Animal physiology is to understand how a process operates within an organism, experiments must be designed to allow the measurement of metabolic rate, blood flow, urine production, muscle contraction) in the animal (or its cells or tissues) while it is in a known state such as resting, exercising, digesting, or sleeping. This kind of experimentation is particularly challenging and requires the use of a variety of techniques and methods. Many of the experimental techniques and measuring devices common in animal physiology are "time-honored By knowing why and how experiments in animal physiology are performed-whether they employ traditional or emerging methods.

MOLECULAR TECHNIQUES- The variety of molecular techniques available have had major implications for biological research in general, and animal physiology has certainly benefited from molecular approaches.

1. **Tracing Molecules with Radioisotopes-** Greater understanding of physiological processes can often be achieved by knowing the movements of molecules within and between cells. For example, we can more easily understand the role of a particular neurohormone in regulating physiological processes if its movements can be traced from its site of synthesis to its site of release and on to its site of action.

   Isotopes have same no. of e^- bur proton and neutrons no. are different

   C= C^7, C^8

   H= H^2, H^3

   O= O^{17} and O^{18}

   N= N15

   $6CO_2 + 6H_2O = C_6H_{12}O_6 + O_2$

   $6CO_2^{17} + 6H_2O^{18} = C_6H_{12}O_6 + O_2^{18}$

Many types of experiments that follow the movement of physiologically important molecules employ radioisotopes, the relatively unstable, disintegrating radioactive isotopes of the chemical elements. The natural disintegration of radioisotopes is accompanied by release of high-energy particles.

.Although radioisotopes occur naturally, those normally used in experimental studies are produced in nuclear reactors. The most commonly used isotopes in biological research are $_{32}P$, $_{125}I$, $_{35}S$, $_{14}C$, $_{45}Ca$, and $_{3}H$. A radioisotope of an element normally present in the molecule of interest can be incorporated ***in*** vitro or in vivo either directly into the molecule or into a precursor molecule that will eventually be converted into the molecule of interest. The resulting radiolabeled molecule has the same chemical and biochemical properties as the unlabeled molecule. An amazing array of so-called radiolabeled biologically active molecules (e.g.,amino acids, sugars, hormones, proteins) are now readily available (at a substantial price) from companies that specialize in their production.

Once a molecule has been radiolabeled, the particles emitted from the radioisotope can be used to detect the presence of the molecule, even at very low concentrations.

In one type of tracing experiment, the radiolabeled molecule of interest or its precursor is administered to an animal, isolated organ, or cells growing in vitro culture, and then samples are removed periodically for measurement of particle emission.

Two types of instruments are used to detect emitted particles

2. **Tracing molecules with monoclonal antibodies-** Examination of biological structure in fixed tissue slice on a microscope slide can be doubtful without staining their, cytoplasm, cell membrane and nucleus etc. Antibody staining generally involved covalently linking a fluorescent dye that recognize a specific determinant on an antigen molecules identical antibodies produced in response to an antigen are called monoclonal antibodies.

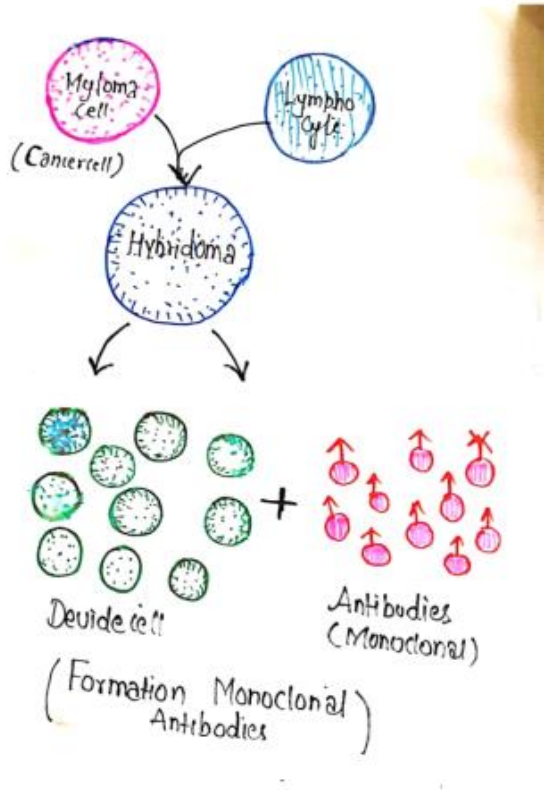
   Production of monoclonal antibodies – Antibodies are produced by immune system against the antigen. Readymade antibodies in some case our immune system antibodies can not produced in that case readymade antibodies are used

a. Orthodox method- BY using animals Snake Venom

b. Hybridoma technique- Mylemo cells or cancer cells are used due to following region

I. Divide rapidly

II. Can divide outside the body



A. Specific protein can detect with the help of hybridome antibodies- Once antibodies that recognized discrete site on s molecule on interest have been produced and linked to a fluorescent dye. It can injected in cell or tissues under the study by using immunoflourescent microscope.

B. Now radiolebelled monoclonal antibodies are also used to identifying of any antigen antibodies complex and detected by auto radiography. This technology has been used to localized some hormone like epinephrine or norepinephrine.

C. Some uses of monocular antibodies (mAbs)

a. **Diagnostic Testing**- Once mAbs are produced for a specific substance, they can be then used to test for the presence of that substance in a vessel. This can include toxins, drugs or hormones.
b. **Pregnancy Testing-** MAbs that have been developed to detect human chorionic gonadotropin (HCG) are now present in pregnancy test kits.

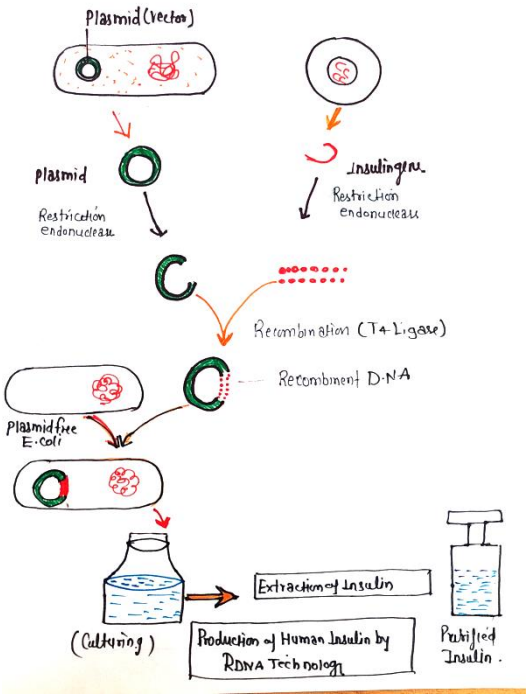
c. **Radioimmunodetection (RID) of Cancer-** An imaging technique used to detect the presence of cancerous or cancer-specific cells has been developed deploying radio-labelled antibodies, which can be produced as mAbs.
d. **Radioimmunotheraphy (RIT) of Cancer-** Similar to RID, RIT uses mAbs to specifically target antigen cells that are associated with tumours, and then blast these with a lethal dose of radiation, whilst minimising the level of radiation absorbed by normal cells.
e. **Treatment of Cancer through Drugs -**Many different drugs are being developed in clinical trials with the ultimate hope of being able to treat various strains of cancer. In fact, some of these are already on the market. In 1997, a drug named Ritoxin was approved by the FDA for commercial use which is based on mAb technology.
f. **Viral Disease Treatment-** Doctors hope that with further research into mAbs and an increased knowledge of their properties, treatments will become available for diseases previously thought to be incurable, such as AIDS.
g. **Identifying Pathogens-** MAbs can now be used to identify strains of a single pathogen, for example *neisseria gonorrhoeae.*
h. **Tracing Specific Cells and their Functions**- Scientists can use mAbs to first identify and then track certain cells or molecules in a living thing, and determine its function. For example, scientists at the University of Oregon are using such practices to determine which proteins are responsible for differentiation amongst cells in the respiratory system.
i. **Organ Rejection-** A certain mAb named OKT3 (developed as an antibody to the T3 antigen) is able to be used to alleviate the effects and likelihood of organ rejection when transplanting new organs into a subject.
j. **Rhesus disease Immunisation-** Anti-rhesus antiserum is becoming increasingly hard to find, and the UK Blood Products laboratory has been researching the possibility of substituting mAb rhesus immunisation, with a view to ultimately replacing the serum

3. **Genetic engineering**- It encompasses various technique for manipulating the gentic material of an organism.This approach is increasingly used in both agriculture and medidicine. Genetic engineering is being with identical structural gene that code for specific protein with in theDNA isolated from an organism

For example Insulin hormone- Gene has uncode and isolated from human DNA. This gene now inserted into clone vector or in bacteria and that bacteria replicate and multiply anf insulin gene also multiply.

A. **Producing Recombinant Insulin**

1. First, scientists synthesized genes for the insulin.
2. They were then inserted into plasmids along with a strong lacZ promoter.
3. So recombinant DNA forms
4. The recombinant DNA or plamid transfer in to plasmid free bacteria or E. coli.
5. Recombinat DNA bacteria cuture
6. The insulin extracted from culture medium
7. Now purified insulin under laboratory conditions.

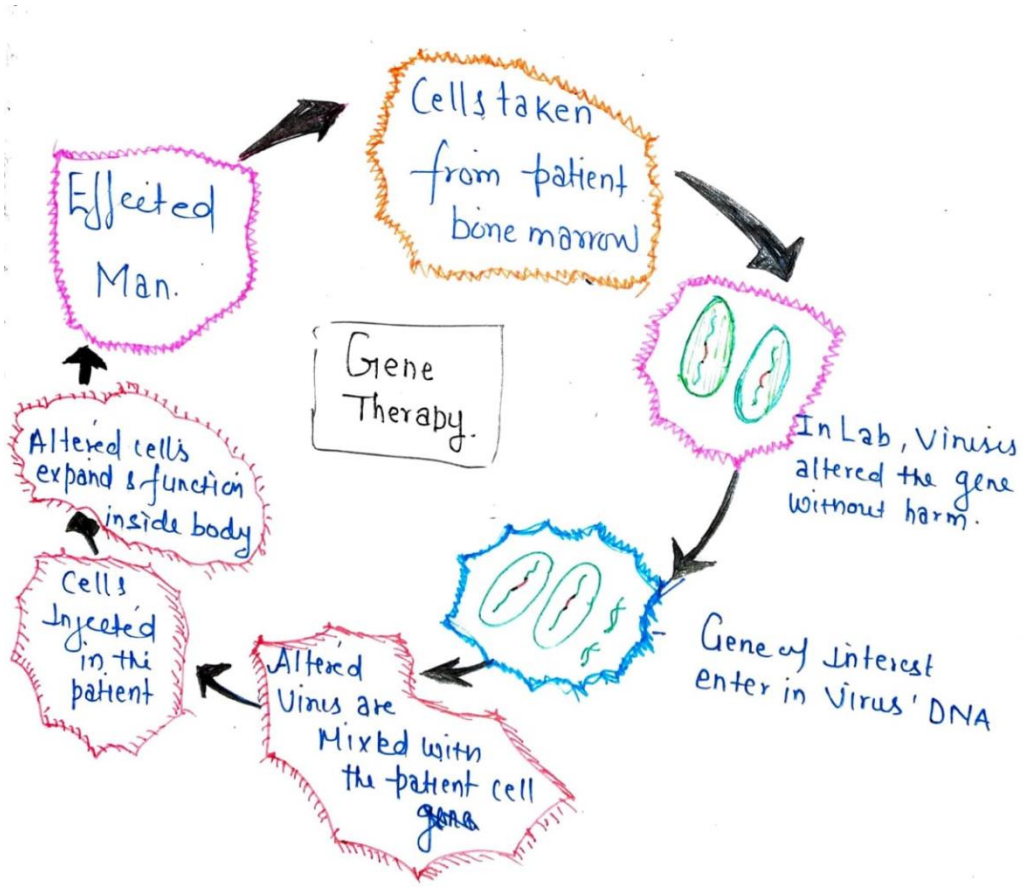


Modern pharmaceutical manufacturing frequently relies upon recombinant drugs.

1. Human Insulin
2. Human Growth Hormone
3. Human blood clotting factors
4. Vaccines
5. Monoclonal Antibodies

6. Interferons
7. Antibiotics & other secondary metabolite.

Gene therapy- Gene therapy replaces a faulty gene or adds a new gene in an attempt to cure disease or improve your body's ability to fight disease. Gene therapy holds promise for treating a wide range of diseases, such as cancer, cystic fibrosis, heart disease, diabetes, hemophilia and AIDS.



Process of gene therapy-

1. Gene therapy is a technique which involves the replacement of defective genes with healthy ones in order to treat genetic disorders.
2. It is an artificial method that introduces DNA into the cells of the human body.
3. The first gene therapy was successfully accomplished in the year 1989. The simple process of gene therapy is shown in the figure below:
4. The the defected gene is replace by retro virus (A retrovirus is a virus that uses RNA as its genetic material. When a retrovirus infects a cell, it makes a DNA copy of its genome that is inserted into the DNA of the host cell. There are a variety of different retroviruses that cause human diseases such as some forms of cancer and AIDS).

5. The virus alters the defect gene
6. Normal cells injected in to patient .(This process is repeated many times)
7. The normal cells forms normal tissue and start normal function.
8. This technique is employed mainly to fight against the diseases in the human body and also to treat genetic disorders..

Types of gene Therapy- Basically, there are two types of gene therapy.

A. **Somatic Gene Therapy-** This type usually occurs in the somatic cells of human body. This is related to a single person and the only person who has the damaged cells will be replaced with healthy cells. In this method, therapeutic genes are transferred into the somatic cells or the stem cells of the human body. This technique is considered as the best and safest method of gene therapy.

B. **Germline gene Therapy-**It occurs in the germline cells of the human body. Generally, this method is adopted to treat the genetic, disease causing-variations of genes which are passed from the parents to their children. The process involves introducing a healthy DNA into the cells responsible for producing reproductive cells, eggs or sperms. Germline gene therapy is not legal in many places as the risks outweigh the rewards.

Application of Gene Therapy

1. It is used in the replacement of genes that cause medical ill-health
2. The method generally destroys the problem causing genes
3. It helps the body to fight against diseases by adding genes to the human body
4. This method is employed to treat diseases such as cancer, ADA deficiency, cystic fibrosis, etc.

Uses of gene therapy

1. Clinical gene transfer application
2. Vaccine development.
3. Production animals
4. Treatment of cancer and AIDS
5. Gene discovery
6. Enhancing resistant in plants and animals
7. Genetically modified organism

4. **Mutations** in the genes can be induced or could arise spontaneously. Induced mutations are generated when an entity is exposed to a mutagen or an agent causing mutations. These mutations usually tend to have higher frequencies than spontaneous mutations. Spontaneous

mutations are the naturally occurring mutations which appear in all cells. Comprehension of the gene mutation mechanism requires DNA analysis and also the analysis of protein molecules.

A. Gene Mutation- A gene mutation can be described as a change or alteration in the sequence of nucleotides in a DNA polymer which is a series of nucleotides joined together. It can be altered in many ways and can have effects on the health of an individual. It can cause genetic disorders depending upon the site at which they occur and whether they can alter functions of essential proteins. While some other mutations could be beneficial, making individuals better adapt to their surroundings.

B. Types of Mutations

a. Substitution Mutation- It is a mutation that switches a single chemical letter or base for another. It places the wrong nucleotide in the wrong position. It could result in a silent mutation. In such a mutation, a codon is altered to another that encodes the corresponding amino acid resulting in no change in the protein that is generated or it could alter an amino-acid coding codon to a single-halt codon resulting in the formation of an incomplete protein which could be non-functional. Also, it could cause an alteration in a codon to one that encodes another amino acid causing a small change in the protein synthesized.

b. **Insertion**- It causes an alteration in the number of DNA bases in a gene by inserting a piece of DNA. Consequently, the protein produced may not be functional.

c. **Deletion**- A change in the number of bases of DNA is caused due to the deletion of a piece of DNA. Any number of nucleotides can be deleted or removed. Small deletions may cause the dismissal of a few base pairs within a gene, while larger deletions can discard a whole gene or neighbouring genes producing an alteration in the functions of resulting proteins.

d. Frameshift Mutation- It occurs when a deletion or addition of DNA bases causes an alteration in the reading frame of a gene. There are three groups of base pairs wherein each code for one amino acid in a reading frame. This type of mutation causes a shift in the organization of these bases and modifies the code for amino acids**.**

Transgenic Animals- Transgenic animals are the animals with the modified genome. A foreign gene is inserted into the genome of the animal to alter its DNA. This method is done to improve the genetic traits of the target animal. The transgenic animals are genetically engineered and are

also known as genetically modified organisms. The first genetically modified organism was engineered in the year 1980. Let us have a detailed look at the process, importance and applications of transgenic animals.

Methods for Creating Transgenic Animals- The transgenic animals are created by the following methods:

1. **Physical Transfection-** In this method, the gene of interest is directly injected into the pronucleus of a fertilized ovum. It is the very first method that proved to be effective in mammals. This method was applicable to a wide variety of species. Other methods of physical transfection include particle bombardment, ultrasound and electroporation.
2. **Chemical Transfection-** One of the chemical methods of gene transfection includes transformation. In this method, the target DNA is taken up in the presence of calcium phosphate. The DNA and calcium phosphate co-precipitates, which facilitates DNA uptake. The mammalian cells possess the ability to take up foreign DNA from the culture medium.
3. **Retrovirus-Mediated Gene Transfer-** To increase the chances of expression, the gene is transferred by means of a vector. Since retroviruses have the ability to infect the host cell, they are used as vectors to transfect the gene of interest into the target genome.
4. **Viral Vectors-** Viruses are used to transfect rDNA into the animal cell. The viruses possess the ability to infect the host cell, express well and replicate efficiently.
5. **Bactofection-** It is the process by which the gene of interest is transferred into the target gene with the help of bacteria.

Examples of Transgenic Animals- Following are the examples of transgenic animals:

1. **Dolly Sheep-** Dolly the sheep was the first mammal to be cloned from an adult cell. In this, the udder cells from a 6-year-old Finn Dorset white sheep were injected into an unfertilized egg from a Scottish Blackface ewe, which had its nucleus removed. The cell was made to fuse by electrical pulses. After the fusion of the nucleus of the cell with the egg, the resultant embryo was cultured for six to seven days. It was then implanted into another Scottish Blackface ewe which gave birth to the transgenic sheep, Dolly.
2. **Transgenic Mice-** Transgenic mice are developed by injecting DNA into the oocytes or 1-2 celled embryos taken from female mice. After injecting the DNA, the embryo is implanted into the uterus of receptive females.

Applications of Transgenic Animals- The transgenic animals are created because of the benefits they provide to the man. Let us discuss a few of them here.

1. **Normal Physiology and Development-** In transgenic animals, a foreign gene is introduced due to which the growth factor is altered. Hence, these animals facilitate the study of gene regulation and their effect on the everyday functions of the body.
2. **Study of Diseases**- Transgenic animals are specially designed to study the role of genes in the development of certain diseases. Moreover, in order to devise a cure for these diseases, the transgenic animals are used as model organisms. These transgenic models are used in research for the development of medicines. For example, we have transgenic models for diseases such as Alzheimer's and cancer.
3. **Biological Products-** A number of biological products such as medicines and nutritional supplements are obtained from transgenic animals. Research for the manufacture of medicines to treat diseases such as phenylketonuria (PKU) and hereditary emphysema is going on. The first transgenic cow, Rosie (1997), produced milk containing human protein (2.4 grams per litre). This milk contains the human gene alpha-lactalbumin and could be given to babies as an alternative to natural cow milk.
4. **Vaccine Safety-** Transgenic animals are used as model organisms for testing the safety of vaccines before they are injected into humans. This was conventionally done on monkeys.