SHAMBHUNATH INSTITUTE OF PHARMACY

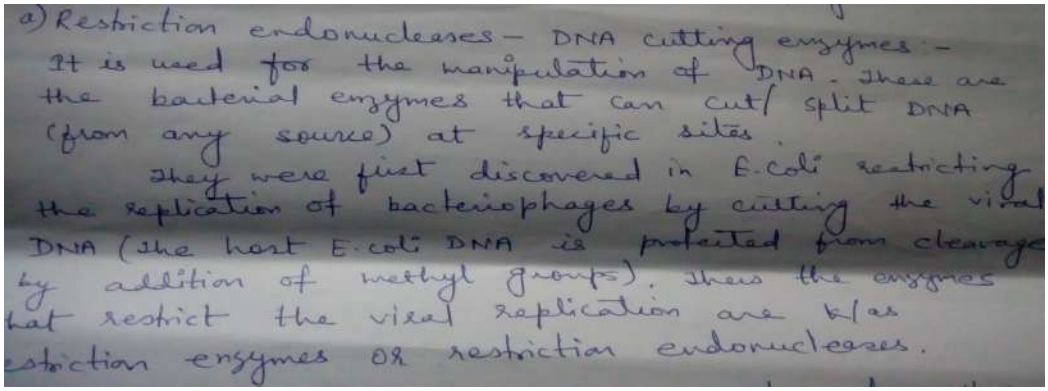
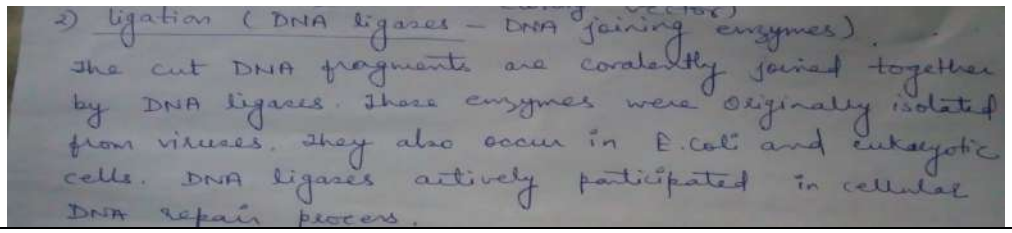
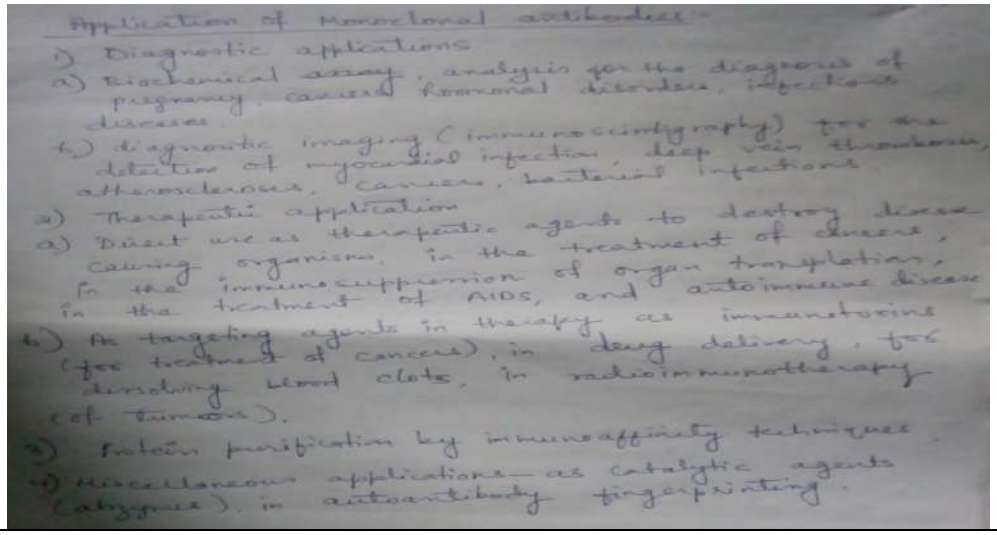
Subject Code: RPH-840 Subject: PHARMACEUTICAL BIOTECHNOLOGY

B.Pharm.-8th SEMESTER

Solution

SECTION – A

1. Attempt all questions in brief.

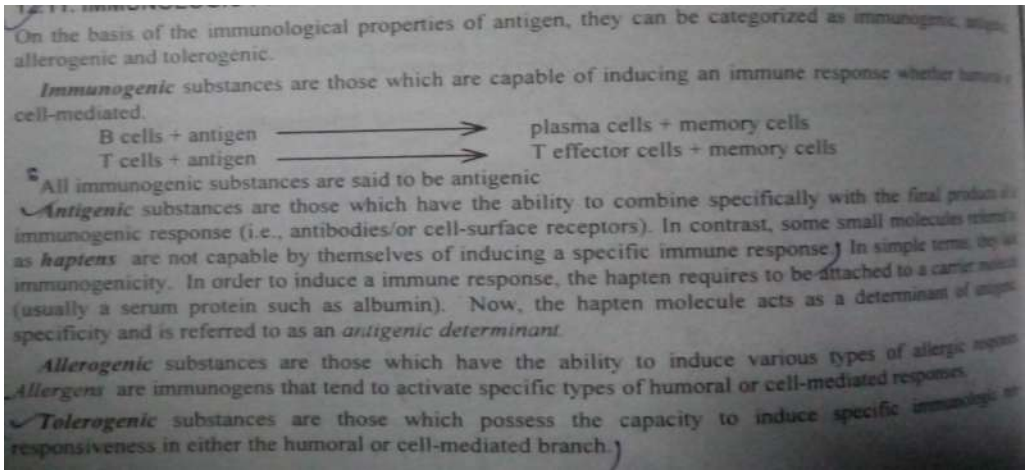
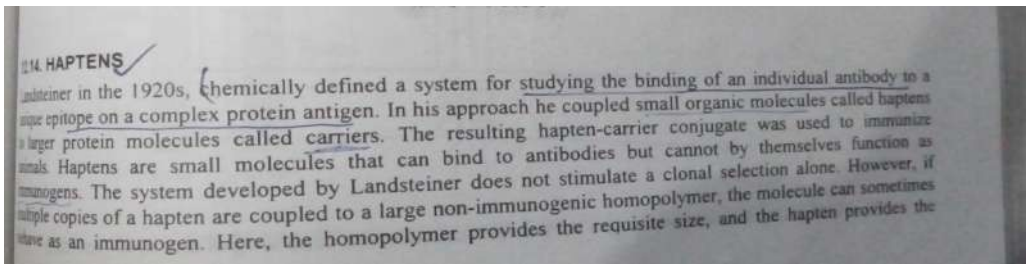
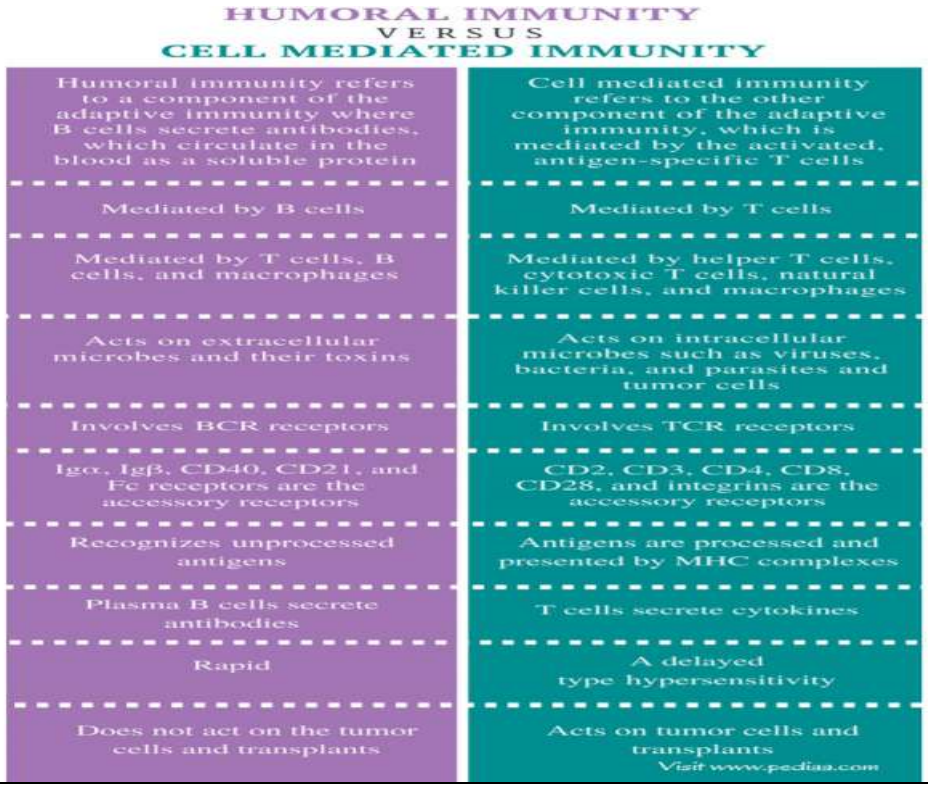
Q. No.	QUESTION
a.	Define role of restriction endonuclease.  a) Restriction endonucleases – DNA cutting enzymes :- It is used for the manipulation of DNA. These are the bacterial enzymes that can cut/ split DNA (from any source) at specific sites. They were first discovered in E.coli restricting the replication of bacteriophages by cutting the viral DNA (the host E.coli DNA is protected from cleavage by addition of methyl groups). Thus the enzymes that restrict the viral replication are k/as restriction enzymes OR restriction endonucleases.
b.	Define role of DNA ligase.  2) ligation (DNA ligases – DNA joining enzymes). The cut DNA fragments are covalently joined together by DNA ligases. These enzymes were originally isolated from viruses. They also occur in E.coli and eukaryotic cells. DNA ligases actively participated in cellular DNA repair process.
c.	State the use of monoclonal antibody.  Application of Monoclonal antibodies:- 1) Diagnostic applications a) Biochemical assay, analysis for the diagnosis of pregnancy, cancer, hormonal disorders, infectious diseases. b) diagnostic imaging (immunoscintigraphy) for the detection of myocardial infection, deep vein thrombosis, atherosclerosis, cancers, bacterial infections. 2) Therapeutic application a) Direct use as therapeutic agents to destroy disease causing organisms, in the treatment of cancer, in the immunosuppression of organ transplation, in the treatment of AIDS, and autoimmune disease. b) As targeting agents in therapy as immunotoxins (for treatment of cancers), in drug delivery, for dissolving blood clots, in radioimmunotherapy (of tumors). 3) Protein purification by immunoaffinity techniques. 4) Miscellaneous applications – as catalytic agents (catalytic antibody), in autoantibody fingerprinting.
d.	Write two application of r-DNA.

	Application of Recombinant DNA - Some of the area where R-DNA technology is having a considerable impact — ① Genetically modified Organisms (GMOs) — Transgenic animals as experimental models in biomedical research. — Transgenic fruit flies (Drosophila melanogaster) as model organism used in biomedical research to study the effects of genetic changes on develop ② Agriculture — Better crops which are resistant to insects, pests herbicide and harsh environmental conditions suc as heat or cold. — crops with increased nutritional value. — Green revolution is the outcome of r-DNA technol ③ Medicine — Recombinant vaccine (hepatitis B) — Production of clotting factors. — Production of Insulin
e.	Write two application of hybridoma technology. ➢ Dose determination of a medicine ➢ To detect allergies & viral disease ➢ to detect certain type of cancer; to monitor the presence or appearance of malignant cells after surgical or radio-therapeutic treatments. ➢ For purification of complex biological mixture. ➢ Envisaged for the labelling & precise identification of some specialized cell such as neurone for gaining better knowledge of the way of association & operation. ➢ For purification of structural cell membrane protein. ➢ Mab has a great role in serotherapy

SECTION - B

2. Attempt any <u>TWO</u> of the following.

Q. No.	QUESTION
a.	Explain Humoral immunity. 12.8 HUMORAL IMMUNITY Emil von Behring and Shibasaburo Kitasato in 1890 were the first to present to the World the insights of mechanism involved in immunity. They demonstrated that serum from animals, previously immunized when administered to non-immunized animals, provided immunization to these animals. Since the immunity was mediated by antibodies contained in body fluids (in early days it was known as *humors*), the kind of immunity was named *humoral immunity*. Humoral immunity or response is carried by a special group of cells called B cells. These B cells are responsible for the production of antibodies. The humoral response, which involves in the elimination of extracellular pathogens, is well classified by the production of a large number of antibody molecules. B cells which are produced in the bone marrow, mature and migrate into the lymphoidal organ, where they encounter antigen (Fig. 12.10). When an appropriate antigen contacts the antigen receptor antibodies on a B cell, the B cell proliferates into a large clone of cells. This phenomenon is known as clonal selection (discussed in the early part of this chapter). Sometimes, the production of antibodies by a B cell depends on other cells e.g., production of antibodies against T-dependent antigens requires the help of certain macrophages and T cells. The above discussion makes it clear that humoral immune system involves antibodies that are found in blood plasma and lymph.

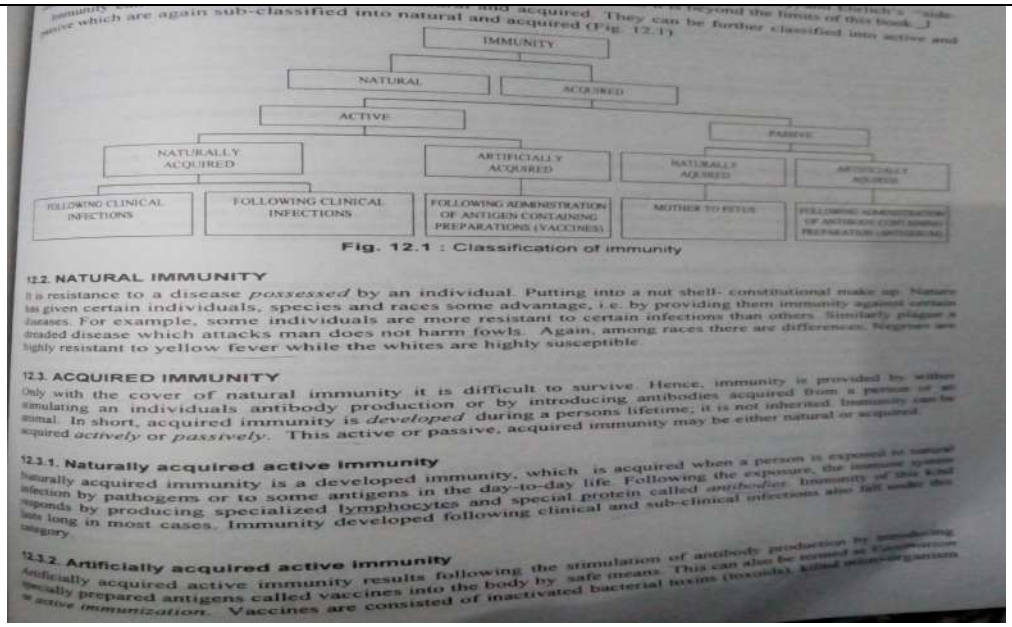
b.	Write a note on Antigen.  On the basis of the immunological properties of antigen, they can be categorized as immunogenic, allergenic and tolerogenic. ***Immunogenic*** substances are those which are capable of inducing an immune response whether humoral or cell-mediated. B cells + antigen → plasma cells + memory cells T cells + antigen → T effector cells + memory cells All immunogenic substances are said to be antigenic ***Antigenic*** substances are those which have the ability to combine specifically with the final product of immunogenic response (i.e., antibodies/or cell-surface receptors). In contrast, some small molecules referred as ***haptens*** are not capable by themselves of inducing a specific immune response. In simple terms, they lack immunogenicity. In order to induce a immune response, the hapten requires to be attached to a carrier molecule (usually a serum protein such as albumin). Now, the hapten molecule acts as a determinant of antigen specificity and is referred to as an *antigenic determinant*. ***Allerogenic*** substances are those which have the ability to induce various types of allergic responses. *Allergens* are immunogens that tend to activate specific types of humoral or cell-mediated responses. ***Tolerogenic*** substances are those which possess the capacity to induce specific immunologic unresponsiveness in either the humoral or cell-mediated branch.
c.	Write a note on Haptens.  **12.14. HAPTENS** Landsteiner in the 1920s, chemically defined a system for studying the binding of an individual antibody to a unique epitope on a complex protein antigen. In his approach he coupled small organic molecules called haptens to larger protein molecules called carriers. The resulting hapten-carrier conjugate was used to immunize animals. Haptens are small molecules that can bind to antibodies but cannot by themselves function as immunogens. The system developed by Landsteiner does not stimulate a clonal selection alone. However, if multiple copies of a hapten are coupled to a large non-immunogenic homopolymer, the molecule can sometimes behave as an immunogen. Here, the homopolymer provides the requisite size, and the hapten provides the
d.	State the difference between cellular and humoral immunity.  **HUMORAL IMMUNITY VERSUS CELL MEDIATED IMMUNITY** Visit www.pediaa.com

Humoral immunity refers to a component of the adaptive immunity where B cells secrete antibodies, which circulate in the blood as a soluble protein	Cell mediated immunity refers to the other component of the adaptive immunity, which is mediated by the activated, antigen-specific T cells
Mediated by B cells	Mediated by T cells
Mediated by T cells, B cells, and macrophages	Mediated by helper T cells, cytotoxic T cells, natural killer cells, and macrophages
Acts on extracellular microbes and their toxins	Acts on intracellular microbes such as viruses, bacteria, and parasites and tumor cells
Involves BCR receptors	Involves TCR receptors
Igα, Igβ, CD40, CD21, and Fc receptors are the accessory receptors	CD2, CD3, CD4, CD8, CD28, and integrins are the accessory receptors
Recognizes unprocessed antigens	Antigens are processed and presented by MHC complexes
Plasma B cells secrete antibodies	T cells secrete cytokines
Rapid	A delayed type hypersensitivity
Does not act on the tumor cells and transplants	Acts on tumor cells and transplants

SECTION - C

3. Attempt any ONE part of the following :

Q No.	QUESTION
a.	Explain in detail the principles of Immunology.



which are again sub-classified into natural and acquired. They can be further classified into active and

Fig. 12.1 : Classification of immunity

12.2. NATURAL IMMUNITY

It is resistance to a disease *possessed* by an individual. Putting into a nut shell- constitutional make up. Nature has given certain individuals, species and races some advantage, i.e. by providing them immunity against certain diseases. For example, some individuals are more resistant to certain infections than others. Similarly plague a dreaded disease which attacks man does not harm fowls. Again, among races there are differences. Negroes are highly resistant to yellow fever while the whites are highly susceptible.

12.3. ACQUIRED IMMUNITY

Only with the cover of natural immunity it is difficult to survive. Hence, immunity is provided by either stimulating an individuals antibody production or by introducing antibodies acquired from a person or an animal. In short, acquired immunity is *developed* during a persons lifetime; it is not inherited. Immunity can be acquired *actively* or *passively*. This active or passive, acquired immunity may be either natural or acquired.

12.3.1. Naturally acquired active immunity

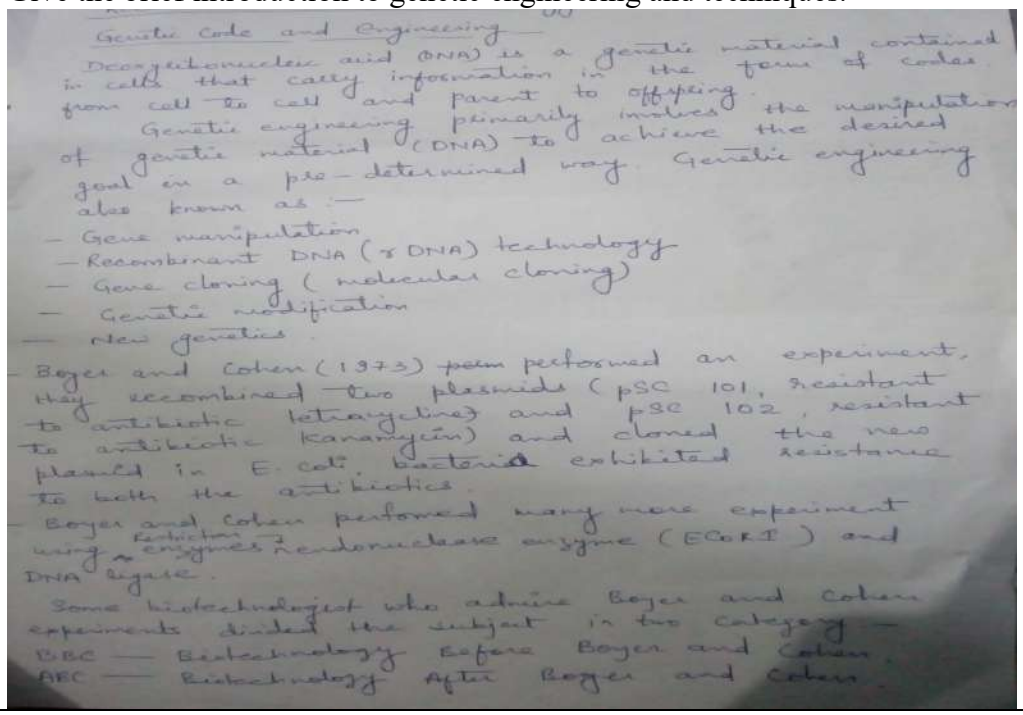
Naturally acquired immunity is a developed immunity, which is acquired when a person is exposed to natural infection by pathogens or to some antigens in the day-to-day life. Following the exposure, the immune system responds by producing specialized lymphocytes and special protein called *antibodies*. Immunity of this kind lasts long in most cases. Immunity developed following clinical and sub-clinical infections also fall under this category.

12.3.2. Artificially acquired active immunity

Artificially acquired active immunity results following the stimulation of antibody production by introducing specially prepared antigens called vaccines into the body by safe means. This can also be termed as 'Immunization or *active immunization*. Vaccines are consisted of inactivated bacterial toxins (toxoids), killed microorganisms

b.

Give the brief introduction to genetic engineering and techniques.



Genetic Code and Engineering

Deoxyribonucleic acid (DNA) is a genetic material contained in cells that carry information in the form of codes from cell to cell and parent to offspring.

Genetic engineering primarily involves the manipulation of genetic material (DNA) to achieve the desired goal in a pre-determined way. Genetic engineering also known as :—

- Gene manipulation
- Recombinant DNA (r DNA) technology
- Gene cloning (molecular cloning)
- Genetic modification
- New genetics

- Boyer and Cohen (1973) performed an experiment, they recombined two plasmids (pSC 101, resistant to antibiotic tetracycline) and pSC 102, resistant to antibiotic kanamycin) and cloned the new plasmid in E. coli bacteria exhibited resistance to both the antibiotics.
- Boyer and Cohen performed many more experiment using restriction endonuclease enzyme (EcoRI) and DNA ligase.

Some biotechnologist who admire Boyer and Cohen experiments divided the subject in two category :—

BBC — Biotechnology Before Boyer and Cohen.
ABC — Biotechnology After Boyer and Cohen.

Techniques of Genetic Engineering :-

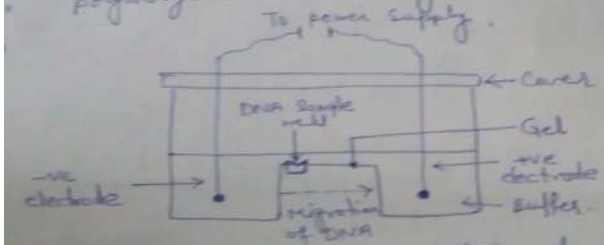
There are several techniques used in recombinant DNA technology or gene manipulation. The most frequently used methods are listed.

1. Agarose gel electrophoresis
2. Isolation and purification of nucleic acids
3. Isolation of chromosomes
4. Nucleic acid blotting techniques
5. DNA sequencing
6. Alternate method of DNA sequencing
7. chemical synthesis of DNA
8. Methods of gene transfer
9. Polymerase chain Reaction
10. Production of monoclonal antibodies
11. Construction of gene library
12. Radiolabeling of nucleic acid

Agarose Gel Electrophoresis :-

Electrophoresis refers to the movement of charged molecules in an electric field. Gel electrophoresis is a routinely used analytical technique for the separation / purification of specific DNA fragments

The gel is composed of either polyacrylamide or agarose (polysaccharide)

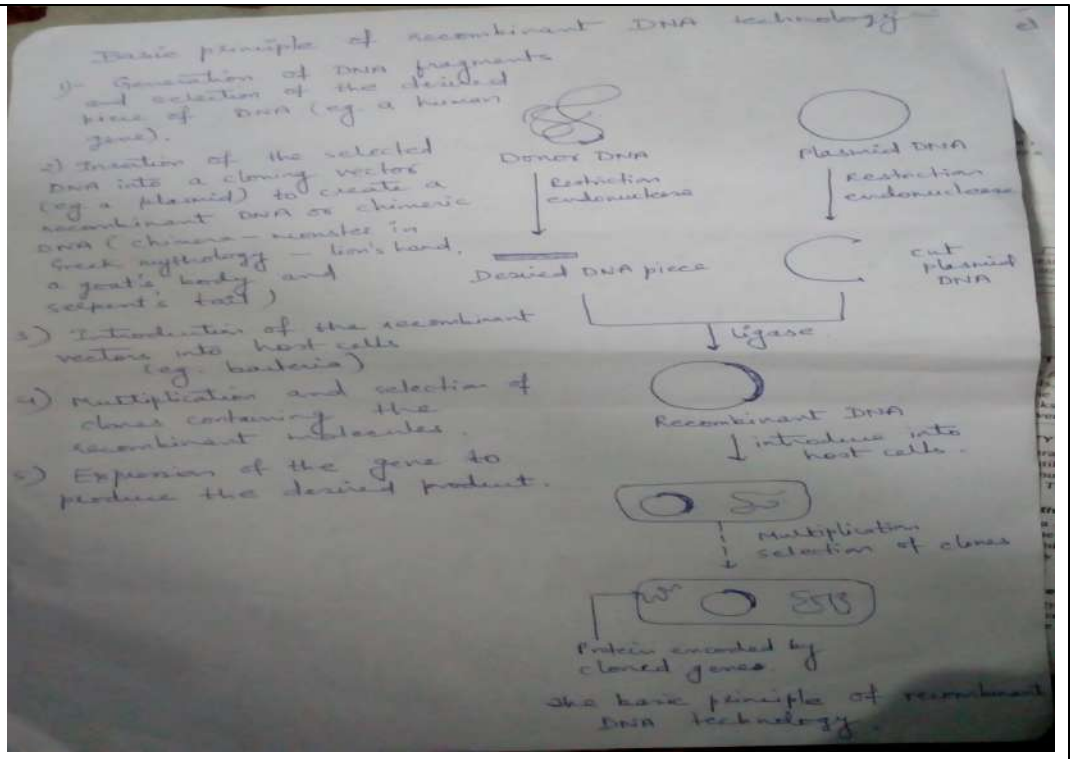
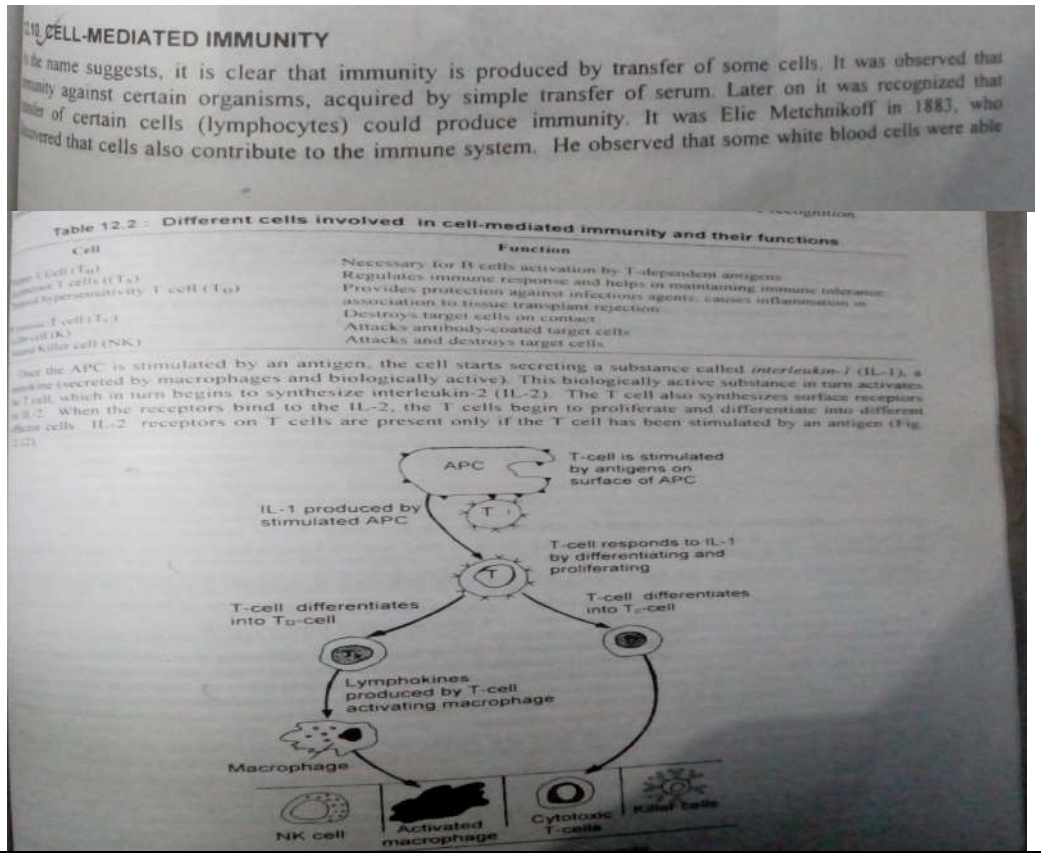


A diagrammatic representation of agarose gel electrophoresis system.

The DNA samples are placed in the wells of the gel surface and the power supply is switched on. As the DNA is negatively charged DNA fragments move through the gel towards the positive electrode. The rate of migration of DNA is dependent on the size and shape. Smaller linear fragments move faster than the larger ones. This technique is used for the separation of a mixture of DNA fragments based on their size.

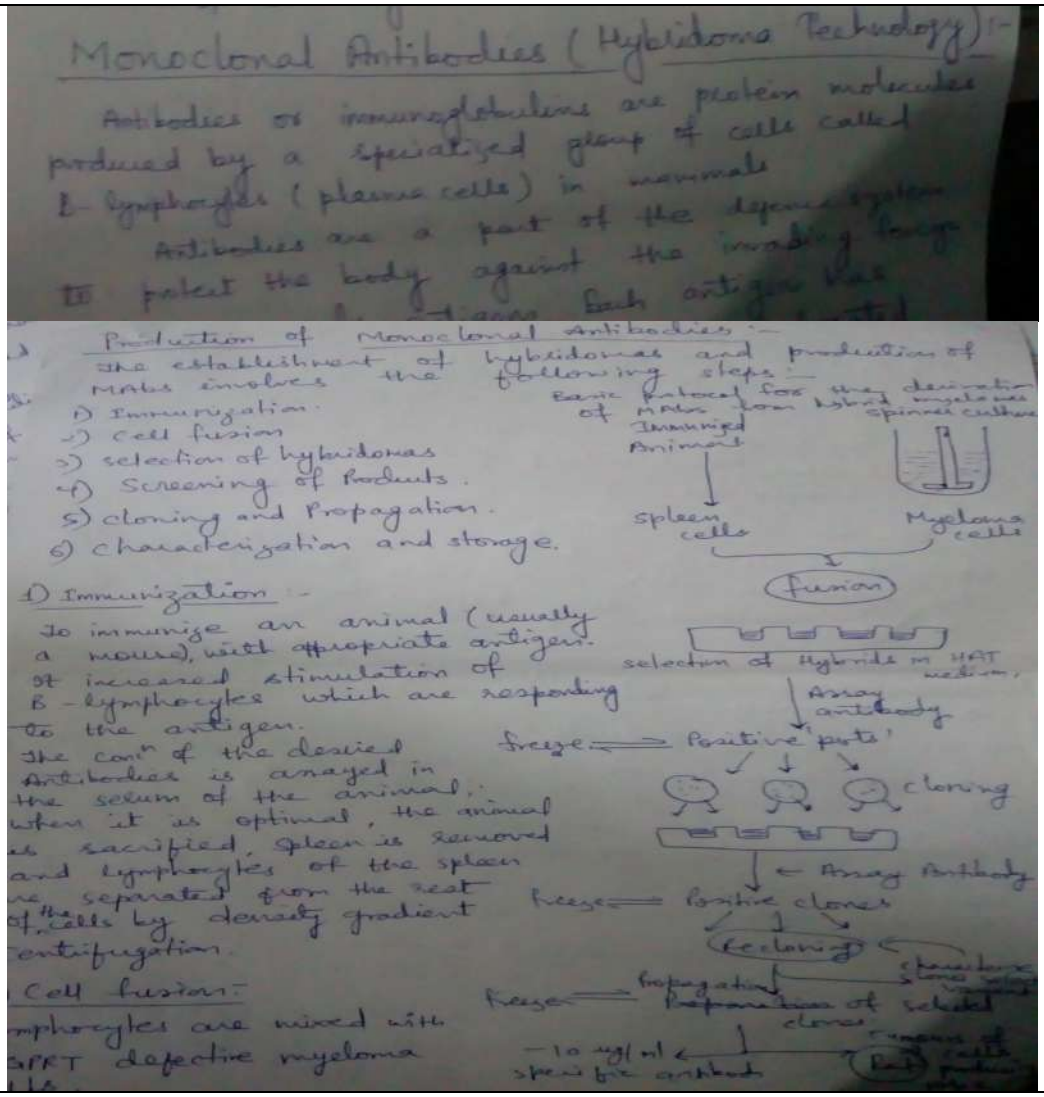
4. Attempt any ONE part of the following :

Q. No.	QUESTION
a.	State the procedure for recombinant DNA technology.

	
b.	Explain cellular immunity. 

5. Attempt any ONE part of the following :

Q. No.	QUESTION
a.	Explain the development of hybridoma for monoclonal antibody.

	
b.	Write a note on immune system.

12.7. THE CELLS OF THE IMMUNE SYSTEM

Generation of an effective immune response involves a number of organs and several different cell types that can accurately and specifically recognize non-self antigens on micro-organisms and to eliminate them. Two major groups of cells fall under this category.

1. Lymphocytes
   - B Lymphocytes
   - T Lymphocytes
     - Helper
     - Cytotoxic
     - Suppressive

2. Antigen presenting cells (APC)

Among the two different kinds of lymphocytes, T cells differentiate initially in the thymus where it acquires differentiate in fetal liver, spleen and in bone marrow. In addition to these cells, non-B or non-T cells or null population cells/null cells are also present. Further, a number of auxiliary cells are involved in generating immunity against invading organism.

12.7.1. Lymphoidal cells

12.7.1.1. B lymphocytes or B cells

Classically, the B lymphocytes are defined by the presence of endogenously produced immunoglobulin (antibody). That is the B cell receptor is an antibody molecule, a membrane-bound glycoprotein. They mature in the bone marrow and leave the marrow with a unique antigen-binding receptor on the membrane (Fig. 12.7).

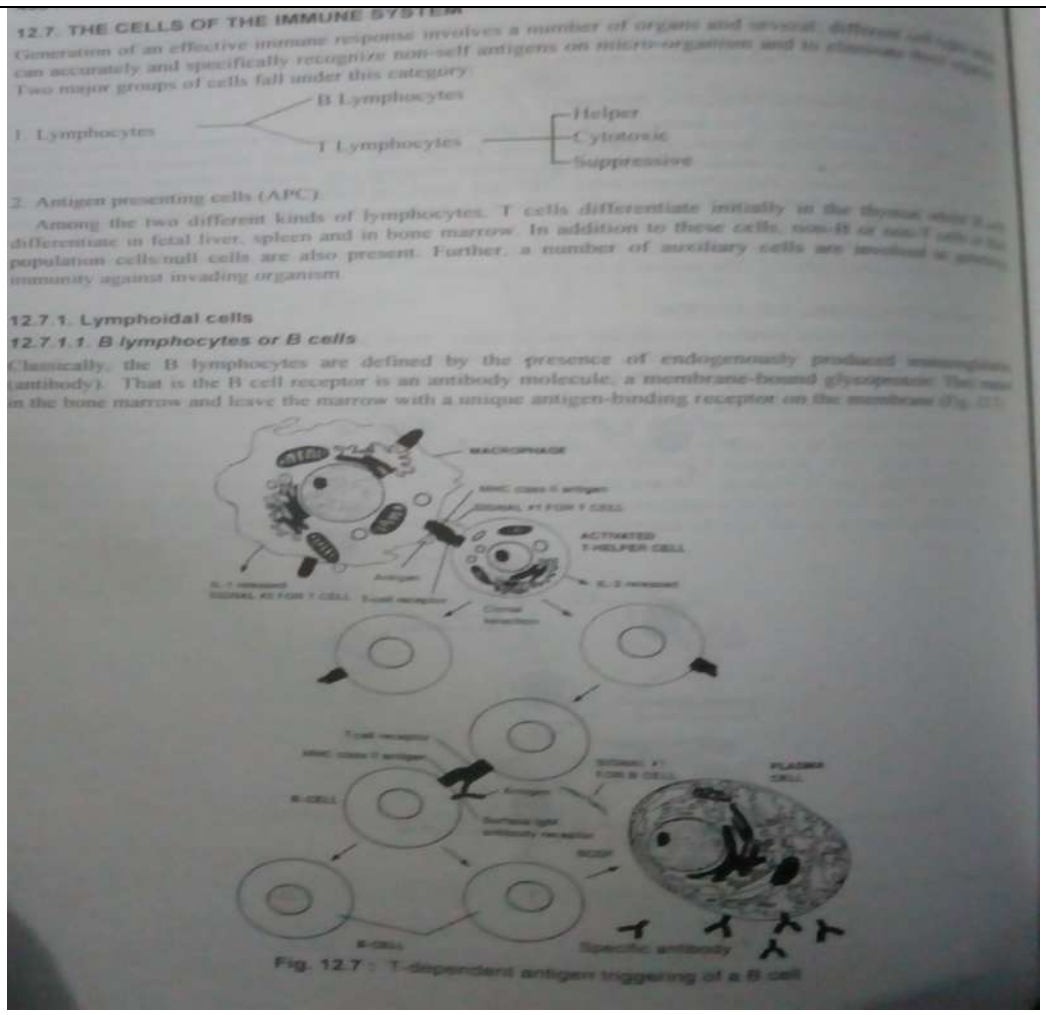


Fig. 12.7 : T-dependent antigen triggering of a B cell