Chapter-02

ERYTHROPOIESIS AND LABORATORY TECHNIQUES

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ABSTRACT

Erythropoiesis is the formation and production of erythrocytes. The journey of erythropoiesis starts from bone marrow. Hematopoietic stem cells differentiate into erythroid progenitors, eventually maturing into fully functional red blood cells. Erythropoiesis is essential for unraveling the complexities associated with hematologic disorders and anemia. Erythropoiesis at a molecular, cellular, and systemic level and Molecular biology tools, such as polymerase chain reaction (PCR) and gene expression analysis, allow researchers to dissect the genetic regulation of erythroid differentiation. Flow cytometry enables the precise characterization and isolation of specific erythroid cell populations, facilitating in-depth studies of their maturation and function. In the diagnosis and monitoring of erythropoietic disorders, these methods offer precise and reliable assessments of red blood cell parameters, contributing to the early detection and management of various hematologic conditions.

Keywords: *Erythropoiesis, Hematopoiesis, Hemocytometer, Myeloid Progenitor Stem Cell.*

2.1 INTRODUCTION

The term erythropoiesis means the formation or production of erythrocytes in the bone marrow (spongy tissue that present in the medullary cavities of bones). Up to the third month, erythropoiesis has in the yolk sac. From the third month to the fifth month, erythropoiesis has in the spleen and liver. This stage of erythropoiesis is called hepatic stage. And the formation or productions of erythrocytes through myeloid progenitor stem cells (Peschle C., 1980).

2.2 MECHANISM OF ERYTHROPOIESIS

Erythropoiesis is the process by which erythrocytes are produced. This process occurs primarily in the bone marrow, specifically in the red marrow of flat bones such as the sternum, ribs, and pelvis. The mechanism of erythropoiesis involves several stages, from the differentiation of hematopoietic stem cells to allow the erythrocytes into the bloodstream *(Aljurf M, et al. 1996).*

There are various steps of erythropoiesis:

1. **Multipotent Hematopoietic Stem Cell:** Erythropoiesis begins with hematopoietic stem cells that are pluripotent cells capable of generate into various blood cell types. These cells are found in the bone marrow.

2. **Proerythroblast Stage:** Under the influence of specific growth factors, particularly erythropoietin (EPO), mother cells differentiate into committed erythroid progenitor cells called proerythroblasts.

3. **Erythroblast Stages:** Proerythroblasts mature into erythroblasts, which are characterized by a reduction in cell size and the appearance of hemoglobin in the cytoplasm. As erythroblasts continue to mature, they go through several stages (basophilic erythroblast, polychromatophilic erythroblast, and orthochromatic erythroblast) with changes in nuclear and cytoplasmic morphology.

4. **Nuclear Ejection:** The nucleus of the orthochromatic erythroblast is extruded from the cell, resulting in a cell without a nucleus known as a reticulocyte. This process is necessary for the cell to become a functional red blood cell.

5. **Reticulocyte Maturation:** Reticulocytes are allowing to flow into the bloodstream and circulate for about 1-2 days.

6. **Erythropoietin (EPO) Regulation:** EPO is a hormone produced by the kidneys in response due to low oxygen levels in the blood and plays a crucial role in regulating erythropoiesis. In hypoxia, EPO production increases and stimulating the bone marrow to release lots of erythrocytes.

7. **Iron Metabolism:** Iron is a component of hemoglobin. During erythropoiesis increased demand of iron. The body regulates iron absorption in the small intestine and regulate from storage sites (such as the liver and spleen) to ensure an adequate supply for hemoglobin synthesis.

2.3 STRUCTURE OF ERYTHROCYTES

- Erythrocytes, commonly known as red blood cells (RBCs), are specialized cells in the blood that responsible for transporting oxygen from the lungs to the all-body tissues and carbon dioxide from the tissues back to the lungs. The structure of erythrocytes is adapted to their specific function (Gregory C. J. and Evaes A.C., 1997**)**.

- Erythrocytes have a distinctive biconcave disc shape, which provides a large surface area for gas exchange and flexibility to navigate through narrow capillaries.

- It lacks a nucleus, mitochondria, and other organelles, allowing more space for hemoglobin, the protein responsible for oxygen transport. The cytoplasm of erythrocytes is rich in hemoglobin, giving the cells their characteristic red color. Erythrocytes have a limited lifespan, typically around 120 days.

Figure-2.1: Hemocytometer (Source: Chinchilla Life Sciences)

2.4 DETERMINATION OF ERYTHROCYTE COUNT BY HEMOCYTOMETER

Erythrocyte counts are a must in hematology (the study of blood). Erythrocyte ***count*** is the estimation of the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). DLC is a manual and quantitative method (Bunn H. F., 1972).

2.4.1 Principle

The blood specimen is diluted 1:200 with the RBC diluting fluid and the erythrocytes counted by the compound microscope (at 40x objectives) by a counting chamber.

2.4.2 Method

- Erythrocyte count and each method have its advantages and applications and some major methods used for erythrocyte counting.
- **Hemocytometry Method:** A Hemocytometer is a specialized microscope slide with a grid etched on it. Blood is diluted with a specific solution, and a known volume of the dilution is kept at the ***Hemocytometer (Figure 2.1).*** Then cells are counted under a microscope and the count is extrapolated to determine the number of erythrocytes per microliter of blood.

2.4.3 Specimens

- When collecting blood specimens for erythrocyte analysis, it's important to follow proper procedures to ensure accurate and reliable results. some general method for collecting blood specimens for erythrocyte analysis.
- Capillary blood (the specimen need not be a fasting sample), EDTA blood.
- Use tubes containing anticoagulants like EDTA (ethylene diamine tetra acetic acid) or heparin. These anticoagulants prevent blood clotting and preserve the integrity of erythrocytes for analysis.

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2.4.4 Requirements

- Estimating the erythrocyte count is a component of a CBC. The RBC count measures the number of erythrocytes per microliter of blood. To estimate RBC, count accurately, certain requirements -

    (i) Microscope

    (ii) Improved Neubauer chamber

    (iii) RBC pipette or micro pipette (10, 50 ul)

    (iv) RBC diluting fluid

    (v) RBC diluting fluid, including components prepared as:

        (a) Sodium citrate, 3 g

        (b) Formalin, 1 ml

        (c) Distilled water, 100 ml

- Mix well and this solution keeps at room temperature (26 °C) for at least one year.

2.4.5 Procedure

i. Take 3.95 ml of RBC diluting fluid in a clean, grease-free test tube.

ii. Add 20 ul of blood specimen to the diluting fluid with micropipette or RBC pipette.

iii. Take out the counting chamber and clean it gently.

iv. Take the RBC pipette and fill it with the diluted specimen. Mix the solution well and then discard one to two drops from the pipette before loading the chamber.

v. Then touch the tip of the pipette with the hanging drop against the edge of the coverslip, making an angle of approximately 40 to 45^0.

vi. Then, allow a small amount of fluid from the pipette to fill the chamber without air bubbles.

vii. Then count the erythrocyte under the microscope at 40x objective.

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2.4.6 Calculations

- While there isn't a specific "calculation formula" for erythrocytes, you may be referring to parameters associated with erythrocytes in a blood test. Commonly measured parameters include.
- The total erythrocytes in a microliter (μL) of blood.
- Hemoglobin is the conjugated protein in red blood. It is often measured in grams per deciliter (g/dL) of blood.
- Hematocrit is the ratio of the volume of erythrocytes to the total volume of blood. It is usually calculated in percentage.
- RBC count Cu mm (ul) = *total number of cells counted x 10,000.*

2.4.7 Normal Ranges

- Normal values for erythrocytes may vary in the laboratory and the population being considered. However, here are general reference ranges for erythrocyte parameters
- **Male:** 4.5 to 6.0 million/cu mm (ul)
- **Female:** 4.0 to 4.5 million/cu mm (ul)

2.4.8 Clinical Significance

- **Increase:** Increase erythrocytes are several potential reasons and strategies. However, it's crucial to note that self-diagnosis and treatment can be risky. Always consult with a healthcare professional for personalized advice tailored to your specific situation.
- RBC rose in conditions such as hemoconcentration due to burns and dehydration. RBC also increases in chronic heart disease and conditions of decreased lung function, such as emphysema, and increases in polycythemia. The common causes of anemia are iron deficiency. Consuming iron-rich foods like red meat, poultry, fish, lentils, beans, and fortified cereals can help. Iron supplements may be prescribed by a doctor if necessary. Vitamin B12 is responsible for the production of red blood cells. Foods rich in B12 include meat, fish, dairy products, and fortified cereals. In some cases, B12 supplements or injections may be recommended. Folate (vitamin B9) is another important nutrient for erythrocyte

production. Foods high in folate include leafy green vegetables and citrus fruits and fortified cereals. Folate supplements may be prescribed in some cases.

- **Decrease:** A decrease in erythrocytes, or red blood cells, can be caused by various medical conditions, nutritional deficiencies, or environmental factors.
- Anemia is a condition characterized by a lower-than-normal number of red blood cells or a decrease in the amount of hemoglobin in the blood. Common types include iron-deficiency anemia, vitamin B12 deficiency anemia, and anemia of chronic disease. Some chronic disorders such as chronic kidney disease and inflammatory disorders, can affect the production of erythrocytes. Diseases affecting the bone marrow.

2.5 CONCLUSION

Erythropoiesis is a complex and finely regulated process that maintains the body's oxygen homeostasis. Understanding the various factors and stages of erythropoiesis is critical for understanding both normal physiological processes and pathological conditions involving red blood cell production. Erythrocyte counts are determined as MCV and MCH).

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