is also called "Electrogenic pump".

b) **Sodium potassium leak pump**

Gap between protein molecules allow the sodium potassium ions to pass through. Membrane is 100 time more permeable for K^+ ions.

c) **Voltage Gate**

i) **Sodium Voltage Gate**

has two gates, on outer side the *activation gate* that can open outside and an *inner inactivaton* gate that opens inside.

- At rest (-90 mV) all gates will be closed when *membrane potential* becomes less negative (-70m V to –50mV) the activation gate flip opens allow more sodium to flow in (5000 time faster).
- When membrane potential reaches positive inside then inactivation gate closes stopping flow of sodium ions. This gate will not open till the nerve fibre is repolarised.

Potassium Voltage gate

- When membrane potential rises from –90 mV towards zero the voltage gate opens allowing K^+ ions to flow out. This channel is very slow. Most of the potassium ions flow out.

Saltatory Conduction of Nerve Impulse

- Nerves covered with myclinated sheath are exposed to exterior at nodes only.
- Even though ions cannot flow significantly through the thick myclinated sheath it can flow with considerable ease through nodes of Ranvier.
- Action potential occurs only at nodes.
- The electrical current flows through surrounding extra cellular fluids into nodes.
- Thus nerve impulse jumps from node to node down the fibre. This is termed as "salutatory conduction".

Advances

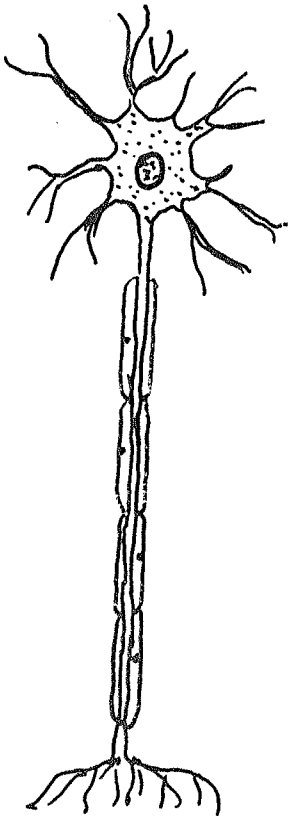
1. *Increases velocity* of conduction of impulse.
2. It *reduces excess expenditure of energy.*
3. Repolarisation *becomes easy and fast.*

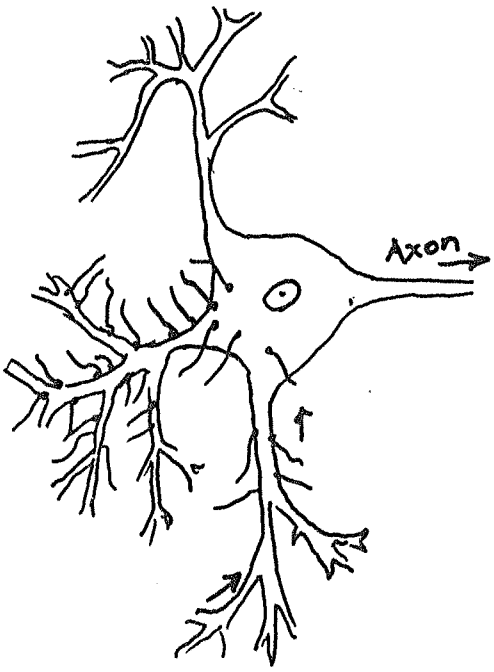
Speed of Nerve Impulse

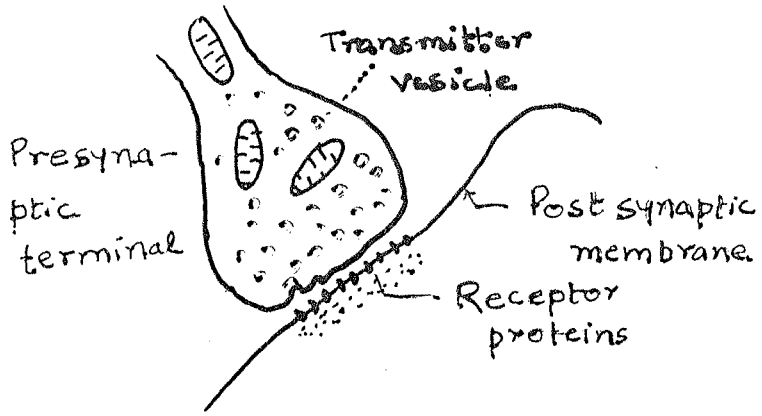
1.	Small non-myclinated nerve fibres	0.5 m/ sec
2.	Large myclinated nerve	100m/sec

Velocity increases with thickness.

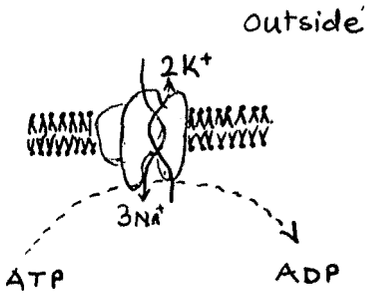
Direction of flow of impulse




Axon

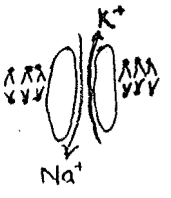

Transmitter vesicle
Presynaptic terminal
Post synaptic membrane
Receptor proteins

Extra Cellular fluid.	Intra Cellular fluid
Na^+ 140 :	Na^+ 10
K^+ 4 :	K^+ 140
Ca^{++} 2·4 :	Ca^{++} 0·0001
Cl^- 103 :	Cl 4
Proteins 5 :	40

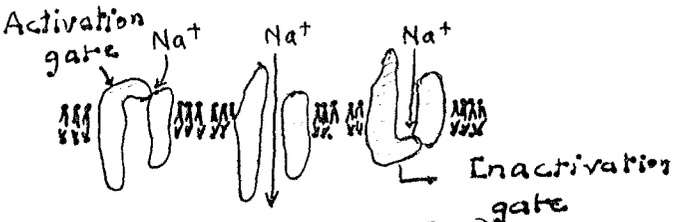


Na-K- Pump.

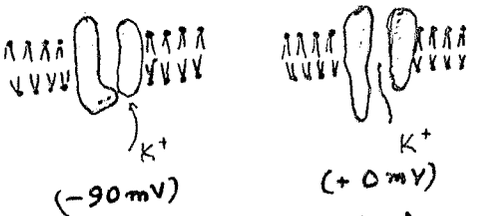
electrogenic pump



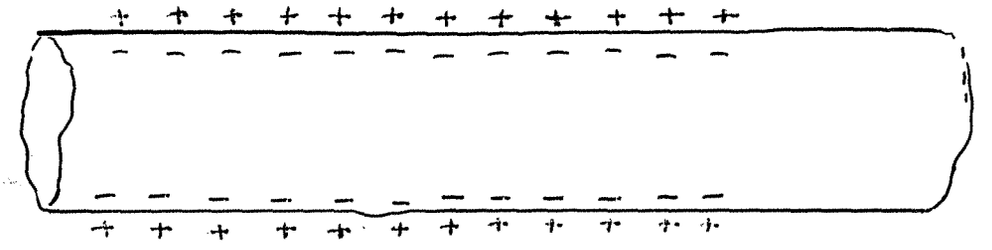
Leak channel



Voltage gated Na channel

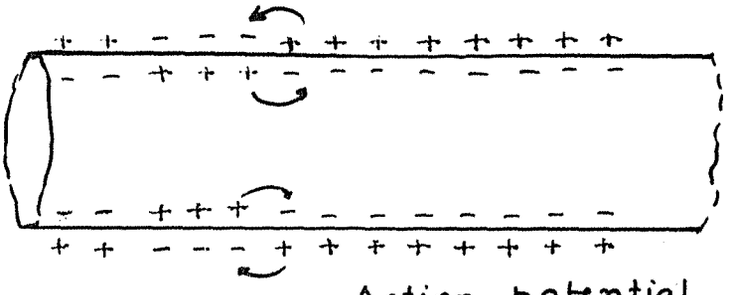


Voltage (charged) gated K channel:



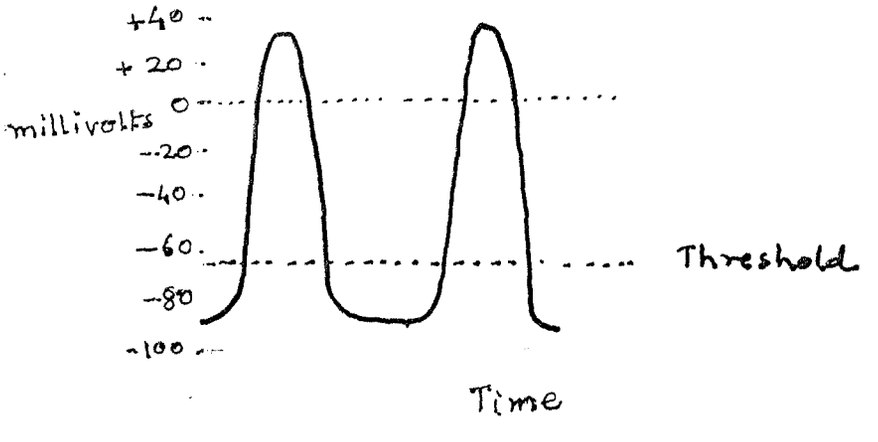
Resting potential.

Polarised



Action potential

Depolarised.



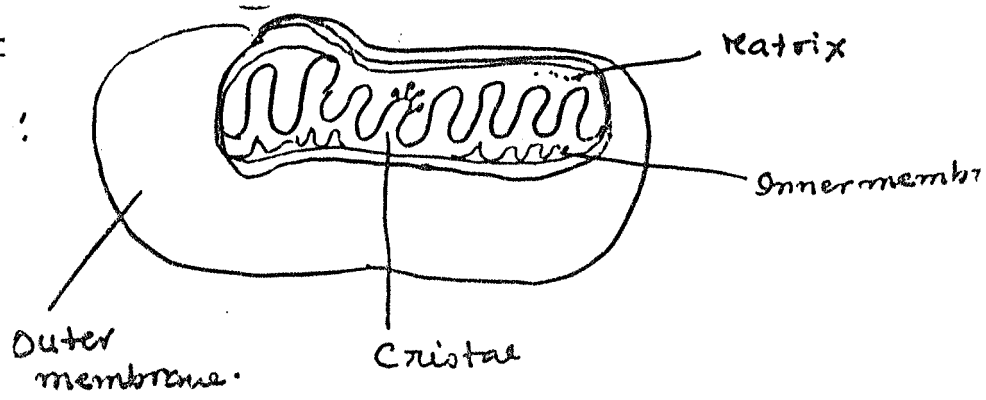
ELECTRON TRANSPORT

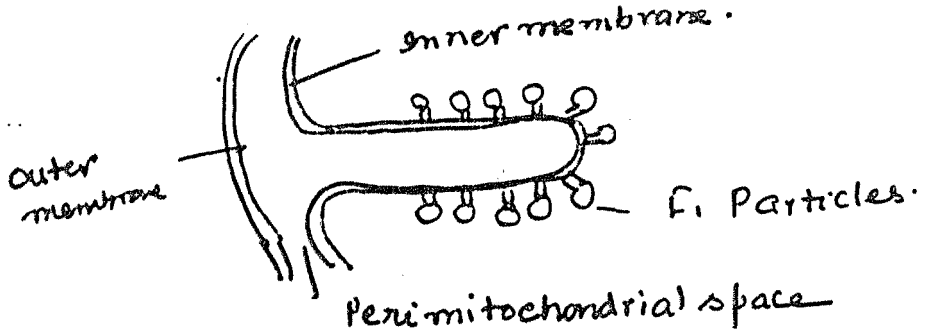
a) At the end of citric acid cycle
Glucose molecules is completely oxidized. The removal of e^- from compounded called "Oxidation".

Electron transport takes place on the Cristae"

b) This is usually accompanied by removal of hydrogen . H^+
c) The additional of electrons to a compound is also called reduction. This is usually accompanied by addition e^+ of hydrogen H^+.

Mitochondria:-





Terminal oxidation:- It is the last step of aerobic respiration NAOH and $NADH_2$ are formed during aerobic respiration. Then, the protons (H^+) and electrons(e^-) are released from these NADH and $FADH_2$ by oxidation. Finally the H^+ and e^- combine with molecular oxygen to form water. This reaction is called terminal oxidation. Water formed during terminal oxidation is called metabolic water.

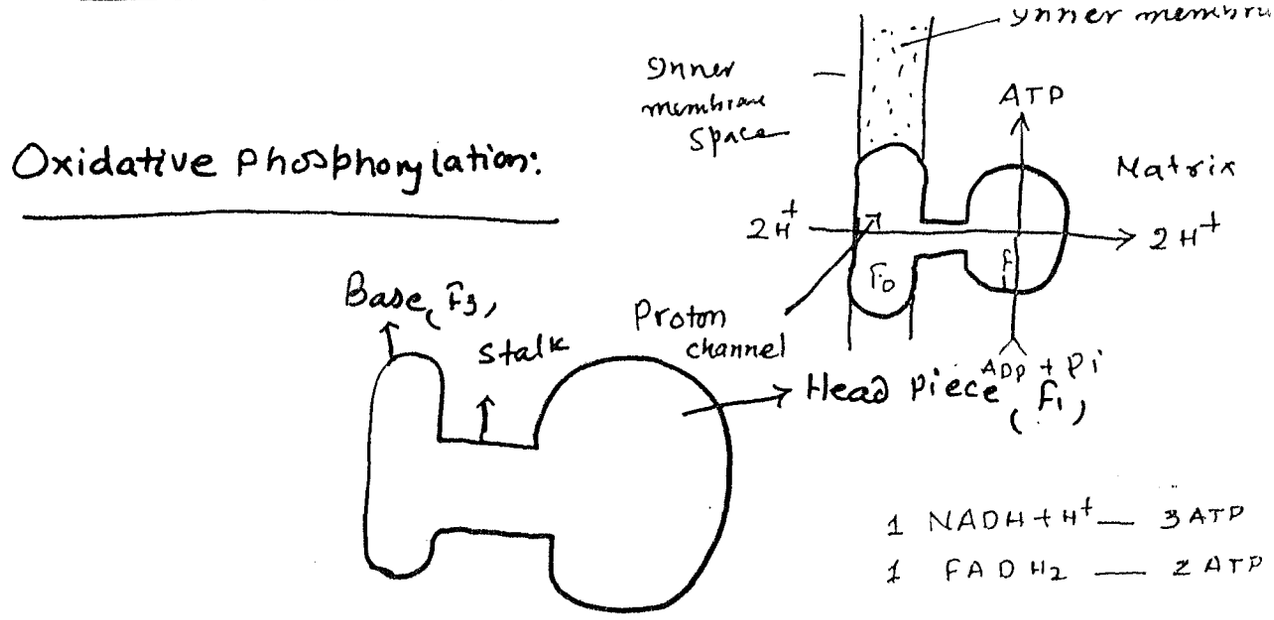
$$NADH + H^+ \rightarrow NAD^+ + 2H^+ + 2e^-$$

$$FADH_2 \rightarrow FAD + 2H^+ + 2e^-$$

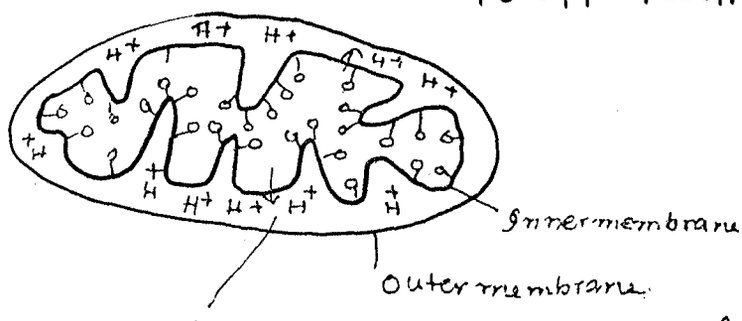
$$2H^+ + 2e^- + \tfrac{1}{2} O_2 \rightarrow H_2O \text{ (metabolic water)}$$

Terminal Oxidation has two steps.
They are electron transport system and Oxidative Phosphorylation.

Oxidative Phosphorylation:-



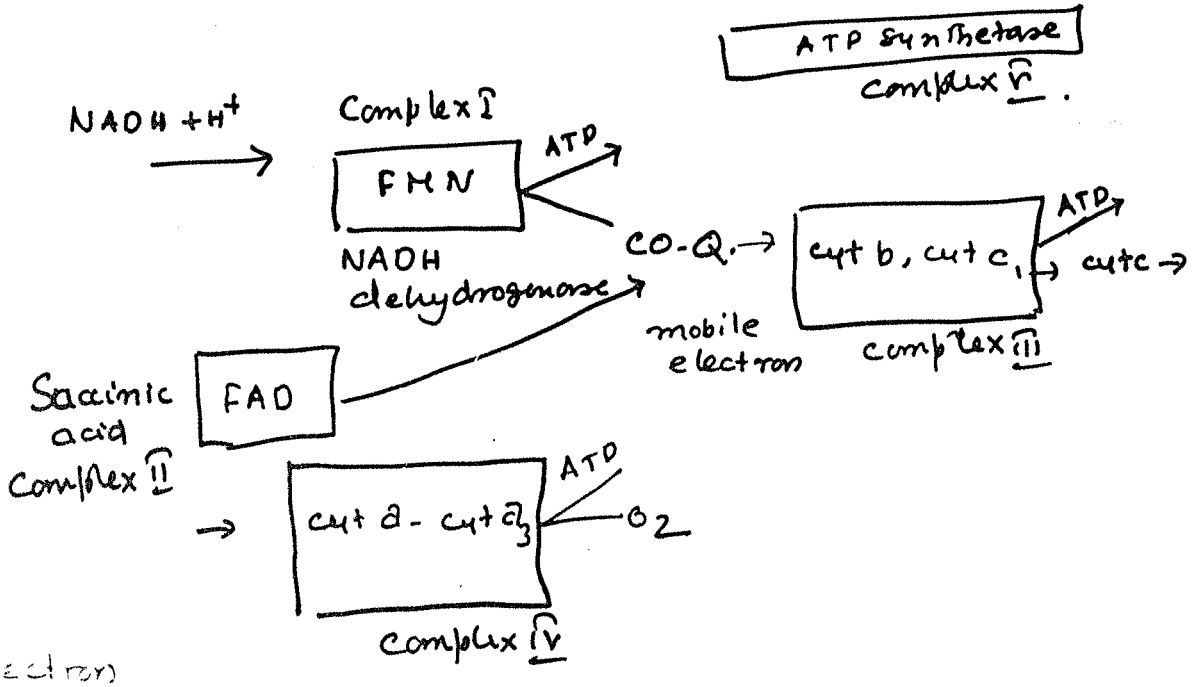
Fo - F1 Particle (Oxysome)

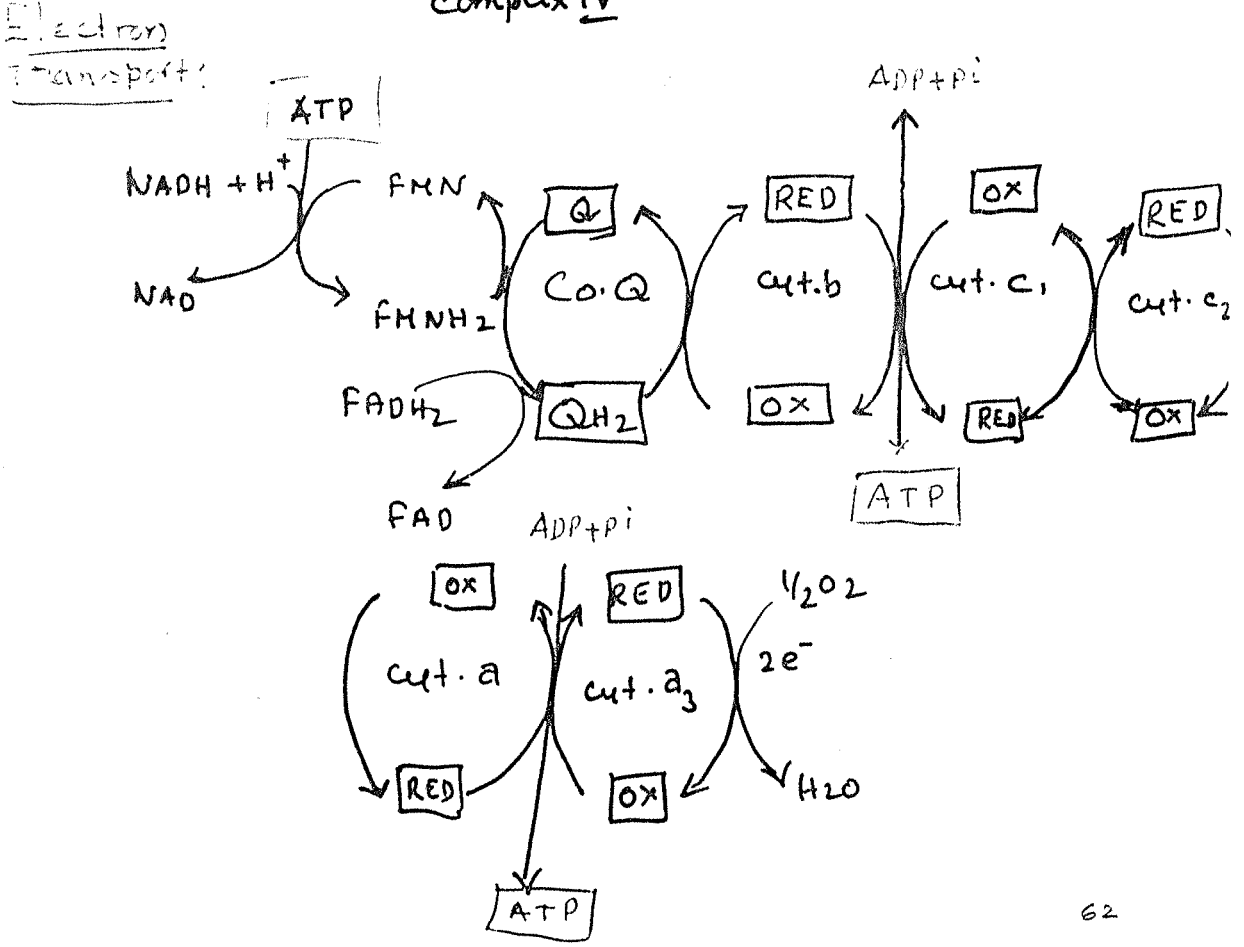


Inner membrane space with high Proton concentration.

Events occuring in the final phase of cell respiration.

1 Electron transport system (ETS)





According to Chemo-osmotic theory, during ETS, the oxidation of one molecules of NADH + H^+ plus 3 protons pairs ($2H^+$, $2H^+ \cdot 2H^+$) in to inner membrane space. $FADH_2$ push 2 proton pairs ($2H^+ \cdot 2H^+$)

As a result of the proton gradient, an electron (electrochemical potential difference occurs across the inner mitochondria),matrix. But the inner mitochondrial membrane is impermeable to protons except in the region of elementary particle ($F_0 - F_1$) particle except. $F_0 - F_1$ particle functions as portion channel. The flow of protons back to mitochondrial matrix. But the inner mitochondrial membrane is impermeable to protons except in the regions of elementary particle (F_0-F_1 particle) (F_0-F_1) particle functions as proton channel.

The flow of protons back to mitochondrial matrix is driven by electrochemical proton gradient.

During the black flow of protons the energy released is used for the synthesis of ATP from ADP and inorganic phosphate in F_1 as a result of 2 protons ($2H^+$) transported back through F_0 from inner membrane space to mitochondrial matrix.

PLANT TISSUE CULTURE

Concept of Cell Culture

German Botanist Gottlieb Haberlandt (1902) developed the concept of invitro cell culture. He was the first to culture isolated, fully differentiated cells in a nutrient medium containing glucose, peptone and knops salt solution.

Additional Guidelines for washing and handling of Glassware:

1. Reusable glassware for tissue culture should be emptied immediately after use and soaked. Never allow media or agar to dry on the glassware.
2. Segregate all glassware containing corrosive chemicals or fixatives from the rest of the tissue culture glassware.
3. All glassware contaminated or coming into contact with micro-organisms should be decontaminated before washing.
4. Remove labels and marking ink before washing. Marking ink may be wiped out by abrasive cleanser or acetone and immediate rinsing.
5. Melted agar in the culture vessels should be poured into a collecting sieve and discarded.
6. Rubber-lined screw caps should be soaked only in distilled water. Never soak caps in detergent or soap solution.
7. Glassware for growing cells should be acid cleaned if proteinaceous deposits are not removed by conventional washing.
8. Silicone-treated glassware should be permanently labeled and placed separately from the rest of the glassware.

Glass Washing

1. Tissue culture glassware should be soaked in 5% detergent solution for a minimum of one hour.

2. The automatic washer is used only as a rinsing unit and no detergent is added to the washer. Glassware should be treated with detergent prior to its transfer into the washing unit.
3. The automatic washer is programmed for no less than six times tap water rinses and a 1 min. distilled water rinse.
4. The glassware that does not fit into an automatic unit is rinsed by hand. Hand-rinsing requires a minimum of six times thorough tap water rinses and four rinses in quality water.
5. If an automatic dish-washing facility is not available, a simple washing machine with brushes of different sizes ma be used for manual washing.

Acid-Cleaning

1. Personnel concerned should wear a full-face mask and acid-resistant apron and gloves.
2. Handling of acid and processing of glassware in the acid should be done in a fume hood.
3. Never add water to acid. To dilute acid, add acid slowly to water while stirring.
4. After acid-cleaning, if the solution in the acid bath becomes dark coloured, it should be discarded.

Equipment commonly used in a Laboratory for many Tissue Culture Techniques

Preparation of Media

1. Gas, water and electricity supplies.
2. Provision for compressed air and vacuum lines.
3. Water heater or small stove.
4. Hot plate with magnetic stirrer.

5. Glass or stainless steel containers for heating and dissolving media.
6. Autoclave or pressure-steam sterilizer.
7. pH meter
8. Centigram balances or digital balances
9. Graduated measuring cylinders, flasks, beakers, petridishes and pipettes with teats.
10. Culture tubes, bottles and other glassware with table closures (such as cotton plugs, aluminium foil, plastic film or metal caps).
11. Small transfer instruments such as spatulas, scalpels, forceps or dissecting needles.
12. Hot-air oven for rapid heating of media and agar mixtures (microwave oven will also do rapid thawing of frozen items).
13. Water de-ioniser, distilled or double-distilled water units.
14. Chemicals for preparing culture media or commercially available powdered culture media, growth hormones and other organic constituents.
15. Filter membranes with holders and hypodermic syringes to filter-sterilise solutions.
16. Detergents, disinfectants
17. Pipette washers (acid proof)
18. Drying and draining racks
19. Storage space for chemicals, glassware, nutrient media, sterile water and other items.

Isolation of Cultures

1. Laminar air flow cabinet
2. Spirit lamp or Bunsen burner in the inoculation cabinet.
3. Stereo-microscope.

4. Ethyl alcohol (96%) for sterilization and flaming of small metal instruments.
5. Tiles or glass plates for use during sterile cutting.

General Equipment

1. Refrigerator, deep-freeze.
2. Automatic dish-washer
3. Glassatomiser
4. Dispensing devices (e.g. wire mesh baskets, trolleys with trays and metal racks for holding test tubes or culture vials in the autoclave).
5. Acid proof baths for cleansing glassware.
6. Microscopes (e.g. compound, inverted) with microphotographic equipment.
7. Markers, labels (similar material for wrapping, culture vessels, glassware, tiles and other lab. ware).

Culture Room

1. Temperature control (17 – 27°C)
2. Electricity supply essential for lighting, cooling and heating.
3. Shelves for culture racks.
4. Fluorescent tubes for lighting
5. Timer for regulating day – length.
6. Racks for culture vials
7. Rotary shaker
9. Observations table

Transplantation Facility (Green House)

A small area where high humidity, light and temperature can be controlled in the form of a green house or a small room.

Nutritional Components of some Plant tissue Culture Media

Components	Amount (mg l^{-1})					
	Whites	MS	B_5	Nitseh's	N_6	E_1
Macronutrients						
$MgSO_4.7H_2O$	750	370	250	185	185	400
KH_2PO_4	-	170	-	68	400	250
$NaH_2PO_4,.H_2O$	19	-	150	-	-	-
KNO_3	80	1900	2500	950	2830	2100
NH_4NO_3	-	1650	-	720	-	600
$CaCl_2.2H_2O$	-	440	150	-	166	450
$(NH_4)_2.SO_4$	-	-	134	-	463	-
Micronutrients						
H_3BO_3	1.5	6.2	3	-	1.6	3
$MnSO_4.4H_2O$	5	22.3	-	25	4.4	-
$MnSO_4.H_2O$	-	-	10	-	3.3	10
$ZnSO_4.7H_2O$	3	8.6	2	10	1.5	2
$Na_2MoO_4.2H_2O$	-	0.25	0.25	0.25	-	0.25
$CuSO_4.5H_2O$	0.01	0.025	0.025	0.025	-	0.025
$CoCl_2.6H_2O$	-	0.025	0.025	0.025	-	0.025
KI	0.75	0.83	0.75	-	0.8	0.8
$FeSO_4.7H_2O$	-	27.8	-	27.8	27.8	-
$Na_2EDTA.2H_2O$	-	37.3	-	37.3	37.3	-
EDTA Na ferric salt	-	-	43	-	-	43
Sucrose (g)	20	30	20	20	50	25
Organic Supplements						
Vitamins						
Thiamine HCl	0.01	0.5	10	0.5	1	10
Pyridoxine HCl	0.01	0.5	1	0.5	0.5	1
Nicotinic acid	0.05	0.5	1	5	0.5	1
Others						
Glycine	3	2	-	2	-	-
Folic acid	-	-	-	0.5	-	-
Biotin	-	-	-	0.05	-	-
PH	5.8	5.8	5.5	5.8	5.8	5.5

Whites (1953; Am. J. Bot. 40 : 517-524)
MS Murashinge and Skoog, 1962; Physiol. Plant, 15 : 473)
B_5 (Gamborg et al. 1968; Exp : Cell Res. 50 : 151)
Nitsch's (Nitsch and Nitsch 1969; Science, New York, 163 : 85)
N_6 (Chu, 1978; Proc. Symp. Plant tissue Culture, Science Press, Peking, p.43).
E_1 (Gamborg et. al. 1983; Plant cell Rep. 2 : 209)

Stock Solutions for MS bases medium

Constituents	Amount (mg l^{-1})
Stock Solution I	
$MgSO_4 . 7H_2O$	7400
KH_2PO_4	3400
KNO_3	38000
NH_4NO_3	33000
$CaCl_2 . 2H_2O$	8800
Stock Solution II	
H_3BO_3	1240
$MnSO_4.4 H_2O$	4460
$ZnSO_4. 7H_2O$	1720
$Na_2MoO_4. 2H_2O$	50
$CuSO_4.5H_2O$	5
$CoCl_2.6H_2O$	5
Stock Solution III	
$FeSO_4. 7H_2O$	5560
$Na_2.EDTA.2H_2O$	7460
Stock Solution IV	
Gnositol	20000
Thiamine HCl	100
Pyridoxine HCl	100
Nicotinic acid	100
Glycine	400

To prepare one litre of medium take 50 ml of stock solution and 1.5 ml each of stock solutions II – V. Dissolve $FeSO_4. 7H_2O$ and $Na_2.EDTA.2H_2O$ separately in 450 ml distilled water by heating and constant stirring. Mix the two solutions. Adjust the pH to 5.5 to 6.0 and add distilled water to make up the final volume to one litre.

PREPARATION OF MEDIA FOR MICROBIOL CULTURE

Objective : To prepare potato-dextrose agar media.

Definitions

Medium : Material on, or in which microorganisms are growr in the abora tory is called medium.

Culture : Cultivation of a micro-organism in group. This group may be homogeneous or heterogeneous.

Preculture: When the cultivation is only homogeneous or all similar or of only one kind organism, it is called pure culture.

Requirements: For the preparation of 1000 ml. of potato-dextrose-agar media (P.D.A.):-

Agar - 20 g
Dextrose (or Sugar) - 20 g
Potato - 50 g
Petri dishes in pair
Conical flask (500 ml) - 2
Conical flask (1000 ml) - 1
Beaker (500 ml) - 2
Glass Rods 10-20 - 2
Non-absorbant cotton - 100 g
Pressure Cooker of 10 litre capacity.
Gas burner or electrical heater.
Distilled water
Oil paper
Thread

Procedure :

1. Weigh 50 g of potato. Clean it but do not peel.
2. Cut the potato into small pieces (1-2 cm) and put in a beaker with 200 ml of water. Heat it slowly for about 1/2 hr till water turns turbid. Filter the whole material through a piece of cloth and pour the filtrate into a 1000 ml conical flask.

   Dissolve 20 g of agar in not more than 100 ml water in a beaker. Agar may not dissolve at room temperature. Boil the solution carefully.

4. Dissolve Dextrose (Sugar) in another 100 ml of water.

5. Mix the agar and dextrose solution with the filtrate of boiled potato in the flask. Add water to make 1000 ml.

6. Plug the flask tightly with cotton. Cover plug with oil paper and tie with thread.

7. Sterilise it in the pressure cooker for minimum 1/2 hr at 15 lb pressure and 120°C temperature.

8. Sterilisation of petridishes:-

(i) Clean desired number of pairs of petridishes.
(ii) Wrap them in newspaper or any ordinary paper and tie them.
(iii) Put individual pairs in a wire basket.
(iv) Put the basket in the pressure cooker. The basket should be placed on raised platform above water level.
(v) Close the pressure cooker properly.
(vi) Sterilise for a minimum of half an hour.
(vii) When the pressure cooker comes to normal temperature, take out the petridishes. Do not remove the wrapper, open the wrapping in the inoculation chamber only.

9. Pour 5 ml of media in each petridish only in the inoculation chamber.

NOTE:- If inoculation is done immediately, put the inoculated petridishes in culture chamber after making them with glass marking pencil. Resterilise the unused medium.

Precaution: Put sufficient water in the pressure cooker keeping in mind the time of sterilization.

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CULTURE AND STAINING OF BACTERIA

A : CULTURE

Aim :- To culture bacteria on a simple media and

Requirements:

1. Potato/Carrot
2. Sheet of blotting paper
3. A long glass rod.
4. Paired petridishes
5. Beaker
6. Heater/burner

Procedure:

1. Cut cubical pieces of carrot or potato about 1-2 cm^3.
2. Boil them in a beaker till half cooked.
3. Line the base of petridishes with wet blotting paper and place the half boiled vegetable cubes and cover with lid.
4. Observe after 3-4 days.

B. STAINING

Aim:- To demonstrate single staining and gram staining of bacteria.

Requirements

1. Metnylene blue
2. Crystal violet stain:

Soln A	Crystal violet (90% dye content)	2 g
	Ethanol (95%)	- 20 ml.
Soln B	Ammonium oxalate	0.8 g
	Distilled water	80 ml

Mix solutions A and B before use

3. Safranine stain

Safranine - 2.25 g
Ethanol (95%) - 225 ml.

4. Iodine solution

Iodine - 2 g
Potassssium iodide -
Distilled water - 100 ml.

5. Alcohol

6. Glycerine

7. Slides and coverslip

8. Microscope

Procedure :

(a) Single staining

1. Make a smear of bacteria on a slide.
2. Dry the smear and flood with methylene blue for one minute.
3. Wash gently under running water.
4. Mount in glycerine and observe under high power of the microscope.

(b) Gram's staining

Gram staining is used to distinguish between gram positive and gram negative bacteri

1. Flood a dry bacterial smear with crystal violet solution.
2. Wash gently under running water.
3. Stain with iodine solution.
4. Wash with alcohol
5. Stain with saffranine for a minute.
6. Wash gently under running water.
7. Mount in glyserine and observe under high power.

Those bacteria which are stained with crystal violet colour, and are not decolourised after washing with alcohol are termed gram positive. Those which stain pink red with saffranine because the crystal violet stain had been washed out, are called gram negative bacteria.

Aim : To see the effect of different concentration of antibiotics on bacterial growth.

Requirements:

Petridishes 20 pairs
Antebiotic 1 vial
Measuring cylinder 10 ml.
Scissors
Glass marking pencil
P.D.A. media (see RCEB/DBT/MICROBIOL/2)

Procedure

1. Prepare P.D.A. medium and pour into petriplates as descsribed in RCEB/DBT/MICROBIOL/2.
2. Cut circular pieces of blotting paper of 1 cm diameter.
3. Prepare solutions of different concentrations of antibiotic.
4. Soak a piece of circular blotting paper in each concentration of antibiotic, and place each in a separate petridish.
5. Bacterial inoculation is not necessary as there is normal contamination. However to be very sure a diluted culture of bacteria may be spread on the plate.
6. Observe bacterial growth after 48 hrs. If the bacteria is sensitive to the antibiotic there will be a clear zone round the blotting paper. The greater the concentration the wider the zone.

.....

Ex. No.
Date :

COMPARATIVE STUDY OF BLOOD SMEARS OF MAN AND FROG

AIM : To compare and contrast the blood corpuscles of man and frog.

MATERIALS REQUIRED : Glass slides, sterilized needles, spirit lamp & microscope.

CHEMICALS REQUIRED : Leishman's stain, 100% alcohol & distilled water.

PROCEDURE :

1. Prick the finger with a sterilized needle, wipe away the first drop of blood. Take the second drop on the end of a clean slide. Incase of frog's blood, it has to be dissected open and two drops of blood was transferred on a clean slide.
2. Place the slide on a smooth surface holding it steady with the left hand. Hold a second slide or at an angle of 45° just in front of the drop of the blood, draw the slide in such a way that the blood spreads along its edge. Push the second or drawing slide forward at 45° on the first slide without exerting any pressure. Thus prepare a blood smear. Dry the blood film in air.
3. Stain the blood film with Leishman's stain (or Wright's stain).
4. Wait and after one minute carefully add distilled water to the stain, the water should be twice the volume of the stain used. Mix the water and stain well by sucking the mixture in and out a pipette. After a time a greenish metallic scum forms on the surface of the mixture. Allow the diluted stain to act from 7 to 10 minutes.
5. Drain off the stain and wash film for 10 second with distilled water. The film should be rose pink in color, if it is too purple, then wash again with distilled water.
6. Examine it under microscope and record the observations.

PRECAUTIONS:

i) Only one time the slide should be passed/smeared.
ii) Sterilized needles and cotton should be used.
iii) The smears should be submerged in stain or D.W during staining.

OBSERVATION TABLE

S n.	Cell type	Characters	Human Blood		Frog's Blood	
			Dia-gram	Obser-vation	Dia-gram	Obser-vation
1.	ERYTHROCYTES	a) Shape b) Size c) Color d) Nucleus (✓ / ×)				
2.	LEUCOCYTES					
A.	**Granulocytes**	a) Shape b) Size c) Color				
	i) Neutrophil	d) Nucleus (✓/×) e) No. of lobes of the nucleus f) Size and amount of granules				
	ii) Eosinophils	a) Shape b) Size c) Color d) Nucleus (✓/×) e) No. of lobes of the nucleus f) Size and amount of granules				

	iii) Basophils	a) Shape b) Size c) Color d) Nucleus (✓/×) e) No. of lobes of the nucleus f) Size and amount of granules				
B.	**Agranulocytes** i) Monocytes	a) Shape b) Size c) Color d) Nucleus (✓/×) e) No. of lobes of the nucleus				
	ii) Lymphocytes	a) Shape b) Size c) Color d) Nucleus (✓/×) e) No. of lobes of the nucleus				
3.	THROMBOCYTES	a) Shape b) Size c) Color d) Nucleus (✓/×) e) No. of lobes of the nucleus f) Size and amount of granules				

INFERENCE / CONCLUSION

i)

ii)

iii)

iv)

v)

SKILLS DEVELOPED

i)

ii)

iii)

iv)

v)

SIGNIFICANCE OF THE STUDY

How this study is useful to us? (or)

Why should I do this study ? (write in one paragraph)

*****

Ex.no:
Date:

ENUMERATION OF WHITE BLOOD CORPUSCLES

Aim: To determine the I) total number of WBC present in cubic millimeter of blood and II) the relative proportion of WBCs of blood in man and frog.

Objectives:

On completion of this experiment, students will be able to :

Knowledge level:

- Recall the structure of WBC
- Recognize the different types of WBC

Understanding level:

- Describe the Haemocytometer
- Classify the WBC
- Discriminate between different types of WBC
- Describe the procedure to count WBC
- Give reason for use of dilution fluid
- State the formula to calculate WBC
- Compare the number of WBC of frog and man
- Explain the significance of the study

Application level:

- Interpret the recordings
- Suggests simple projects.

Skill level:

- Dilutes the blood accurately
- Charges the chamber without air bubble
- Count and calculate the number of cells correctly.
- Handle the instrument safely
- Draw scientific diagram an label them

Synthesis level:

- Synthesis the facts and give possible explanations for the observation.

Attitude level:

- Objective in approach
- Honest in collecting and recording scientific data
- Observe cleanliness in activity
- Maintain time (punctual).

HAEMOCYTOMETER

It is an instrument used for counting blood cells. It consists of two pipettes and a counting chamber. The counting chamber that is used most commonly is the **Levy Chamber** with improved **NEUBAUER'S** ruling. It has two ruled stages separated with a small gutter. The two stages in turn are separated from the two ridges one on either side by a gutter. The surface of the two ridges is 1/10 mm above the surface of the stage. When a cover slip is placed on them, there is a space of 1/10 mm (in which the sample is loaded) between the bottom of the cover slip and the top of the chamber stage.

a) Improved NEUBAUER'S ruling:

- There is a large ruled area of approximately 9 sq.mm. on each chamber stage.
- It is divided into 9 squares of 1 sq. mm. each.
- The corner squares are divided into 16 small squares, which are used for counting of WBCs.
- The central square area is divided into 25 squares, each of which is further divided into 16 small squares.
- Each squares measures 1/400 sq.mm in area. Five of the squares with a total of so small squares are used for counting RBCs.

b) Micro-pipettes:

The Haemocytometer includes two graduated micro-pipettes in which dilution of blood is done. One micropipette with red bead is used for counting RBCs, while the other with white for counting WBCs. Each micropipette bears at least 3 graduation; those on the micro pipette for RBCs are 0.5, 1 and 101 and for WBCs are 0.5, 1 and 11. The beads are assisting in mixing the blood.

Note:

Draw the scientific diagram of counting chamber,NEUBAUER'S ruling, RBC and WBC micro-pipettes in your record note.

*****

I. TOTAL COUNT

Apparatus Required : WBC pipette, NEUBAUER's counting chamber, coverslip, needle, microscope.

Reagents Required : Alcohol, distilled water, WBC dilution fluid.

Procedure :

A. Dilution of Blood

- Prick the finger with sterilized needle, wipe away the first drop of blood, take the second drop on a clean slide. Incase of frog's blood, it has to be dissected open and two drops of blood was transferred on a clean slide.
- In the WBC pipette, blood is drawn up to the mark 0.5 and then the diluting fluid is drawn up to the mark 11.
- The pipette is rotated rapidly between the fingers to allow the fluid to mix well. The glass bead assists the mixing.
- Care should be taken till mixing is over to see that the fluid does not run out off the pipette and avoid air bubbles in the pipette.
- The volume the bulb is 10, hence 0.5 ml volume of blood is taken into this 10, it gives a dilution of 1/20. So dilution of the blood content is 1/20 and not 1/21.

B. Charging the counting chamber

- Counting chamber should be clear and dry.
- Cover slip should be clean and dry and it has to be applied carefully.
- Mix the suspension of RBC well.
- Discard first a few drops from the micro-pipette.
- Seal one end of the tube with index finger and place the tip on platform adjacent to the edge of the cover slip.
- Hold the pipette slightly inclined and gently releases the pressure with finger and allow a small volume of the fluid to fall down. This will run under the cover slip by capillary action.
- Allow the cells to settle for 5 minutes and start counting.
- Record your observation and calculate the number of RBC/cu.mm. of blood.

Precautions

The filling should be done in one application on the tip of the pipette.
There should be no air bubble under the cover slip.
The fluid should not overrush into the gutters.

Functions of WBC diluting fluid :

The fluid contains,

Glacial acetic acid	:	1.5 ml
1% solution of Gentian violet in water	:	1 ml
Distilled water	:	97.5 ml

- This is isotonic, so that haemolysis does not occur.
- Glacial acetic acid breaks down red cells.
- Gentian violet slightly stains the nucleus of leucocytes so that they can be observed easily under microscope.

Calculation:

Total No. of WBCs/cu.mm =

$$\frac{Number\ of\ \ cells\ counted\ \ (N)}{Dilution\ Factor\ \frac{1}{200} \times Chamber\ \ Depth\ Factor\ \frac{1}{10} \times Chamber\ area\ \ counted\ \ 4}$$

It is commonly expressed as :

$$\frac{No.\ of\ \ cells\ \ counted \times Dilution\ \ factor \times Depth\ Factor}{Area\ \ counted}$$

$$= \frac{N \times 20 \times 10}{4} = 50N$$

= N x 50

Result :

Total number of WBCs/cu.mm of blood is ————————

II. DIFFERENTIAL COUNT

Materials required: Glass slides, sterilized needles, spirit lamp & microscope.
Chemicals required: Leishman's stain, 100% alcohol & distilled water.

Procedure :

- Smearing and staining procedure is followed as in study of blood smears.
- Count 100 or 200 WBCs noting on a paper each type of WBC encounted and calculate the percentage.

Calculation :

$$\frac{Number\ of\ \ particular\ \ type\ cells}{Total\ \ number\ of\ WBC\ \ counted} \times 100$$

Observation Table :

Sl.No.	Cell Type	No. of cells counted	Percentage
1.	Neutrophils		
2.	Eosinophphis		
3.	Basophils		
4.	Lymphocytes		
5.	Monocytes		

Result :
Discussion :
Significance of the Study:
Project:
Suggest a possible, fessible, objective and rationale project based on above study (at UG level) and write the brief outline of procedure.

*****

Ex.no:
Date:

ENUMERATION OF RED BLOOD CORPUSCLES

Aim : To determine the number of RBCs present in cubic millimeter of blood of man and frog.

Objectives:

On completion of this experiment, students will be able to :

Knowledge level:

- Recall the structure of RBC
- Recognize the RBC

Understanding level:

- Describe the Haemocytometer
- Describe the procedure to count RBC
- Give reason for use of dilution fluid
- State the formula to calculate RBC
- Compare the number of RBC of frog and man
- Explain the significance of the study

Application level:

- Interpret the recordings
- Suggests simple projects.

Skill level:

- Dilutes the blood accurately
- Charges the chamber without air bubble
- Count and calculate the number of cells correctly.
- Handle the instrument safely
- Draw scientific diagram and label them

Synthesis level:

- Synthesis the facts and give possible explanations for the observation.

Attitude level:

- Objective in approach
- Honest in collecting and recording scientific data
- Observe cleanliness in activity
- Maintain time (punctual).

Apparatus Required : RBC pipette, NEUBAUER's counting chamber, cover slip, needle and microscope.

Reagents required : Alcohol, distilled water, RBC dilution fluid.

Procedure :

A. Dilution of Blood:

* Prick the finger with sterilized needle, wipe away the first drop of blood, take the second drop on a clean slide. Incase of frog's blood, it has to be dissected open and two drops of blood was transferred on a clean slide.
* In the RBC pipette, blood is drawn into the mark 0.5 and then the diluting fluid is drawn up to the mark 101.
* The pipette is rotated rapidly between the fingers to allow the fluid to mix well. The glass bead assists the mixing.
* Care should be taken till mixing is over to see that the fluid does not run out off the pipette and avoid air bubbles in the pipette.
* The volume the bulb is 100, hence 0.5 ml volume of blood is taken into this 100, it gives a dilution of 1/200. So dilution of the blood content is 1/200 and not 1/201.

B. Charging the counting chamber

* Counting chamber should be clear and dry.
* Cover slip should be clean and dry and it has to be applied carefully.
* Mix the suspension of RBC well.
* Discard first a few drops from the micro pipette.
* Seal one end of the tube with index finger and place the tip on platform adjacent to the edge of the cover slip.
* Hold the pipette slightly inclined and gently releases the pressure with finger and allow a small volume of the fluid to fall down. This will run under the cover slip by capillary action.
* Allow the cells to settle for 5 minutes and start counting.
* Record your observation and calculate the number of RBC/cu.mm. of blood.

Precautions

The filling should be done in one application on the tip of the pipette.
There should be no air bubble under the cover slip.
The fluid should not overrush into the gutters.

Functions of RBC diluting fluid :

The fluid contains,

Sodium citrate	:	3 gms
Formalin	:	1 ml
Distilled water	:	100 ml

* This is isotonic, so that haemolysis does not occur.
* Formalin fixes the cells and retards bacterial and fungal growth in fluid.
* Sodium citrate prevents the coagulation and provides proper shape of RBC.
* Dissolves the WBCs.

Calculation:

Number of RBCs/cu.mm =

$$\frac{Number\ of\ \ cells\ counted\ \ (N)}{Dilution\ Factor\ \frac{1}{200} \times Chamber\ \ Depth\ Factor\ \frac{1}{10} \times Chamber\ area\ \ counted\ \frac{1}{5}}$$

It is commonly expressed as :

No. of cells counted × dilution factor × chamber depth factor × chamber area counted.

= N × 200 × 10 × 5

= **N × 10,000**

Result :

Number of RBCs / mm^3 of blood is = ————————

Discussion :

→
→
→

Significance of the Study:

→
→

Project:

Suggest a possible, fessible, objective and rationale project based on above study (at UG level) and write the brief outline of procedure.

*****

Ex.no:
Date:

ENUMERATION OF RED BLOOD CORPUSCLES

Aim : To determine the number of RBCs present in cubic millimeter of blood of man and frog.

Objectives:

On completion of this experiment, students will be able to :

Knowledge level:

- Recall the structure of RBC
- Recognize the RBC

Understanding level:

- Describe the Haemocytometer
- Describe the procedure to count RBC
- Give reason for use of dilution fluid
- State the formula to calculate RBC
- Compare the number of RBC of frog and man
- Explain the significance of the study

Application level:

- Interpret the recordings
- Suggests simple projects.

Skill level:

- Dilutes the blood accurately
- Charges the chamber without air bubble
- Count and calculate the number of cells correctly.
- Handle the instrument safely
- Draw scientific diagram and label them

Synthesis level:

- Synthesis the facts and give possible explanations for the observation.

Attitude level:

- Objective in approach
- Honest in collecting and recording scientific data
- Observe cleanliness in activity
- Maintain time (punctual).

Apparatus Required : RBC pipette, NEUBAUER's counting chamber, cover slip, needle and microscope.

Reagents required : Alcohol, distilled water, RBC dilution fluid.

Procedure :

A. Dilution of Blood:

* Prick the finger with sterilized needle, wipe away the first drop of blood, take the second drop on a clean slide. Incase of frog's blood, it has to be dissected open and two drops of blood was transferred on a clean slide.
* In the RBC pipette, blood is drawn into the mark 0.5 and then the diluting fluid is drawn up to the mark 101.
* The pipette is rotated rapidly between the fingers to allow the fluid to mix well. The glass bead assists the mixing.
* Care should be taken till mixing is over to see that the fluid does not run out off the pipette and avoid air bubbles in the pipette.
* The volume the bulb is 100, hence 0.5 ml volume of blood is taken into this 100, it gives a dilution of 1/200. So dilution of the blood content is 1/200 and not 1/201.

B. Charging the counting chamber

* Counting chamber should be clear and dry.
* Cover slip should be clean and dry and it has to be applied carefully.
* Mix the suspension of RBC well.
* Discard first a few drops from the micro pipette.
* Seal one end of the tube with index finger and place the tip on platform adjacent to the edge of the cover slip.
* Hold the pipette slightly inclined and gently releases the pressure with finger and allow a small volume of the fluid to fall down. This will run under the cover slip by capillary action.
* Allow the cells to settle for 5 minutes and start counting.
* Record your observation and calculate the number of RBC/cu.mm. of blood.

Precautions

The filling should be done in one application on the tip of the pipette.
There should be no air bubble under the cover slip.
The fluid should not overrush into the gutters.

Functions of RBC diluting fluid :

The fluid contains,

Sodium citrate	:	3 gms
Formalin	:	1 ml
Distilled water	:	100 ml

* This is isotonic, so that haemolysis does not occur.
* Formalin fixes the cells and retards bacterial and fungal growth in fluid.
* Sodium citrate prevents the coagulation and provides proper shape of RBC.
* Dissolves the WBCs.

Calculation:

Number of RBCs/cu.mm =

$$\frac{Number\ of\ \ cells\ counted\ \ (N)}{Dilution\ Factor\ \frac{1}{200} \times Chamber\ \ Depth\ Factor\ \frac{1}{10} \times Chamber\ area\ \ counted\ \frac{1}{5}}$$

It is commonly expressed as :

No. of cells counted × dilution factor × chamber depth factor × chamber area counted.

= N × 200 × 10 × 5

= **N × 10,000**

Result :

Number of RBCs / mm^3 of blood is = ————————

Discussion :

→
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→

Significance of the Study:

→
→

Project:

Suggest a possible, fessible, objective and rationale project based on above study (at UG **level) and write the brief outline of procedure.**

*****

Ex.no :
Date :

BLOOD PRESSURE – MEASUREMENT AND ANALYSIS

Aim : (i)To measure the blood pressure and (ii) to find it's relation with sex, age, height, weight, food habits, play habits (out door games or any exercise other than regular work) and socio-economic status (profession and annual income of parents).

Objectives :

On completion this experiment students will be able to,

Knowledge:

- Define blood pressure and pulse pressure

Understanding:

- Differentiate systolic and diastolic pressure.
- Describe the sphygmomanometer and stethoscope.
- Explain the method to measure the blood pressure
- Explain the significance of the study

Application :

- Interpret the recordings (data) in terms of:
  a) comparing the amount of BP
  - between boys and girls.
  - between Vegetarian and Non-vegetarian
  - between players and non-players
  b) Correlate the BP through illustration (like – table, graph, diagram)
  - with height
  - with weight
  - with socio-economic status.
- Suggest simple projects (at UG level).

Skill :

- Measure BP correctly.
- Operate the instrument carefully.
- Draw scientific diagram of sphygmomanometer & stethoscope and label the parts.

Synthesis :

- Synthesize the facts and give possible explanations for the observation.

Attitude :

- Objective in approach
- Honest in collecting and recording scientific data
- Observe cleanliness in activity
- Maintain time (punctual)

Blood Pressure :

Arterial blood pressure is the force of pressure, which the blood is exerting against the walls of the blood vessels in which it is contained. The blood pressure in the arteries varies during the **cardiac cycle.** During ventricular systole, when the left ventricle is forcing blood into the aorta the pressure rises to a peak, referred to as **systolic pressure.** During diastole the pressure falls, the lowest value it reaches is referred to as **diastolic pressure.**

The systolic blood pressure is produced by the heart muscles, which drive the contents of the ventricle into the already stretched arteries. During diastole the arteries are kept partly distended because the peripheral resistance of the arterioles prevents all the blood running off into the issues. Thus, the blood pressure depends partly on the force and volume of blood pumped by the heart and partly on the contraction of the muscles in the walls of the arterioles. This contraction is maintained by vasoconstrictor nerves, which are controlled by the vasomotor centre in the medulla oblongata of the brain.

The vasomotor centre adjusts the peripheral resistance to maintain the blood pressure relatively constant. It, however, changes slightly in physiological variations of exertions as during exercise, with mental changes of anxiety and emotion, in sleep and when eating. For this reason, it is always suitable to measure blood pressure when a person is relaxed and resting. The measurement of blood pressure is very significant in correctly estimating the state of blood circulation and thus the working of heart.

(I) MEASURING THE BLOOD PRESSURE

Blood pressure is measured by a instrument called **sphygmomanometer** and with the use of stethoscope.

Description of sphygmomanometer :

It consists of a long glass manometer capillary tube. The upper end of this tube is open when its lower end is slightly bent. The manometer is mounted in the middle of a **dark scale** having markings on its both sides. The lower bend end of manometer is connected to the ventral side of a large **bulb filled with mercury.** The upper open end of the manometer is exposed to atmospheric pressure but is guarded by a valve to check the spilling of mercury from the open end. The upper end of the bulb is connected with a **nozzle** and the nozzle is connected with a rubber tube having narrow lumen. The free end of rubber tube is connected to a flexible rubber bag enclosed in a **cuff.** The cuff is further connected to a **rubber pumping bulb** with a rubber tube. The neck of the bulb is provided with an **adjusting screw** ; the tightening or untightening of this screw ensures the flow of air into the cuff (inflation) or out of the cuff (deflation).

Method of using sphygmomanometer :

Open the adjusting screw at the proximal end of the pumping bulb so that air is removed from the cuff and tubes. Then wrap the cuff around the upper arm just above the elbow joint and fix it with the help of attached hook in a manner that neither it is too light nor too loose. While wrapping the cuff care should be taken that the tubes attached to it must face toward the lower end. Then tighten the adjusting screw and pump the air by pumping rubber bulb rapidly so that mercury is raised upto 110-120 mm Hg mark in the manometer. Observe the sound of arterial blood in brachial artery with the help of a stethoscope. Then again pump the air slowly and keep on observing the sound of arterial blood in the brachial artery with stethoscope to disappear. As and when sound is not felt, note the mercury level in the manometer and mark the reading. This is the **diastolic pressure.** Then start loosing the adjusting screw slowly and keep on observing carefully the return of normal arterial sounds through the stethoscope. As and when it is felt, close the adjusting screw and mark the reading of mercury in manometer. Pump a little more air

and again note the proper arterial sound to reappear. This would be the **systolic pressure.** Now, loose the screw, remove all air and open the cuff and keep the apparatus in a safe place.The difference in pressure between systole and diastole is called the **pulse pressure** which is normally 30-50 mm Hg. The lower limit of systolic pressure in a normal adult is estimated about 105 mm Hg and the upper limit about 150 mmHg.

Normal Blood Pressure Range (in mm Hg)

Period	Diastolic	Systolic
In infants	50	70 – 90
In childhood	60	80 – 100
During adolescent stage	60	90 – 110
In young adult	60 – 70	110 – 125
As age advances it is increased	80 – 90	130 - 150

(II) ANALYSIS AND INTERPRETATION

(Finding the relation between Hb content and various parameters)

- Record the observed BP of 20 or 40 students in observation table.

Observation Table

Sl. No.	Name	Sex	Age	Height (cm)	Weight (kg)	Food habit V/NV	Play habit Pl/Npl	so.ec.status				BP	
								F		M		D	S
								P	I	P	I		

(F-Father, M- Mother, P-Profession, I-Income, Pl- player, Npl- Non-player, D- Diastole, S- Systole.)

Analyze your recording on following directions:

Find out the mean difference:

- Is there any difference in 'Mean BP' between male and female ?
- Is there any difference in 'Mean BP' between vegetarian and non-vegetarian ?

Find out the correlation:

- When the age increases, what happens to BP ?
- When the weight increases, what happens to BP ?
- When the height increases, what happens to BP ?
- When the socio-economic status increases, what happens to BP ?

Statistical techniques :

To answer the above questions the following simple statistical techniques may be adopted :

*** The mean difference between boys and girls:**

Number of Boys (NB) = ——— Total BP of boys = ——— i) Systole : ——— ii) Diastole : ——— Average BPof Boys : $= \frac{Total\ BP\ (B)}{NB}$ Mean BP of boys = ...	No. of Girls (NG) = ——— Total BP of girls = ——— i) Systole : ——— ii) Diastole : ——— Average BP of girls : $= \frac{Total\ BP(G)}{NG}$ Mean BP of girls = ...

- Repeat the same way of analysis for
  - vegetarian and non-vegetarian.
  - Players and non-players

- Find out the correlation (illustrative, like – table, graph, diagram) between
  - height vs BP
  - weight vs BP
  - age vs BP and
  - socio-economic status vs BP

To find the correlation by tabulation method, write the values of height / weight / age / soc. ec.status in ascending order in first column. Then write the corresponding values of BP in the second column and analyze.

To find the correlation by graphical method, plot the values of height / weight / age / soc. ec.status in X axis and corresponding values of BP on Y axis. Construct and interpret the graph.

Discussion / Interpretation:

→
→
→

Significance of the Study:

→
→

Project:

Suggest a possible, fessible, objective and rationale project based on above study (at UG level) and write the brief outline of procedure.

*****

Ex.no :
Date :

ESTIMATION & COMPARISON OF HAEMOGLOBIN CONTENT

Aim : (i)To estimate the haemoglobin content of human blood and (ii) to find it's relation with sex, age, height, weight, food habits, play habits (out door games or any exercise other than regular work), socio-economic status and (profession and annual income of parents) the amount of RBC.

Objectives:

On completion of this experiment, students will be able to :

Knowledge :

- Recall the structure and position of of Hb

Understanding :

- State the functions of Hb
- Describe the Haemoglobinometer
- Explain the principle involved in estimation o Hb
- Describe the procedure to estimate Hb
- Give reason for using of 0.1n Hcl fluid
- Explain the significance of the study

Application :

- Interpret the recordings (data) in terms of:
  a) comparing the amount of Hb
  - between boys and girls,
  - between Vegetarian and Non-vegitarian
  - between players and non-players
  b) Correlate the amount Hb through illustration (like – table, graph, diagram)
  - with height
  - with weight
  - with socio-economic status
  - with number of RBCs
- Suggests simple projects.

Skill :

- Collect the blood accurately
- Stir the acid-haematin mixture carefully
- Read the HB content correctly.
- Handle the instrument safely
- Draw scientific diagram and label them

Synthesis :

- Synthesize the facts and give possible explanations for the observation.

Attitude :

- Objective in approach
- Honest in collecting and recording scientific data
- Observe cleanliness in activity
- Maintain time (punctual).

I) ESTIMATION OF Hb

Apparatus required : Sahli's haemoglobinometer (pipette with a 20 cu.mm mark, prickling needle, dropper, stirrer,comparator).
Reagents required : 100% Alcohol, distilled water, 0.1N HCl (1ml con Hcl +99 ml DW).

Principle :

Haemoglobin is a complex substance that gives red color to RBC. It is an iron containing protein made of large protein molecule called globin and four-ring like structure. Each contains one atom of iron heam. It helps in carrying O2 to the tissues and CO_2 away from the tissues. Hence the oxygen carrying capacity of blood depends on the amount of haemoglobin it.

Haemoglobin is converted to acid haematin by the addition of N/10 or 0.1 HCl and the resulting brown color is compared to the standard brown glass in haemoglobinometer. The intensity of the brown color depends on the acid haematin which in turn depends on the amount of haemoglobin in the blood sample.

The Sahli's haemcglobinometer consists of a standard brown glass on a comparator and a graduated tube. A special pipette is used to measure 20 cu mm of blood.

Procedure :

- Place N/10 or 0.1N HCl in the graduated tube upto the 2% mark.
- Draw blood upto20 cu mm mark in the pipette and transfer it to the acid in the tube and mix the acid and blood using glass stirrer provided.
- Allow it to stand for about 10 minutes to develop brown color. Complete color will be developed only after 10 minutes so it is very essential to wait.
- Then dilute the solution by using distilled water by adding two drops at a time until the color matches with the glass plates in the comparator.
- When once it matches stop adding distilled water.
- Then take out the tube and record the level of solution (amount of Hb) in the tube.
- Repeat the procedure 3 times and calculate the average concentration of Hb.
- Report the concentration of haemoglobin in terms of gms/100 ml of blood.

Precaution :

- Observe the color with the help of a constant (natural) light source.
- Concentration of acid should be proper (0.1 N HCl).
- Add distilled water two drops at a time (drop by drop toward the end), so that it does not exceed the match point.
- Stir the fluid after adding water drops.
- Don't rest the stirrer on table or other places during adding DW and hold it in left hand.

II) ANALYSIS AND INTERPRETATION

(Finding the relation between Hb content and various parameters)

- Record the observed haemoglobin content of 20 or 40 students in observation table.

Observation Table

Sl.no	name	sex	age	height (cm)	weight (kg)	food habit V/NV	play habit Pl/Npl	so.ec.status				amount of RBC	Hb content (gm)
								F		M			
								P	I	P	I		

(F-Father, M- Mother, P-Profession, I-Income,Pl- player, Npl- Non-player)

Analyze your recording on following directions:

Find out the mean difference:

- Is there any difference in 'Mean of Hb content' between male and female ?
- Is there any difference in 'Mean Hb content ' between vegetarian and non-vegetarian ?

Find out the correlation:

- When the age increases, what happens to Hb content ?
- When the weight increases, what happens to Hb content ?
- When the height increases, what happens to Hb content ?
- When the socio-economic status increases, what happens to Hb content ?
- When the number of RBC increases, what happens to Hb content ?

Statistical techniques :

To answer the above questions the following simple statistical techniques may be adopted :

- **The mean difference between boys and girls:**

Number of Boys (NB) = ———	No. of Girls (NG) = ———
Total Hb of boys = ———	Total BP of girls = ———
Average HB of Boys :	Average Hb of girls :
$= \frac{Total\ Hb\ (B)}{NB}$	$= \frac{Total\ Hb(G)}{NG}$
Mean Hb of boys = ...	Mean Hb of girls = ...

- Repeat the same way of analysis for
  - vegetarian and non-vegetarian.
  - Players and non-players

- Find out the correlation (illustrative, like – table, graph, diagram) between
  - height vs ofHb content
  - weight vs ofHb content
  - age vs ofHb content
  - socio-economic status vs ofHb content and
  - number of RBC vs ofHb content.

  To find the correlation by tabulation method, write the values of height / weight / age / soc. ec.status / number of RBC in ascending order in first column. Then write the corresponding values of Hb content in the second column and analize.

  To find the correlation by graphical method, plot the values of height / weight / age / soc. ec.status / number of RBC in X axis and corresponding values of Hb on Y axis. Construct and interpret the graph.

Discussion / Interpretation:

→

Significance of the Study:

→

Project:

Suggest a possible, fessible, objective and rationale project based on above study (at UG level) and write the brief outline of procedure.

*****

AIM: To study the stages of mitotic cell division using onion root-tips.

MATERIALS REQUIRED: Onions, 50 ml beakers-3 to 4 nos, spirit lamp, Carnoy's fixative, 70% alcohol, 1N HCl, 2% aceto-carmine stain, micro-slide, cover glass, blotting paper, match box, microscope, needles, blade, paraffin wax, n-butanol, DPX mountant.

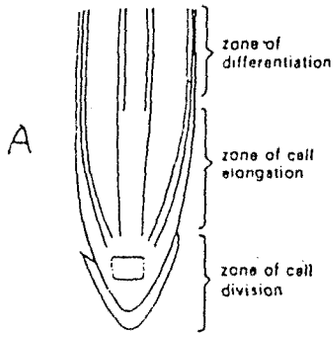
THEORETICAL BACKGROUND

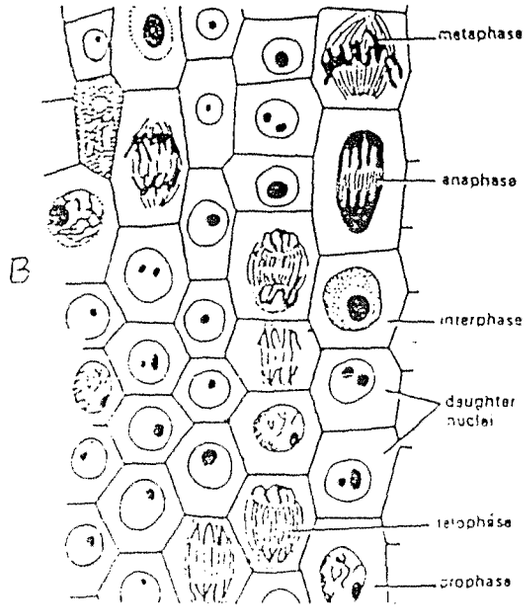
Living cells of eukaryotes undergo 2 kinds of cell divisions, mitosis and meiosis. Mitosis generally takes place in the somatic cells. Actively dividing meristems such as root tips and shoot-tips are suitable materials for studying mitosis. Mitotic cell division has different stages; prophase, metaphase, anaphase and telophase. At the end of mitosis, 2 daughter cells are formed, each having the same chromosome number as that of the parent cell.

PROCEDURE

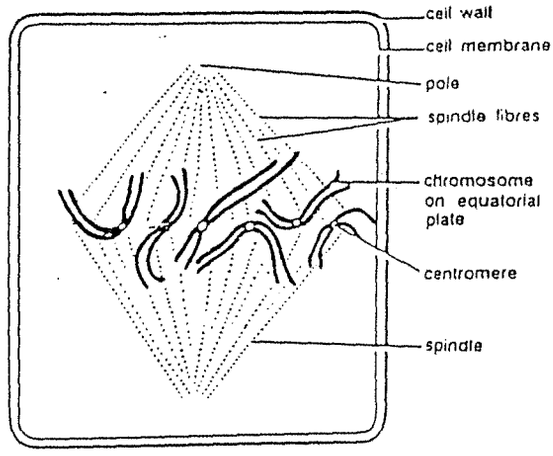
1. Fill 3-4 50ml beakers with water and place an onion bulb in each, in such a way that the root forming portion of onion bulb dips in water.
2. Root initiation occurs after a day. After 2-3 days cut the distal 1 cm tips of all the roots with a blade.
3. Place the root-tips in Carnoy's fixative for 24-48 hours.
4. Wash the root-tips with water and transfer them to 70% alcohol.

5. Select 5-6 root tips and transfer them to 1N HCl taken in a watch glass. Gently warm the watch glass on a flame for 2-3 minutes.
6. Transfer 2-3 root-tips on to a micro-slide and cut their extreme 2-3 mm tips. Retain these tips on the slide and discard rest of the root
7. Put 3-4 drops of acetocarmine stain and crush the root tips with the tip of a needle.
8. Gently warm the slide on a flame for 2-3 minutes, add a few more drops of stain.
9. Put a cover glass on the material. Wrap the slide in a blotting paper and gently tap the blotter and thereby the cover glass with the blunt end of a pencil.
10. Wipe the sides of cover glass and observe carefully Examine each cell for stages of cell division.

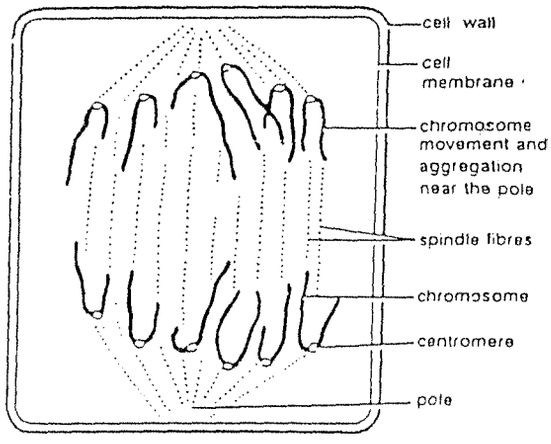




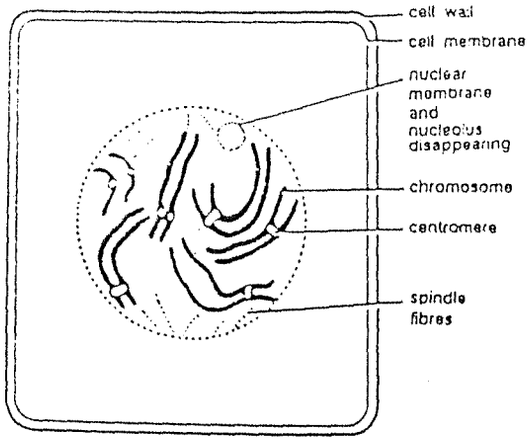
A - ROOT TIP OF ONION.
B - A FEW CELLS OF ROOT TIP SHOWING STAGES OF MITOSIS.



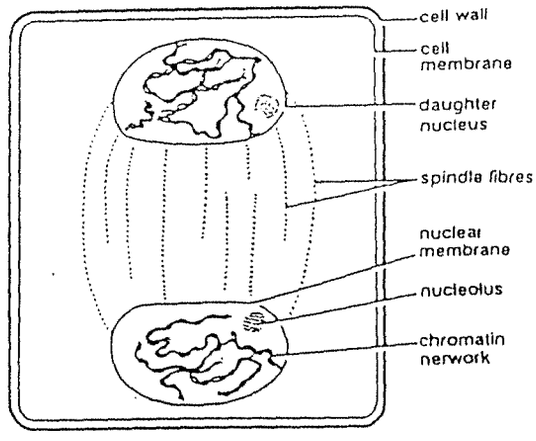
METAPHASE



ANAPHASE



PROPHASE



TELOPHASE

A. Plastidal Pigment

i) **Extraction of the chloroplast pigments :** Take about 50 g of leaves and spread them in an oven at 50°C and allow them to dry. Grind them in a Waring blender or simply chop them into small pieces.

Place the leaf powder or chopped leaves in a small conical flask containing 95% ethyl alcohol (or 80% acetone) and a pinch of $CaCO_3$. After a considerable period, the alcohol (or acetone0 will dissolve out the chlorophyll and a deep green solution results. The extraction can also be made by heating the flask in a water-bath after plugging the mouth of the flask with cotton. Filter the solution on a Buchner flask. The filtrate is an alcohol or acetone extract of chlorophyll - a, chlorophyll-b, carotene and xanthophylls as well as other alcohol or acetone soluble compounds in the leaf tissues.

ii) Separation of the green and yellow pigments – Shake gently about 20ml of the acetone extract [obtained in experiment (i)] with 40 ml of the either in a separating funnel. Then pour 60 ml of distilled water down the side of the separating funnel. The mixture separates into two layers. Now draw off the lower acetone-water layer and discard by opening the stop clock of the separating funnel. The pigments are all now in the ether layer. Wash this solution again with water, draw off and discard the lower layer again.

Take about 10 ml of this ether solution of pigments in a small flask and add 5 ml of 30% methyl alcoholic potassium hydroxide solution and shake. Allow it to stand, when at first, the disappearance of the green color and shortly its reappearance will be observed. Add now 20ml of distilled water and 5ml o more ether. On shaking the flask, the two layers will be separated out of which the lower aqueous alkaline layer contains green pigments and the upper ethereal layer contains yellow carotenoid pigments.

iii) Complete separation of four chloroplast pigments

a) Separation of chlorophyll –a and –b : In a separating funnel add 15 ml of petroleum ether and 10 ml of acetone extract of leaf pigments [obtained in expt. (i)]. Shake these two liquid gently by rotating the separating funnel. Add now excess of distilled water (about 20ml) gently down the side of the funnel and shake it again. Two layers are formed of which the upper layer is deep green. Draw off and discard the lower acetone-layer layer. Repeat this washing with water again and discard the lower layer.

Now add 10 ml of 92% methyl alcohol in this petroleum-ether solution. Shake the funnel and allow it to stand, after which two years will be observed. The upper petroleum-ether layer contains chlorophyll-a (together with carotene) and the lower methyl alcohol layer contains chlorophyll-b (together with xanthophylls).

Draw off the two layers into two separate flasks and save them for further work.

b) Separation of two yellow pigments : Carotene and Xanthophyll – Take 10 ml of methyl-alcohol solution in a separating funnel and to it add 10 ml of ethyl ether. Shake the mixture and pour small portion of distilled water (about 5 ml) down the side of the funnel, two layers will be observed of which discard lower methyl-alcohol layer. The pigments should be in the upper ether layer. Repeat the process for about 5 times to wash off the mixtures and similarly discard the lower methyl-alcohol layer.

Now take two 25ml flasks. To one add 10 ml of petroleum-ether solution [obtained from experiment (iii)[a] and to other add 10ml of ether solution. About 5 ml of freshly prepared 30% methyl alcoholic potassium hydroxide solution was poured down the walls of each flask. Then add about 10ml of distilled water, shake each flask and allow them to form two layers.

Of the two layers in the petroleum-ether flask, upper petroleum-ether solution contains chlorophyll-a and the lower methyl alcoholic layer contains carotene. In the other flask, the upper ethereal layer contains xanthophylls and the lower aqueous alkaline layer contains chlorophyll-b.

(iv) Separation of leaf pigments by filter paper chromatography – Take some fresh leaves in a small beaker. Add sufficient boiling water to immerse the leaves. Clean all dirts from the leaves and decant off the water. now grind the material thoroughly with about 1g quartz sand and 10-15 ml of 50; 1 petroleum ether : methyl alcohol solution. Decant approximately 1ml of the pigment extract into a hard glass test tube.

Cut a strip of filter paper with its width slightly less than the inside diameter of the large test tube and its length should be such that it can hang freely as shown in the figure. Cut notches in one end of the strip paper. Now pierce a paper clip into both the sides of the cork.

Now using a fine pointed pipette, place a drop of the above extract between the notches and allow it to dry. Four such drops of extract were added on the same point and dried. Pour some solvent (8% acetone, 92% petroleum ether) into the test tube, so that the level of the solvent lies behind the notches of the filter paper (below the spot). Hang the paper through the chip of the cork and place the tube in an upright position. When the upper edge of the solvent reaches near the clip, remove the cork and hold the filter paper until the solvent has dried. It will be observed that the yellow zones (chiefly carotene) go well in advance of the green zone and the other pigments (chlorophyll-a, -b and xanthophylls) are adsorbed near the point of contact where the upper bluish –green zone (chlorophyll –b) can b distinguishable. Xanthophyll can be 'visible' as yellow band above the bluish-green zone of chlorophyll –a.

REGIONAL INSTITUTE OF EDUCATION, MYSORE.

Training of key Resource Persons in the Transaction of the NewBiology Textbooks of Kerala at +2 Level
(13th September 2004 to 17th September 2004)

Day & Date	9 to 11.15 am		11.30 to 1 pm		2 to 3.30 pm		3.45 to 5.15
Monday 13.9.04	Registration & Inauguration	T	Circulatory system, Blood – SPK	L	Haematology hands on Experiences (SPK)	T	Conduction of nerve impulse Dr.S.P.Kulkarni
Tuesday 14.9.04	Human Genome Project Dr. Ramachandra	E	Chromosomes Dr.L.Srinkantappa	U	Taxonomy Dr.G.V.Gopal	E	Ecology Prof A L N Sarma Dr.A. Sukumar
Wednesday 15.9.2004	Reproduction in plants Dr. Geetha G. Nair	A	Recombinant DNA Prof S.R.Ramesh	N C H	DNA finger printing Dr.Ramachandra	A	Modern views of mitosis) Dr.Geetha.G.Nair
Thursday 16.9.04	Tissue culture Dr.G.V.Gopal		Immunology Dr.A.Sukumar		Visit To Bio-Technology Dept. of University of Mysore Dr. S.P.Kulkarni		
riday 17-9-2004	Electron Transport system Dr.G.V.Gopal		Use of Computer Softwares in teaching Biology Dr.A.Sukumar		Tissue Culture Practice (Rachappaji and Niranjan		Valedictory Function TA/DA disbursement

Lecture sessions in Bio Methods Lab.

Programme Coordinator

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