Notes

1

COMPOSITION OF BLOOD AND NORMAL ERYTHROPOIESIS

1.1 INTRODUCTION

Blood consists of a fluid component- **plasma**, and a cellular component comprising of red cells, leucocytes and platelets, each of them with distinct morphology and a specific function. **Erythrocytes** or red cells are biconcave discs. They do not have a nucleus and are filled with hemoglobin which carries oxygen to tissues and carbon dioxide from the tissues to the lungs. **Platelets** are small cells. They also do not have a nucleus and are essential for clotting of blood. **Leucocytes** play an important role in fighting against infection. All these cells arise from a single cell called as the **Hematopoietic stem cell**. The process of formation of these cells is called **Hematopoiesis.** In this lesson we will learn the different stages in the development of red cells. The process of formation of red cells is called **erythropoiesis.**

OBJECTIVES

After reading this lesson, you will be able to:

- explain the composition of blood
- describe various stages in the formation of red cells
- explain the precautions in handling blood and blood products
- explain steps for preventing injury from sharp items.

1.2 SITES OF HEMATOPOIESIS

It begins in the early prenatal period, within the first two weeks, in the **yolk sac** in the form of blood islands and is known as **primitive hematopoiesis**. The red cells formed at this time are nucleated and contain embryonic type of hemoglobin which differs in the type of globin chains from the adult hemoglobin.

Definitive hematopoiesis begins a little later from the mesodermal tissue located in the **aorta-gonad-mesonephros** region and the cell giving rise to all haematopoietic elements is the **Hematopoietic Stem Cell (HSC)**.

In **fetal life (upto the 3rd month)** the major site of hematopoiesis is the **liver** with some contribution from the **spleen.**

From the **4th month of fetal life** hematopoiesis begins in the **bone marrow** throughout the skeleton, and at the time of birth it is the **bone marrow** which is hematopoietically active with only few foci of hepatic hematopoiesis. Till puberty hematopoiesis is actively seen in all bones but in **adults** it is confined to the **axial skeleton** mainly to the ends of long bones and the flat bones such as ribs, sternum and pelvis. After birth hematopoiesis is mainly confined to the bones.

1.3 HEMATOPOIETIC STEM CELL (HSC)

The hematopoietic stem cell (HSC) is a pluripotent cell that is the common progenitor of all cellular elements in the blood i.e. red cells, granulocytes, monocytes, platelets and lymphocytes. The cell has the capacity of **self renewal(form more cells of it's type), proliferation(multiply rapidly) and differentiation** along all cell lineages, and morphologically resembles a small lymphocyte. These HSCs are used for transplantation.

Erythropoiesis

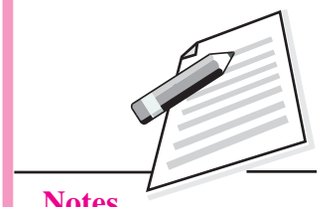
The erythroid progenitors develop from the HSC and give rise to **BFU- E (Burst Forming Units- Erythroid)and CFU –E (Colony Forming Units –Erythroid)** under the influence of a chemical **Erythropoietin (EPO)**.

Erythroblast is a term used for all forms of nucleated RBC. The least mature erythroid precursor cell is called a **proerythroblast.** It is a large cell with a rim of basophilic cytoplasm, a large nucleus which occupies most of the cell. Nuclear chromatin is coarse and prominent nucleoli are seen.

Basophilic/early normoblast This is smaller than the proerythroblast with a smaller nucleus but a more basophilic cytoplasm due to increased numbers of ribosomes in the cytoplasm. These ribosomes are involved in the production of hemoglobin. Nucleoli are not seen at this stage.

Polychromatic/intermediate normoblast This is the last precursor cell capable of mitosis and is smaller than the basophilic erythroblast. Its cytoplasm appears greyer due to the increased acidophilic staining caused by the presence of hemoglobin. Nuclear chromatin undergoes condensation.

Orthochromatic or late normoblast It is the smallest of the precursors and only slightly larger than a mature erythrocyte. Hemoglobinization is complete and so



the cytoplasm appears eosinophilic. The nucleus shrinks and the chromatin is greatly condensed giving the nucleus a homogeneous appearance.

As the proerythroblast matures through stages of basophilic, polychromatophilic and orthrochromatic erythroblasts, the cytoplasm becomes progressively more abundant and its colour changes from deep blue (due to high RNA content) to pink due to the increase in hemoglobin. The nucleus becomes smaller and dark staining(pyknotic).

Reticulocyte The cell is larger than a mature RBC, does not have a nucleus and has remnants of cytoplasmic ribosomal RNA which appear as a fine reticulum when stained with dyes such as new methylene blue and brilliant cresyl blue. On staining with Romansky stains it appears uniformly blue (polychromatophil). After its release from the marrow it remains in the spleen to undergo further maturation As the reticulocyte matures to an adult RBC it loses its ability to synthesize Hb.

The **mature RBC** is an anucleate biconcave disc lacking cytoplasmic organelles such as nucleus, mitochondria or ribosomes. Its major (90%) constituent is hemoglobin (Hb) which carries oxygen to tissues and carbon dioxide from the tissues. Besides Hb, it also contains enzymes required for energy production and for maintenance of Hb in a functional reduced state. The red cells are highly **deformable** under normal- physiological condition and this property allows them to pass easily through the sinusoids of the spleen.

The average **life span** of RBC is 100-120 days. After completion of their life span the RBC's are removed by the macrophages of liver and spleen(extravascular). A small fraction of RBCs may undergo destruction within the circulation that is intravascularly.

INTEXT QUESTIONS 1.1

1. Fluid component of blood is .....
2. ..... are filled with hemoglobin
3. ..... are essential for clotting of blood
4. ..... are essential for fighting against infections
5. Blood cells arise from ..... cells
6. The process of formation of blood cells is called .....
7. ..... cell is used for transplantation

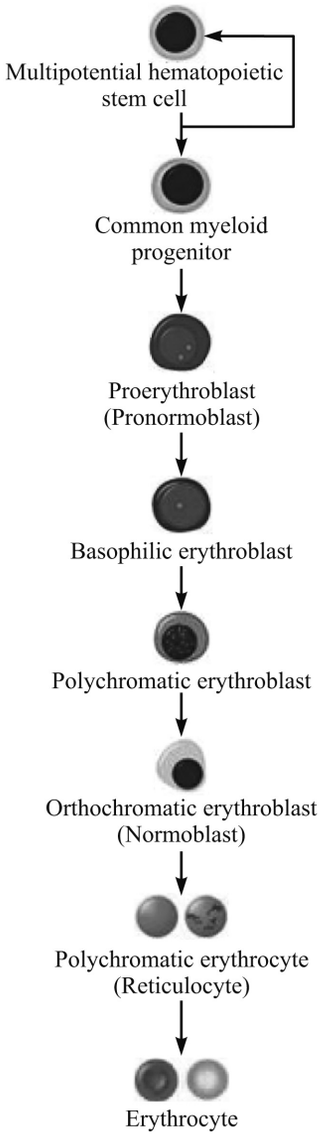
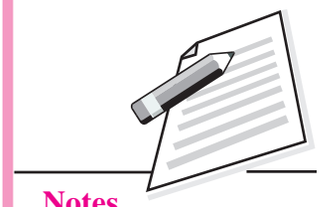


Fig. 1.1: Different stages in the formation of red cells. Note as the cell matures the size decreases, hemoglobinization occurs, the nucleus becomes small and dense

1.4 PRECAUTIONS IN HANDLING BLOOD AND BLOOD PRODUCTS

Human blood components, products made from **human** blood and certain other materials are treated and handled as if known to be potentially infectious and has a risk of transmitting infections such as HIV, HBV and others. Accordingly these are handled using precautions. This is called the **Universal precaution** (UP) approach.



Others which are handled using similar precautions are

- other body fluids containing visible blood, tissues, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid and amniotic fluid.
- any unfixed **tissue** or organ (other than intact skin) from a **human** (living or dead)
- HIV-containing cell or **tissue** cultures, organ cultures and HIV-containing culture medium or other solutions
- blood, organs or other tissues from experimental animals infected with HIV or HBV.

UP does not apply to feces, nasal secretions, sputum, sweat, tears, human breast milk, urine or vomitus unless they contain blood.

The **precautions** used are:

(a) Make full use of appropriate personal protective equipment (PPE).

(i) Use gloves when handling blood, blood products or other fluids for which UP are applicable (UP).

(ii) Use masks, safety goggles and/ or face shields for procedures that may involve splashing of blood or body fluids, creation of aerosols (or droplets of blood or body fluids) or exposure of mucous membranes or eyes, nose or mouth to same. (UP)

(iii) Use gowns, aprons, lab coats, coveralls and other protective body clothing needed to prevent exposure of body parts to blood, blood products or body fluids (UP). Note: Use protective caps, hoods and footwear in order to prevent exposure of head, hair or feet to blood or blood products (as may occur in autopsy) when required.

(iv) Use pocket masks or noncontact resuscitation bags or other regulation devices to resuscitate a patient to minimize exposure that may occur during emergency mouth-to-mouth resuscitation. (UP). Note: Remove contaminated PPE immediately or as soon as possible after completion of tasks or as soon as contaminated. Remove all PPE before leaving the work area.

Hand washing

Hands and other skin surfaces must be washed immediately after contact with blood.

Hands shall be washed each time gloves are removed.

Minimize splashing and splattering during hand washing.

To prevent injury from sharp items

- Use and dispose of needles, scalpels and other sharp items carefully.
- Use and clean instruments or devices after procedures cautiously.
- Do not bend, break or recap needles.
- Dispose off contaminated needles or sharps in a nearby specially designed puncture proof container immediately after use.

Biological Safety Cabinets (BSC)

BSC are required for procedures that may generate an aerosol (vortexing, grinding, blending, etc.).

Dermatitis

Employees who have exudative lesions or weeping dermatitis should refrain from handling blood until the condition resolves.

Exposure Occurrence

If an exposure incident to another person's blood occurs, immediately wash the exposed area with warm water and soap. If the exposed area is in the mouth, rinse mouth with water or mouthwash. If the exposure is in the eyes, flush with warm water or saline if available

Report the incident immediately with the following information:

- how, when and where the incident occurred and with whose blood/ secretions
- Your blood may then be tested for HIV but only with your consent.

The source individual's blood will also be tested if available and the results of the test will be made known to you.

INTEXT QUESTIONS 1.2

1. The average life span of the red cell is

   (a) 100 days (b) 105 days

   (c) 90 days (d) 120 days

2. Most of the red cells are removed after their life span in

   (a) Macrophages (b) The circulation

   (c) Both (d) Either

3. All of the statements are true of orthochromatic normoblast except

   (a) It is capable of mitosis (b) Hemoglobinization is complete

   (c) The nucleus is pyknotic (d) It is the smallest of the precursors

4. ..... is required for procedures that generate aerosol

5. ..... is used while handling blood and blood products

WHAT HAVE YOU LEARNT

- Erythroid cells develop from a hematopoietic stem cell in the marrow.
- The earliest identified erythroid precursor is the **Proerythroblast**.
- As the proerythroblast matures through stages of **basophilic, polychromatophilic and orthrochromatic erythroblasts**, the cytoplasm becomes progressively more abundant and its colour changes from deep blue (due to high RNA content) to pink due to the increase in hemoglobin. The nucleus becomes smaller and dark staining(pyknotic).
- **Reticulocyte** is the next stage. It is larger than the mature RBC, lacks a nucleus and has RNA remnants in the cytoplasm.
- The **mature RBC** is an anucleate biconcave disc which contains Hb, the oxygen carrier.
- The average **life span** of RBC is 100-120 days. After completion of their life span the RBC's are removed by the macrophages of liver and spleen(extravascular). A small fraction of RBCs may undergo destruction within the circulation that is intravascularly.

TERMINAL QUESTIONS

1. Define erythropoiesis
2. Explain the sites of hematopoiesis
3. Describe various stages of erythropoiesis

ANSWERS TO INTEXT QUESTIONS

1.1

1. Plasma
2. Erythrocytes
3. Platelets
4. Leucocytes
5. Hematopoietic
6. Hematopoiesis
7. Hematopoietic stem cell

1.2

1. 120 days
2. Macrophages
3. It is capable of mitosis
4. Biological safety cabinets
5. Personal protective equipements