Model Answer
B Sc (Hons) V Semester Examination 2014-15
LZC-504: Immunology and Biostatistics

Section A

i. a ii. c iii. b iv. d v. c

vi. b vii. d viii. c ix. c x. d

Section B

Ans. 2

Primary lymphatic organs: **Bone marrow and Thymus gland**

Secondary lymphatic organs**: Lymph nodes, Tonsils, Appendix, Spleen**, **Peyer's patches** and **Mucosa Associated Lymphoid Tissue (MALT**).

The immune system stockpiles a huge arsenal of cells, not only lymphocytes but also cell devouring Phagocytes and their relatives. Some immune cells take on all intruders, whereas others are trained on highly specific targets. To work effectively, most immune cells need the cooperation of their comrades. Sometimes immune cells communicate by direct physical contact, and sometimes they communicate releasing chemical messengers Cells of the Immune System..

Lymphocytes

- Small white blood cells which are responsible for much of the work of the Immune System.
- Lymphocytes can be divided into three classes: B cells, T cells and Natural Killer cells (NKC) .
- Mature lymphocytes all have a similar appearance. They are small cells with a deeply basophilic nucleus and scanty cytoplasm.

B cells and T cells

- Also known as *B cell lymphocytes and T cell lymphocytes.*
- T cells or T lymphocytes belong to a group of white blood cells and play a central role in Cell-mediated Immunity. While, B cells are lymphocytes that play a large role in the Humoral Immune Response. B cells is an essential component of the Adaptive Immune System.
- B cells spend their entire early life in the bone marrow. While the T cells, leave the bone marrow at an early age and travel to the thymus, where they mature.

- The principal functions of B cells is to make antibodies against antigens, perform the role of antigen-presenting cells (APCs) and eventually develop into memory B cells after activation by antigen interaction.
- On the other hand, T cells constitutes 65-75% of blood lymphocytes. They can be distinguished from other lymphocytes by the presence of a *T cell receptor* (TCR) on the cell surface.
- Another key feature of B cells and T cells, includes the receptors it has in its surface. T cells recognize a linear sequence of amino acids whereas, B cells the spatial arrangement of proteins, nucleic acids, polyssacharides or lipids.

Helper T-Cell: assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages.

Cytotoxic T-Cell: destroy virally infected cells and tumor cells, and are also implicated in transplant rejection.

Regulatory T-Cell: formerly known as *suppressor T cells*, are crucial for the maintenance of immunological tolerance.

Memory T-Cell: are a subset of antigen-specific T cells that persist long-term after an infection has resolved.

Natural Killer Cells

These cells lack the marker molecules characteristic of B and T cells. They comprise about 10-15% of the lymphocytes of circulating blood.

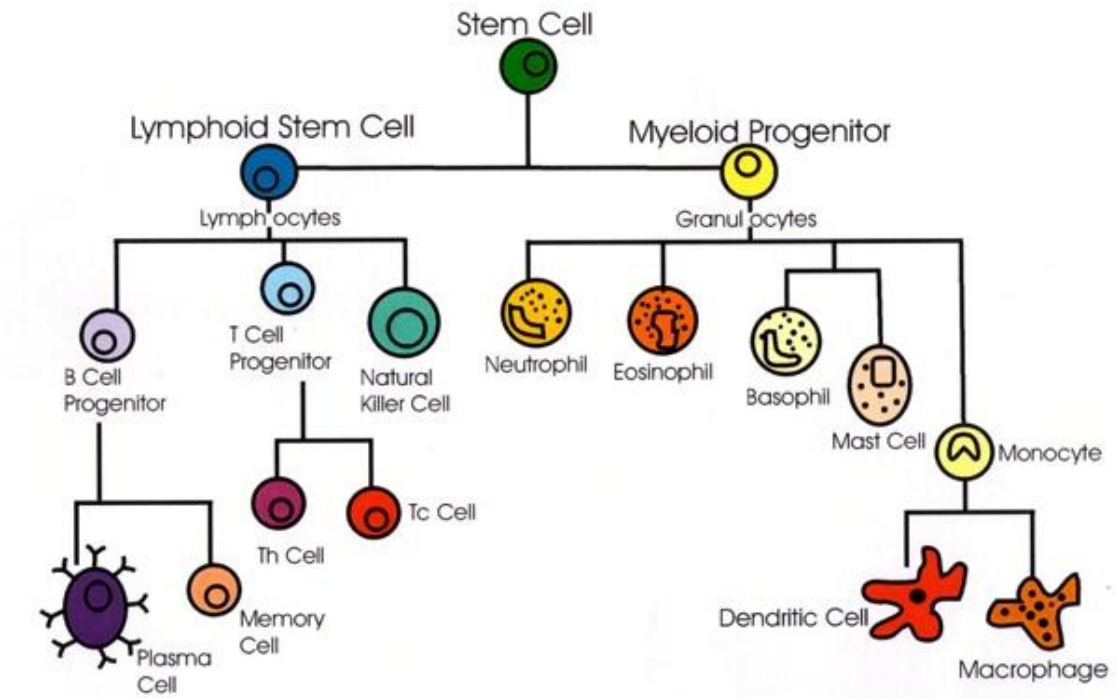
The role NK cells play is analogous to that of cytotoxic T cells in the vertebrate adaptive immune response. NK cells provide rapid responses to virally infected cells and respond to tumor formation, acting at around 3 days after infection.

Macrophages

Macrophages are cells produced by the differentiation of monocytes in tissues. It function in both non-specific defense, Innate Immunity, as well as help initiate specific defense mechanisms, Adaptive Immunity, of vertebrate animals.

Their role is to *"phagocytose"* (engulf and then digest) cellular debris and pathogens, either as stationary or as mobile cells.

They also stimulate lymphocytes and other immune cells to respond to pathogens. They are specialized *phagocytic* cells that attack foreign substances, infectious microbes and cancer cells through destruction and ingestion.



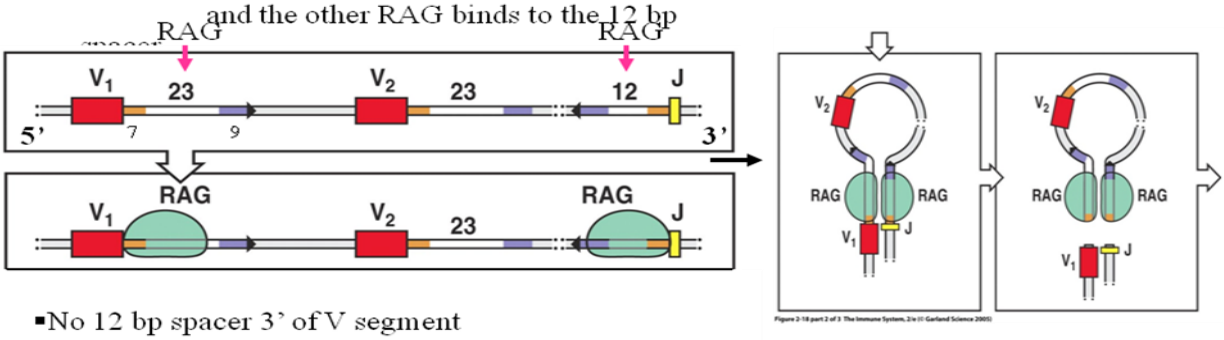
Cell types in immune System

Ans. No 3.

Somatic Recombination

V(D)J recombinase or Recombination Activating Genes (RAG-1 and RAG-2)

- Specific to B and T cells and only present at certain maturation stages
- RAG-1 and RAG-2 interact with each other and with other proteins and form the RAG complex
- 12/23 rule: one RAG binds to the 23 bp spacer and the other RAG binds to the 12 bp



- No 12 bp spacer 3' of V segment
- No 23 bp spacer 5' of J segment
  - –Multiple V segments will not recombine together
  - –Multiple J segments will not recombine together

RAGs associate and DNA is broken at ends of heptamer

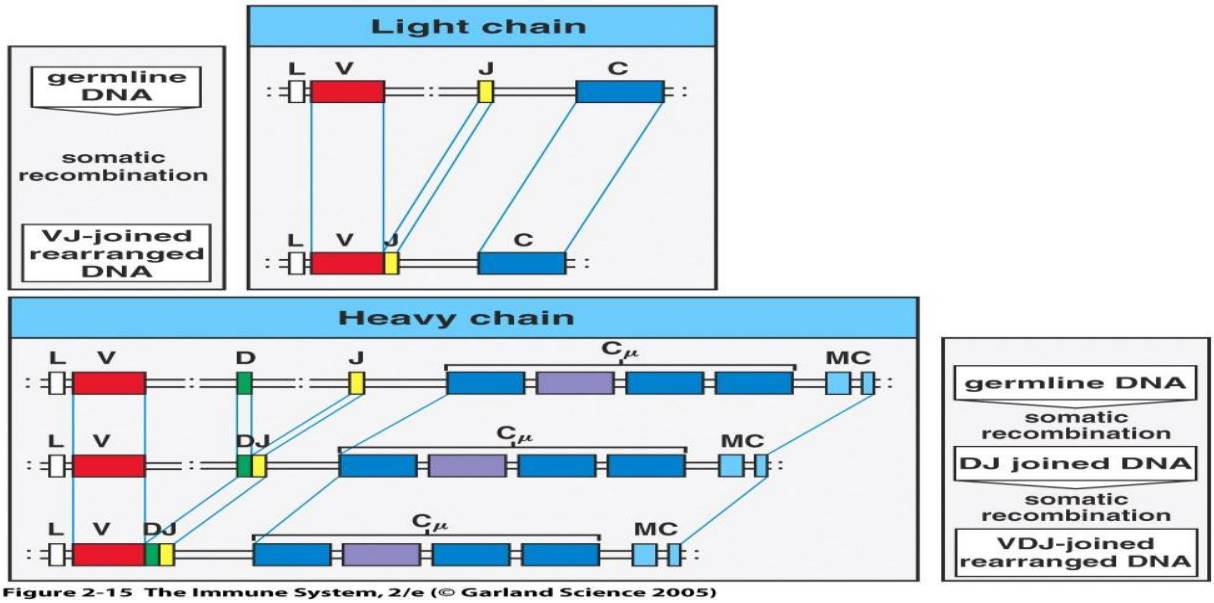


Figure 2-15 The Immune System, 2/e (© Garland Science 2005)

Germline Rearrangement

- Unique organization of Ig genes
- Not a single complete gene but **fragmented** and **unfunctional**
- **Multiple** gene segments sequentially arranged along the chromosome compose the H and L chain locus
  - –inherited through the germline (egg and sperm) - called **germline form** or **germline configuration**
  - –**alternative versions** of the V region encoded by 2 (V_L) or 3 (V_H) gene segments
  - –Leader peptide (L) and the C region do not require gene rearrangement for transcription
- Germline not expressed
  - –Expression depends on **rearrangement** of individual gene segments to assemble a functional gene (occurs in developing B cells)

Generation of Ig Diversity

Segment	Number of gene segments: Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6
Possible recombinations	200	120	10,530

Figure 2-16 The Immune System, 2/e (© Garland Science 2005)

Various copies of each gene segment and random recombination of one V, D or J segment contributes significantly to the generation of antigen diversity.

Answer No.4

The major histocompatibility complex (MHC) is a set of cell surface molecules encoded by a large gene family which controls a major part of the immune system in all vertebrates. The major function of MHCs are to bind to peptide fragments derived from pathogens and display them on the cell surface for recognition by the appropriate T-cells. MHC molecules mediate interactions of leukocytes, also called white blood cells (WBCs), which are immune cells, with other leukocytes or with body cells. The MHC determines compatibility of donors for organ transplant, as well as one's susceptibility to an autoimmune disease via crossreacting immunization.

MHC class I

MHC I glycoproteins are present on almost every cell in the body, acting to present endogenous antigens that originate from the cytoplasm. These antigens include not only self-proteins, but also foreign proteins produced within the cell, such as viral proteins that take over the cell's machinery in order to replicate the virus. When these proteins become degraded, the peptide fragments can be transported to the endoplasmic reticulum, where they can bind to MHC I proteins, before being transported via the Golgi apparatus to the cell surface. Once at the cell surface, the membrane-bound MHC I protein displays the

antigen for recognition by special immune cells known as cytotoxic T cell lymphocytes. MHC I proteins work to present the types of proteins being synthesised within a cell, which can then be monitored by killer T cells as part of a surveillance system that identifies and destroys any cell with over-abundant or unfamiliar peptide antigens, such as malignant cells or those harbouring viruses.

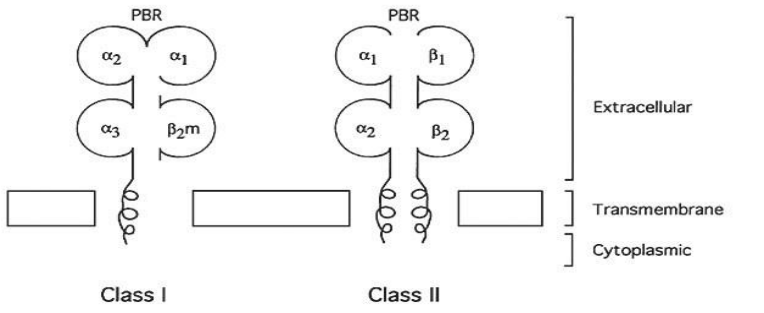
MHC class II

MHC II glycoproteins are only present on specialised antigen-presenting immune cells, including macrophages that engulf foreign particles such as bacteria, dendritic cells that present antigen to T cells, and B cells that produce antibodies. MHC II proteins present exogenous antigens that originate extracellularly from foreign bodies such as bacteria. Upon encountering a pathogenic organism, proteins from the pathogen can be degraded into peptide fragments by the antigen-presenting cell, which then sequesters these fragments into the endosome so they can bind to MHC II proteins, before being transported to the cell surface. Once at the cell surface, the membrane-bound MHC II protein displays the antigen for recognition by a different type of T cell, namely the helper T cell lymphocyte. These helper T cells are activated upon binding to macrophage or dendritic cell MHC II-antigen, causing the release of lymphokines that attract other cells to the area of infection in an attempt to confine and destroy the antigenic material. In addition, the binding of helper T cells to B cell MHC II-antigen stimulates the development of a clone of antibody-producing cells against the antigenic material.

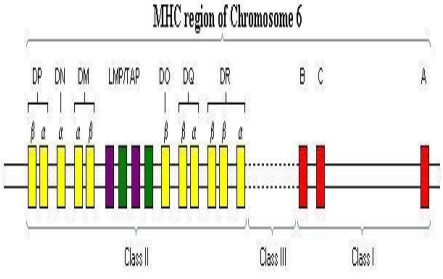
Class III : MHC Class III region has the highest gene density but some of the genes are not involved in the immune system.

Since class III genes encode several components of the complement system (i.e. C2, C4a, C4b, Bf), they are responsible for the levels of components of compliment system.

In addition to components of the complement system MHC III genes code inflammatory cytokines, tumor necrosis factor a and 0 (TNF a and P), two heat shock proteins (HSP) etc. They are not membrane proteins and have no role in Ag presentation. MHC Class III molecules are not structurally related to class I and class II molecules but are important in immune response.



Schematic presentation of the structure of MHC class I and class II molecules. PBR = peptide-binding region. (Reprinted, with permission, from the Annual Review of Genetics, Vol. 32 ©1998 by Annual Reviews, www.annualreviews.org).



Functions:

When disease associated proteins occur in a cell they are broken into pieces by the cells proteolytic machinery. Cell proteins become attached to antigen fragments and transport them to the surface of the cell, where they are "presented" to the bodies defence mechanisms.

These transport molecules are called the Major Histocompatibility Complex (MHC) proteins. Without these, there would be no presentation of internal or external antigens to the T cells. The

importance of MHC proteins is that they allow T cells to distinguish self from non-self. In every cell in your body, antigens are constantly broken up and presented to passing T cells. Without this presentation, other aspects of the immune response cannot occur .
Class I MHC proteins (found on all nucleated cell surfaces) present antigens to cytotoxic T lymphocytes (CTLs) . Most CTLs possess both T-cell receptors (TCR) and CD8 molecules On their surfaces. These TCRs are able to recognize peptides when they are expressed in complexes with MHC Class I molecules

The MHC Class II proteins (found only on B lymphocytes, macrophages, and other cells that present antigens to T cells), which primarily present peptides which have been digested from external sources, are needed for T-cell communication with B-cells and macrophages. Class II MHC proteins presenting antigens are detected by a different group of T cells (called T-helper or TH cells) to Class I MHC proteins (which are detected by CTLs cells). Major Histocompatibility Complex proteins and their associated molecules are fundamental in the process of antigen presentation.

Ans. No. 5.

The basic structural unit of most mammalian antibodies is a glycoprotein (MW ~150,000 daltons) comprising four polypeptide chains—two light chains and two heavy chains, which are connected by disulfide bonds .Each light chain has a molecular weight of ~25,000 daltons and is composed of two domains, one variable domain (V_L) and one constant domain (C_L). There are two types of light chains, lambda (λ) and kappa (κ). In humans, 60% of the light chains are κ, and 40% are λ, whereas in mice, 95% of the light chains are κ and only 5% are λ. A single antibody molecule contains either κ light chains or λ light chains, but never both.

Each heavy chain has a molecular weight of ~50,000 daltons and consists of a constant and variable region. The heavy and light chains contain a number of homologous sections consisting of similar but not identical groups of amino acid sequences. These homologous units consist of about 110 amino acids and are called immunoglobulin domains. The heavy chain contains one variable domain (V_H) and either three or four constant domains (C_H1, C_H2, C_H3 and C_H4, depending on the antibody class or isotype). The region between the C_H1 and C_H2 domains is called the hinge region and permits flexibility between the two Fab arms of the Y-shaped antibody molecule, allowing them to open and close to accommodate binding to two antigenic determinants separated by a fixed distance.

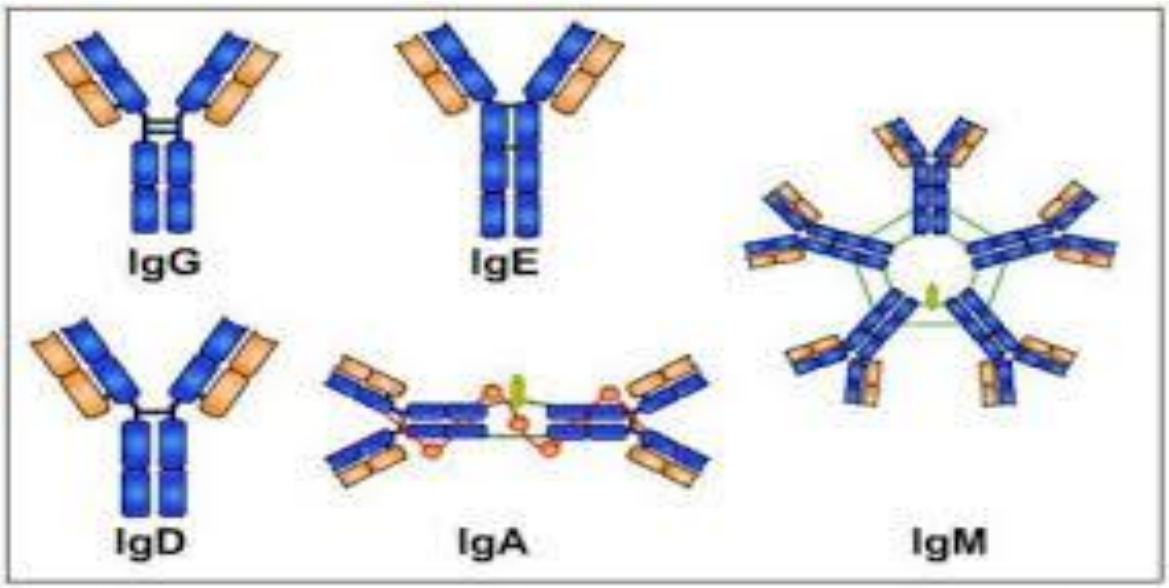
The heavy chain also serves to determine the functional activity of the antibody molecule. There are five antibody classes—IgG, IgA, IgM, IgE and IgD—which are distinguished by their heavy chains γ, α, μ, ε and δ, respectively .The IgD, IgE and IgG antibody classes are each made up of a single structural unit, whereas IgA antibodies may contain either one or two units and IgM antibodies consist of five disulfide-linked structural units. IgG antibodies are further divided into four subclasses (often referred to as isotypes) although the nomenclature differs slightly depending on the species producing the antibody.

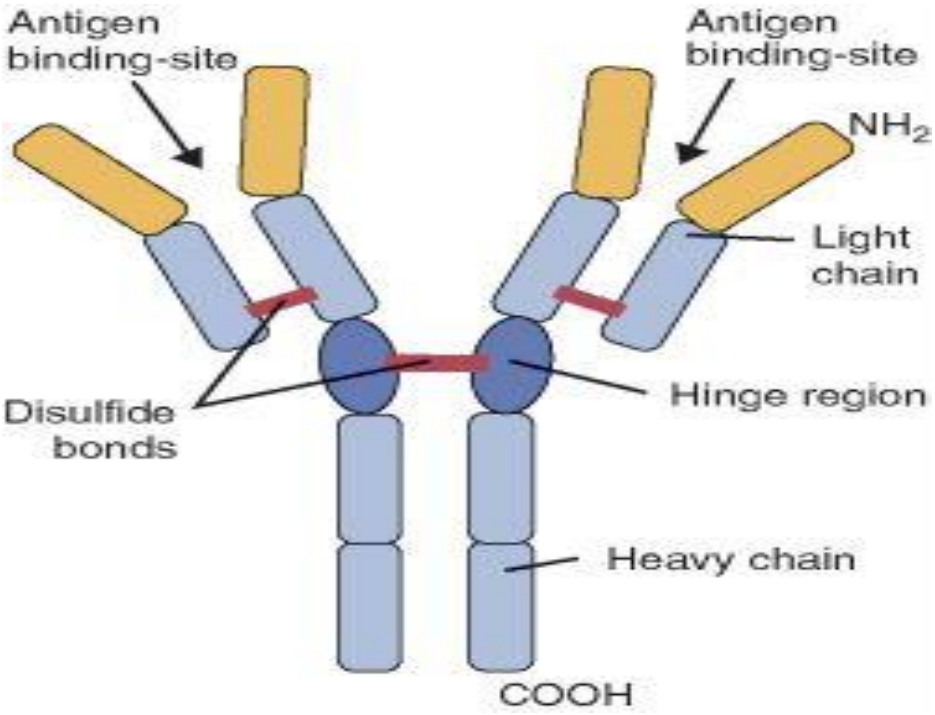
Structure/function studies on IgG have been aided by the discovery that the proteolytic enzymes pepsin and papain cleave the molecule into specific fragments with specific biological properties. Treatment of an IgG molecule with pepsin generates the $F(ab')_2$ fragment, which broadly encompasses the two Fab regions linked by the hinge region. Because the $F(ab')_2$ molecule is bivalent, it is capable of

precipitating an antigen. Papain cleaves the IgG molecule in the hinge region between the C_H1 and C_H2 domains to yield two identical Fab fragments, which retain their antigen-binding ability, and one non-antigen–binding fragment—the (Fc) region.

The Fc region is glycosylated and has many effector functions (e.g., binding complement, binding to cell receptors on macrophages and monocytes), and serves to distinguish one class of antibody from another.

Class	Structure	Properties
IgA	Dimeric and Monomeric	Found in gastrointestinal, respiratory and urogenital tract mucosa. Prevents the colonization by pathogens. Also present in saliva, tears and milk.
IgD	Monomeric	Membrane immunoglobulin. It is part of the membrane receptor of naïve B lymphocytes (BCR).
IgE	Monomeric	Involved in allergic and parasitic processes. Its interaction with basophils and mastocytes causes histamine release.
IgG	Monomeric	Main immunoglobulin of acquired immunity. It has the capacity to cross the placental barrier.
IgM	Monomeric Pentameric	It is part of the membrane receptor of naïve B lymphocytes (BCR). Form found in the serum, secreted early in acquired immune response.





Immunoglobulin	Functions
IgG	Main form of antibodies in circulation: production increased after immunization; secreted during secondary response
IgA	Main antibody type in external secretions, such as saliva and mother's milk
IgE	Responsible for allergic symptoms in immediate hypersensitivity reactions
IgM	Function as antigen receptors on lymphocyte surface prior to immunization; secreted during primary response
IgD	Function as antigen receptors on lymphocyte surface prior to immunization; other functions unknown

Ans. No. 6.

Haematopoiesis also hematopoiesis is the formation of blood cellular components. All cellular blood components are derived from haematopoietic stem cells. In a healthy adult person, approximately 10^{11}–10^{12} new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation.Erythropoiesis - The formation of red blood cells, i.e., the developmental processes of the red blood cell, in the red bone marrow from the descendants of the proerythroblast stem cell by the processes of proliferation and differentiation.

Any immature, undifferentiated, unspecialized cell, capable of repeated cell divisions (proliferation), which can replace itself, and, by differentiation, give rise to one or more specific specialized mature cell types, such as blood cells.

Hemocytoblast = pluripotent hemopoietic stem cell - The primordial stem cell of red bone marrow which is capable of developing into any type of blood cell.

Proerythroblast - The earliest stem cell (derived from the hemocytoblast = pluripotent hemopoietic cell) found in the red bone marrow which is committed to develop into a red blood cell.

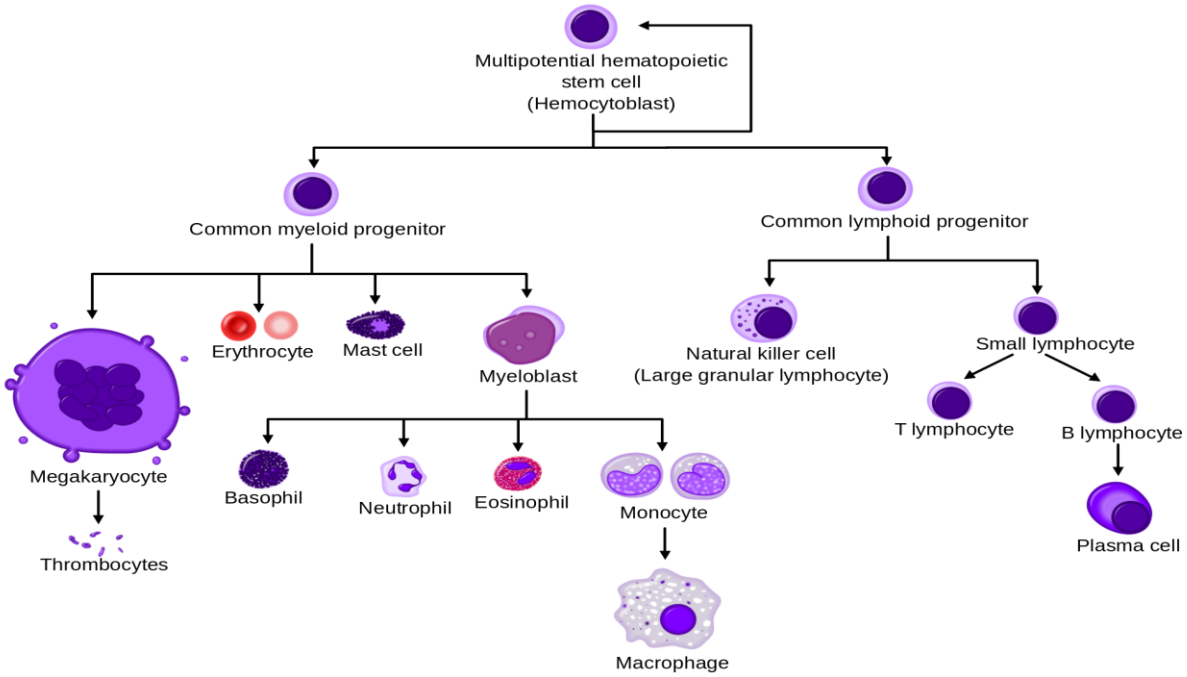
Reticulocyte - The final form of the immature red blood cell which is slightly larger than a mature RBC; it has extruded its nucleus but still contains a network of basophilic (takes up the basic blue-purple stain) filaments, which represent the sites (ribosomes) of final hemoglobin synthesis; primarily they are found in the red bone marrow but a few (0.2-2.0% of total RBCs) are found in the circulating blood; an increase in their numbers in the blood indicates increased RBC proliferation in the red bone marrow.

Megakaryocyte - The largest cell found in red bone marrow, it is the source of blood platelets; it has a characteristic appearance including a lobulate polyploid nucleus; it gives rise to three to four thousand platelets which are membrane-bound fragments shed from its cytoplasm into the blood circulation.

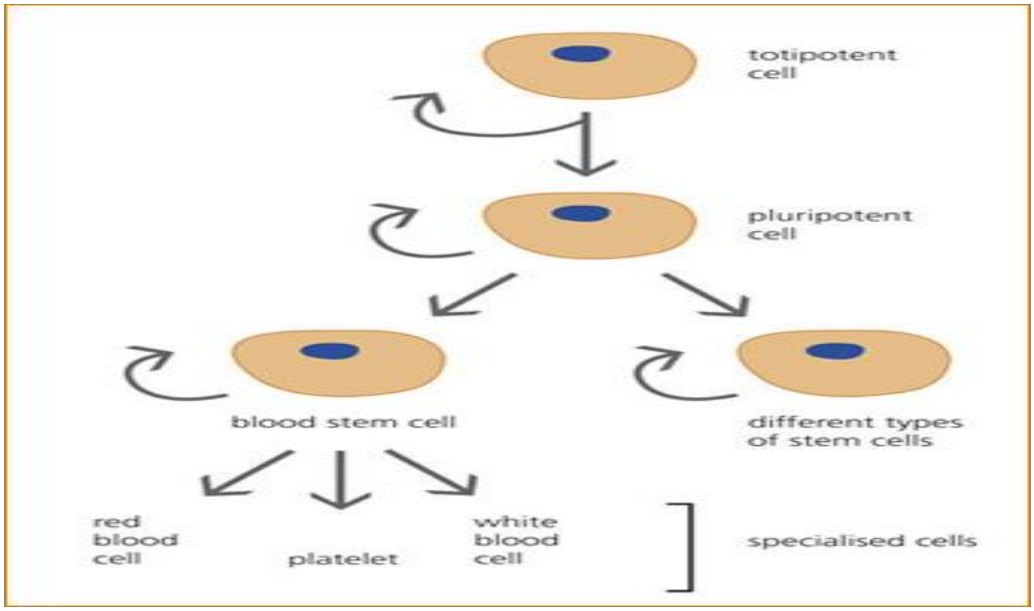
Differentiation:

Cellular differentiation is the process by which a less specialized cell becomes a more specialized cell type. Differentiation occurs numerous times during the development of a multicellular organism as the organism changes from a simple zygote to a complex system of tissues and cell types. Differentiation continues in adulthood as adult stem cells divide and create fully differentiated daughter cells during tissue repair and during normal cell turnover. Differentiation dramatically changes a cell's size, shape, membrane potential, metabolic activity, and responsiveness to signals. These changes are largely due to highly controlled modifications in gene expression. With a few exceptions, cellular differentiation almost never involves a change in the DNA sequence itself. Thus, different cells can have very different physical characteristics despite having the same genome.

A cell that can differentiate into all cell types of the adult organism is known as *pluripotent*. Such cells are called embryonic stem cells in animals and meristematic cells in higher plants. A cell that can differentiate into all cell types, including the placental tissue, is known as *totipotent*. In mammals, only the zygote and subsequent blastomeres are totipotent



Haematopoisis



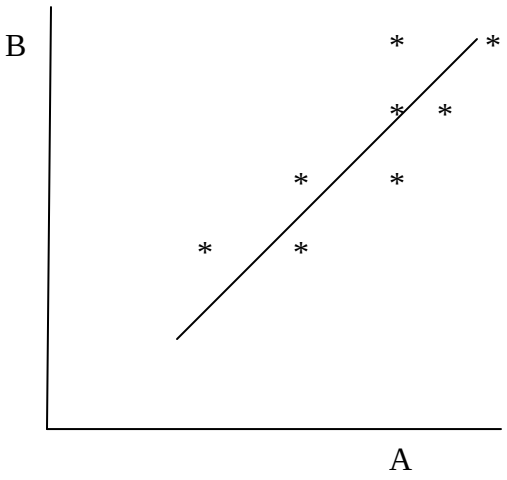
Differentiation

Ans.No. 7

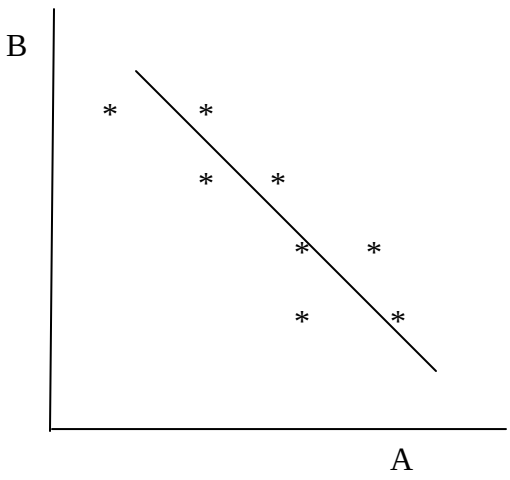
Correlation:

In practice we come across the situations or problems involving the use of two or more than two variates which are to which are to be studied together, like rain fall and humidity, yield and rain fall, yield and fertilizer used, T_4 level and O_2 consumption, height of father and son, glandular activity and environmental factors etc.

If the two quantities vary in such a way that the change in one is accompanied by the change in other, the two quantities are said to be related. The degree of relationship between the variables under consideration is measured through correlation analysis. The measure of correlation is called the Correlation Coefficient or Correlation index (symbolized by 'r').



Positive correlation



Negative correlation

Thus correlation is statistical device which helps in analyzing the covariation of two or more than two variables. The problem of analyzing the relation between two different series should be broken down into three steps-

1. Determining whether a relation exists and if does, measuring it.
2. Testing whether the correlation is significant.
3. Establishing the cause and effect of relation if any.

In general we call the variable, so called the cause, the independent variable and the variable, so called the effect, the dependent variable. At every stage there must be a simultaneous change in both the variable. It should be noted that the detection and analysis of correlation (i. e.

covariation) between the two variables require a relationship of some sort which associates the observations in pairs and one of the each pair being a value of each one of the two variables.

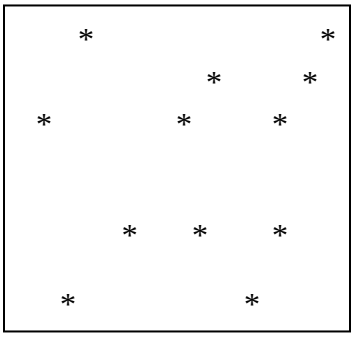
Methods of studying correlation

1. Scatter diagram method
2. Graphic method
3. Karl Pearsonian method

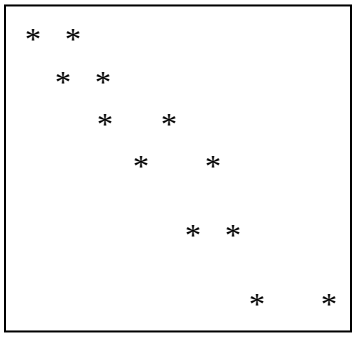
1. Scatter diagram method

Scatter diagram method is also known as dot diagram method. This method ascertains whether the two variables are related. In this method we prepare a dot chart called scatter diagram chart which indicates the scatter of the various points. In this representation the independent variable is plotted on X-axis while the dependent variable on Y-axis and each pair of X and Y values we put a dot and thus we obtain as many points as the number of observations. By looking to the scatter of points we can have an idea whether the two variables are related or not.

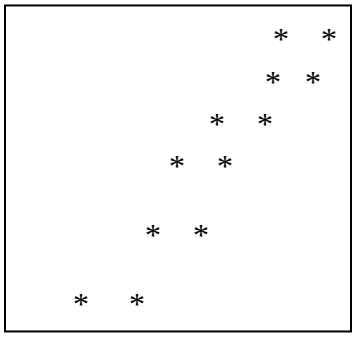
[1] If the points lie on a straight line rising from lower left hand corner to upper right hand corner, the correlation is perfectly positive.
[2] If the points lie on straight line falling from upper left hand corner to lower right hand corner, the correlation is perfectly negative.
[3] If points lie in a band and show rising tendency from left hand corner to right hand corner, the correlation is said to be positive. The width of band indicates the degree of correlation. If the band is narrow, correlation is of higher degree. If the band is wider, the correlation is of lesser degree.
[4] If point lie in a band falling from upper left hand corner to lower right hand corner, the correlation is said to be negative one and again the width of band represents the degree of correlation. If the band is narrow there is high degree of negative correlation. On the other hand if band is wide there is lesser degree of correlation.
[5] If the points lie in a haphazard manner, it shows absence of any correlation.



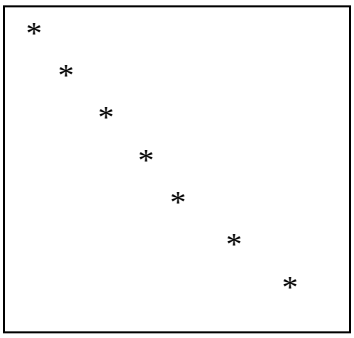
No correlation



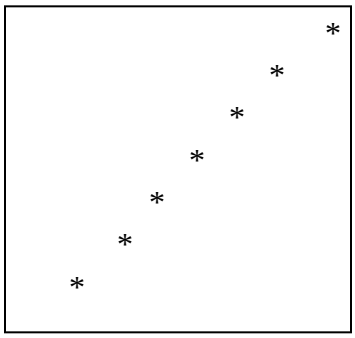
Negative correlation



Positive correlation



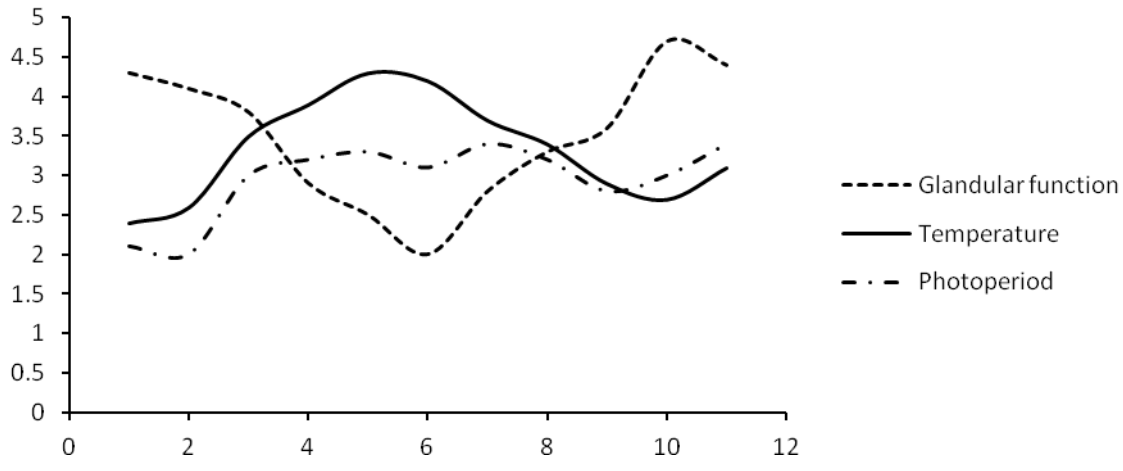
Perfect negative correlation



Perfect positive correlation

2. Graphic method

When graphic method is used the individual value of the variables are plotted on a graph paper. Thus, we obtain different curves for different variables.



Months

By examining the direction and closeness of the curves so drawn, we can infer whether or not the variables are related,

3. Karl Pearson's Method

By examining the direction and closeness of the curves so drawn, we can infer whether or not the variables are related,

4. Karl Pearson's Method

After establishing correlation with the help of scatter diagram and Graphic method we can measure the degree of correlation. There are several mathematical methods of measuring the correlation but the most widely used method is Karl Pearson's Method. Karl Pearson developed a coefficient known as Pearsonian Coefficient of correlation or simply the coefficient of correlation. It is symbolized by 'r'. it is independent of any unit. The formula for computation is –

$$r = \frac{\Sigma x y}{N \sigma_x \sigma_y}$$

Where $x = (X-\overline{X})$

$y = (Y-\overline{Y})$

N = Number of pair observation

σ_x = Standard deviation of series X

σ_y = Standard deviation of series X

Ans. 8

Binomial distribution

It is also known as Bernoulli's distribution as it was derived by the great Swiss Mathematician James Bernoulli. It is very useful distribution for dealing with discrete or discontinuous variates. The binomial distribution is a probability distribution, expressing the possibility of one set of dichotomous event. This distribution has been used to describe a wide variety of processes in business, social science, science as well as other areas. The type of process which gives rise to binomial distribution is usually referred to as Bernoulli trial or Bernoulli process. Bernoulli process is a process where an experiment is performed repeatedly yielding either a success or failure in each trial and where there is absolutely no pattern of occurrence of success or failure.

Bernoulli process is developed under a very specific set of assumptions as below-

1. The experiment is performed under the same condition for a fixed number of time say n
2. In each trial there is only two possible outcome of an experiment, a success or a failure.
3. The probability of success is denoted by 'p' and it remains constant from trial to trial. The probability of failure is denoted by 'q' and is equal to 1-p. If the probability of success is not the same, we will not have a Binomial distribution and the process and experimentation is no more Bernoulli process.
4. The trials are independent that is the outcome of any trial should not affect the outcome of successive trials.

How Binomial Distribution arises

If a coin is tosses, there are only two outcomes, namely Head or Tail.

Probability of head (success) = ½ (p)

Probability of tail (failure) = ½ (q)

Suppose two coins A and B are tossed together, we may have the following 4 probable outcomes

A B	A B	A B	A B
H H	H T	T H	T T
p p	p q	q p	q q
p^2	2pq		q^2

p^2, 2pq, and q^2 are similar to terms of binomial $(q+p)^2$ because $(q+p)^2 = q^2 + 2qp + p^2$

$(q + p)^3 = q^3 + 3q^2p + 3qp^2 + p^3$

$(q + p)n = q^n + {}^nC_1\, q^{n-1}p + {}^nC_2\, q^{n-2}p^2 + {}^nC_3\, q^{n-3}\, p^3 \ldots\ldots\ldots\ldots {}^nC_{n-r}\, q^{n-r}p^r + {}^nCn\, p^n$

0^{th} term 1^{st} term 2^{nd} term 3^{rd} term r^{th} term n^{th} term

Probability of getting four heads when six coins are tossed together-

$$(q + p)^6 = q^6 + 6q^5p + 15q^4p^2 + 20q^3p^3 + 15q^2p^4 + 6qp^5 + p^6$$

Probability of four heads

Probability of getting four heads = $15q^2p^4$

$= 15 \times (½)^2 \times (½)^4$

$= 15 \times ¼ \times 1/16$

$= 15/64$

Probability of getting four heads = 15/64