بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Post Graduate Studies

Evaluation study of Hemoglobin, Red Blood Cells and Red Blood Cells Indices among Workers at Fuel Stations at Khartoum State

دراسة تقييمية للهيموقلوبين ,كريات الدم الحمراء و مؤشرات كريات الدم الحمراء لدى العاملين في محطات الوقود بولاية الخرطوم

A thesis Submitted in Partial Fulfillment for the Requirements for the Degree of M.Sc. In Hematology and Immunohemtology

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بسم الله الرحمن الرحيم

الآية

قال تعالى:

إِنَّ فِي خَلْقِ السَّمَاوَاتِ وَالْأَرْضِ وَاخْتِلَافِ اللَّيْلِ وَالنَّهَارِ لَآيَاتٍ لِأُولِي الْأَلْبَابِ (190) الَّذِينَ يَذْكُرُونَ اللَّهَ

قِيَامًا وَقُعُودًا وَعَلَى جُنُوبِهِمْ وَيَتَفَكَّرُونَ فِي خَلْقِ السَّمَاوَاتِ وَالْأَرْضِ رَبَّنَا مَا خَلَقْتَ هَذَا بَاطِلًا

سُبْحَانَكَ فَقِنَا عَذَابَ النَّارِ (191)

صدق الله العظيم

(سورة آل عمران الآية 190-191)

Dedication

I dedicate this humble work to those who were in my support and encouragement:

My parents, may god preserve and care them.

My dear family and friends.

Special thanks to: Mydear husband

Special thanks to: Honorable supervisor ***Dr. Munsoor Mohammed Munsoor.***

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This research is made possible through the help of ALLAH, then the support from everyone including: parents, teachers, husband, sisters, friends, and in essence, all sentient being. Especially, please allow me to dedicate my acknowledgement of gratitude toward the significant advisors and contributors:

Firstly, I would like to thank the staff of faculty of Medical Laboratory Science of Sudan University of Science and Technology and everyone who contributed to my education.

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Finally, I would like to thank volunteers for giving me the chance to take the samples and data, also ***Al Maali Hospital*** *for their permission to testing the samples.*

Thank you.

Abstract

This study was carried out in Khartoum State in Al Maali hospital, in period from (May to July 2017) to evaluate some hematological parameters among petroleum station workers in Khartoum state, sixty of petrol stations workers in Khartoum,Bahri and Om durman cities were selected and sixty of other healthy individuals were selected as control group. Two and half ml of venous blood was withdrawn from each person, placed in ethylene di amine tetra acetate container, then agitator to mix samples with anticoagulant and that to measure Hb and RBCs indices , SPSS version 21 was used to analyze the results, the results obtained show that the mean values of Hb, RBCs, HCT, MCV, MCH and MCHC were (13.9) g/dl, $(5.16)\times10^{12}$/L, (44%), (85)fl, 27pg, and (33) g/dl respectively. The results showed no significant differences in these parameters when compared to control (P.value > .05) (0.070,0.067,0.060,0.090,0.083,0.095) respectively. The results of workers who were smokers had Hb (14.0)g/dl, RBCs $(5.14)\times10^{12}$/L, HCT (46%), MCV (88)fl, MCH(27) pg and MCHC(30)g/dl, the result showed no significant differences when compared with parameters of nonsmoker workers (p.value >0.05) (0.199, 0.761, 0.998, 0.598, 0.676, 0.480) respectively. According to employment duration from (<5), (5-10) and (>10) years, the results of Hb (14.0) ,(13.5)and(14.1)g/dl respectively. RBCs (5.20), (5.20) and (5.10) $\times$ 10^{12}/l, HCT (47), (47) and (48) %. MCV (90), (88) and (90) fl, MCH (27), (27) and (27) pg, MCHC (30), (30), and (30)g/dl),with no significant differences inregard to controls (P.value > 0.05(0.752, 0.309,0.325,0.376,0.117,0.341) respectively. This work concluded that no significant differences in studied parameters among the workers and control.

ملخص الدراسة

هذه دراسة اجريت في ولاية الخرطوم في مستشفى المعالي في الفترة من (مايو2017 الى يوليو2017) لتقييم بعض معاملات الدم عند السودانيين العاملين في محطات الوقود بولاية الخرطوم.اختير ستون شخص من عمال محطات الوقود في كل من (الخرطوم,الخرطوم بحري وامدرمان) واختير ستون شخص من الاصحاء كمجموعة ضبط.اخذت 2.5 مليلترمن الدم الوريدي من كل شخص ووضعت في وعاء محتوي على مانع تجلط ثنائي امين الايثيلين رباعي حمض الاسيتيت,ومن ثم دور في جهازالخلط لخلط العينات مع مكونات مانع التجلط لقياس الهيموقلوبين ومؤشرات كرات الدم الحمراء,وحللت النتائج بواسطة برنامج الحزم الاحصائيه للعلوم الاجتماعيه اصدار 21 وحسب متوسطات معاملات الدم لعمال محطات الوقود وكانت كلاتي: متوسط الهيموقلوبين (13.9) جرام لكل ديسيلتر,كرات الدم الحمراء (5.16)$\times 10^{12}$لكل لتر,هيماتوكريت (44)في المئه ,متوسط حجم الخليه (85) فيمتوليتر,متوسط هيموقلبوبين الخليه (27) بيكوجرام ومتوسط تركيزهيموقلوبين الخليه (33) جرام لكل ديسيلتر,اظهرت النتائج عدم وجود فروقات ذات دلاله احصائيه بين العمال ومجموعة الضبط (القيمه المعنوية اكبرمن 0.05) 0.095,0.083,0.090,0.060,0.067,0.070) .وفقا للتدخين اظهرت نتائج المدخنين متوسطات كلاتي:هيموقلوبين (14.0)جرام لكل ديسيلتر,كرات الدم الحمراء(5.14)$\times 10^{12}$لكل لتر,هيماتوكريت (46) في المئه,متوسط حجم خلية (88) فيمتولتر,متوسط هيموقلوبين الخليه (27) بيكوجرام ومتوسط تركيزهيموقلوبين الخليه (30) جرام لكل ديسيلتر,كم االظهرت النتائج ايضاعدم وجود فروقات ذات دلالة احصائيه بين المدخنين وغيرالمدخنين (القيمة المعنويه اكبرمن 0.05)(0.480,0.676,0.598,0.998,0.761,0.199) اماوفقا لفترات العمل التي قسمت لثلاثة فئات:- (<5),(5-10)و(>10) سنة المعاملات اظهرت نتائج كلاتي: هيموقلوبين (14.0),(13.5)و(14.1)جرام لكل ديسيلتر,كرات دم حمرا (5.20),(5.20)و(5.10)$\times 10^{12}$ لكل لتر,هيماتوكريت (47),(47)و(48)في المئة,متوسط حجم الخلية (90),(88)و(90) فيمتولتر ,متوسط هيموقلوبين الخلية (27) بيكوجرام لكل فئة ومتوسط تركيز هيموقلوبين الخليه (30)جرام لكل ديسيلترلكل فئه,ايضااظهرت النتائج عدم وجود فروقات ذات دلاله احصائية بين الفئات العمريه لعمال محطات الوقود ومجموعة الضبط (القيمة المعنوية اكبرمن 0.05)(0.752,0.309,0.325,0.376,0.117,0.341).نستنتج ان هذه الدراسة خلصت الى عدم وجود فروقات ذات دلاله احصائية بين العمال ومجموعات الضبط.

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ACGIH: American Conference of Government Industrial Hygiene.

AML: Acute Myeloid Leukemia.

ATP: Adenosine Tri phosphate.

ATSDR: Agency for Toxic Substances and Disease Registry.

CBC: Complete Blood Count.

CSFs: Colony Stimulating Factors.

DNA: De-oxyribo Nucleic Acid.

EDTA: Ethylene Diamine Tetra-Acetate.

EPO: Erythropoietin.

Hb or HBG: Hemoglobin.

HCT: Hematocrit.

HMP: Hexose Mono phosphate.

IDA: Iron Deficiency Anemia.

MCH: Mean Cell Hemoglobin.

MCHC: Mean Cell Hemoglobin Concentration.

MCV: Mean Cell volume.

NAD: Nicotinamide Adenine Dinucleotide.

OCL: Occupational exposure limit.

PCV: Packed Cell Volume.

PPM: Part Per Million.

PPP: Pentose Phosphate Pathway.

RBCs: Red Blood Cell.

RNA: Ribo Nucleic Acid.

ROS: Reactive Oxygen Species.

WBCs: White Blood Cells.

Chapter One

1-Introduction and Literature Review

1-Introduction and Literature Review

1.1General introduction:

Health effects of occupational exposure to Benzene and air pollution from vehicular sources are relatively unexplored among petrol filling workers. Neglect of ventilation in the work place or failure to use personal protective equipment when using petrol containing solvents like benzene will increase the incidence of toxic effects of benzene in humans, which includes haematotoxicity, immunotoxicity, neurotoxicity and carcinogenicity (Handin *et al*., 2007). Acute poisoning can lead to death with higher exposure associated with inflammation of respiratory tract and haemorrhage in the lungs. Various occupational solvents like benzene and atmospheric polluted air are absorbed into the human body either through the respiratory tract or via epidermal contact. These may cause primary respiratory symptoms and impaired pulmonary and dermatological functions (Aksoy *et al*., 2000).

Air pollutants and chemicals with known adverse effects like Benzene, Lead, other heavy metals, and Carbon monoxide (CO) and their metabolites can cause adverse health effects by interacting with molecules crucial to the biochemical or physiological processes of the human body (Aranyi *et al*., 2003). All these have been found to lead to deleterious effect on respiratory, endocrine and haematopoietic systems. The haematopoietic system, as the cells recycle continually, is highly sensitive to most of the air pollutants, which are reaching the blood very fast without being biotransformed. The solvents and air pollutants may interfere in the process of red blood cells proliferation.these changes are reflected in the synthesis of heme and the life expectancy of RBCs. Toxic material from air leads to significant damage to red blood cells causing aplastic anemia (Brief *et al.,* 2001). This would lead to increase erythropoises as compensation.

1.2 Literature Review:

1.2.1 Blood:

Blood is a circulating tissue composed of fluid plasmaand cells. It is composed of different kinds of cells these formed elementsof the blood constitute about 45% of whole blood. Theother 55% is blood plasma, a fluid that is the blood'sliquid medium, appearing yellow in color. The normal pH of human arterial blood is approximately 7.40 (normalrange is 7.35-7.45), a weak alkaline solution. Blood is about 7% of the human body weight, so the average adult has a blood volume of about 5 liters, of which 2.7-3 liters is plasma (Ziser, 2005).

When the formed elements are removed from blood, astraw-colored liquid called plasma is left. Plasma is about 91.5% water and 8.5% solutes, most of which byweight (7%) are proteins.. Some of the proteins inplasma are also found elsewhere in the body, but those confined to blood are called plasma proteins. These proteins play a role in maintaining proper blood osmotic pressure, which is important in total body fluid balance.Most plasma proteins are synthesized by the liver, including the albumins (54% of plasma proteins), globulins (38%), and fibrinogen (7%). Other solutes inplasma include waste products, such as urea, uric acid, creatinine, ammonia, and bilirubin; nutrients; vitamins; regulatory substances such as enzymes and hormones; gasses; and electrolytes. Serum is the liquid remaining after blood clots; it is essentially the same as plasma, except that the clottingfactors and fibrinogen have been removed. The cells of the blood canbe divided into erythrocytes (red blood cells), leukocytes (white blood cells) of various types, and thrombocytes (platelets) (Ziser, 2005).

1.2.2 Blood cell types and function:-

1.2.2.1 Erythrocyte(Red Blood Cell):-

The primary function of erythrocytes is gas exchange. They carry oxygen from the lungs to the tissues and return carbon dioxide (CO2) from the tissues to the lungs to be exhaled. Erythrocytes are anucleate cells containing few organelles; a large proportion of their cytoplasm consists of the ironcontaining oxygen transport molecule hemoglobin.(Handin *et al*., 2007) Erythrocytes are shaped like biconcave disks approximately 7 to 8 μ in diameter. The biconcave disk shape gives red blood cells (RBCs) the flexibility to squeeze their way through capillaries and other small blood vessels. Viewed under the microscope, RBCs look like a circle with a central hole, or central pallor, which is approximately one-third the diameter of the cell. Erythrocytes are the most common cells in blood. The normal RBC count is approximately 4.5 to 6 million cells per microliter. The parameters by which erythrocytes are usually measured are the blood hemoglobin (Hb) in grams per deciliter (g/dL), the hematocrit (Hct) or packed cell volume (volume of RBCs as a percent of total blood volume), and the RBC count (millions of cells per L) (Table 1.2.1.1). The size of red cells is measured as the mean corpuscular volume (MCV), reported in femtoliters (fL; 1 fL = 10–15 L). The normal MCV is ~80 to 100 fL. Red blood cells that are smaller than 80 fL are called microcytic; those that are larger than 100 fL are called macrocytic (Handin *et al*., 2007).Red cells have a life span of approximately 120 days; therefore, approximately 1% of red cells are replaced each day. Young red cells can be identified because they contain ribonucleic acid (RNA).With special stains such as new methylene blue, the RNA aggregates as visible particles called reticulin.Young RBCs containing RNA are designated as reticulocytes, and the number of reticulocytes in the peripheral blood

(reticulocyte count) is the best estimate of RBC production. Reticulocytes cannot be specifically identified on the usual blood smear stains, but they stain slightly moreblue (an appearance that is designated polychromasia) than older RBCs (Handin *et al.,* 2007)

Table (1.2.1.1): Approximate Normal Blood Values

Value	Male	Female
Hemoglobin(g/dL)	14–18	12–16
Hematocrit (%)	42–52	37–47
RBC count (106/L)	4.7–6.1	4.2–5.4
WBC count (103/L)	4.0–10.0	4.0–10.0
Platelet count (103/L)	150–400	150–400

*Values vary between different laboratories and different instruments. (Handin *et al*., 2007)

1.2.2.2 Leukocytes (White blood cell):-

They are a heterogeneous group of nucleated cells that are responsible for the body's defenses and are transported by the blood to the various tissues where they exert their physiologic role, e.g. phagocytosis. WBCs are present in normal blood in smaller number than the red blood cells (4.0-10.0 × 103/µl in adults). Their production is in the bone marrow and lymphoid tissues (lymph nodes, lymph nodules and spleen). There are five distinct cell types each with a characteristic morphologic appearance and specific physiologic role (Handin *et al*., 2007). These are polymorph nuclear leucocytes/granulocytes, neutrophils, eosinophils,basophiles and mononuclear leucocytes,Lymphocytes and Monocytes.

1.2.2.3 Platelets (Thrombocytes):-

These are small, non nucleated, round/oval cells/cell fragments that stain pale blue and contain many pink granules. Their size ranges 1-4μm in diameter. They are produced in the bone marrow by fragmentation of cells called megakaryocytes which are large and multinucleated cells. Their primary function is preventing blood loss from hemorrhage. When blood vessels are injured, platelets rapidly adhere to the damaged vessel and with one another to form a platelet plug. During this process, the soluble blood coagulation factors are activated to produce a mesh of insoluble fibrin around the clumped platelets. This assists and strengthens the platelet plug and produces a blood clot which prevents further blood loss. Normal range: 150-400 x 103 /μl(Handin *et al.,*2007).

1.2.2.4 Functions of blood:-

Blood has important transport, regulatory, and protective functions in the body.

Transportation: Blood transport oxygen form the lungs to the cells of the body and carbon dioxide from the cells to the lungs. It also carries nutrients from the gastrointestinal tract to the cells, heat and waste products away from cells and hormones form endocrine glands to other body cells. (Handin *et al*., 2007)

Regulation: Blood regulates pH through buffers. It also adjusts body temperature through the heat-absorbing and coolant properties of its water content and its variable rate of flow through the skin, where excess heat can be lost to the environment. Blood osmotic pressure also influences the water content of cells, principally through dissolved ions and proteins. (Handin et al., 2007)

Protection: The clotting mechanism protects against blood loss, and certain phagocytic white blood cells or specialized plasma proteins such as antibodies, interferon, and complement protect against foreign microbes and toxins (Handin *et al.,* 2007).

1.2.3 Hemopoieses and hemopoietic factors:

1.2.3.1 Hemopoiesis:

Hemopoiesis/hematopoiesis is refers to the formation and development of all types of blood cells from their parental precursors. In postnatal life in humans, erythrocytes, granulocytes, monocytes, and platelets arenormally produced only in the bone marrow.Lymphocytes are produced in the secondary lymphoid organs, as well as in the bone marrow and thymus gland. There has been much debate over the year's asto the nature of hemopoiesis. According to this theory, the main blood cell groups including the red blood cells, white blood cells and platelets are derived from a pluripotent stem cell (Anthony *et al*., 2009).This stem cell is the first in a sequence of regular and orderly steps of cell growth and maturation. The pluripotent stem cells may mature along morphologically and functionally diverse lines depending on the conditioning stimuli and mediators (colony-stimulating factors, erythropoietin, interleukin, etc.) and may either: Produce other stem cells and self-regenerate maintaining their original numbers, or Mature into two main directions: stem cells may become committed to the lymphoid cell line for lymphopoiesis, or toward the development of a multipotent stem cell capable of granulopoiesis, erythropoiesis and thrombopoiesis (Anthony *et al.*, 2009).

During fetal life, hemopoiesis is first established in the yolk sac mesenchyme and later transfers to the liver and spleen. The splenic and hepatic contribution is

graduallytaken over by the bone marrow which begins at four months and replaces the liver at term (Orkin and Zon,2008).From infancy to adult hood there is progressive change of productive marrow to occupy the central skeleton, especially the sternum, the ribs, vertebrae, sacrum, pelvic bones and the proximal portions of the long bones (humerus andfemurs).Hemopoiesis occurs in a microenvironment in the bone marrow in the presence of fat cells, fibroblasts and macrophages on a bed of endothelial cells. An extracellular matrix of fibronectin, collagen and laminin combine with these cells to provide a setting in which stem cells can grow and divide. In the bone marrow, hemopoiesis occurs in the extravascular part of the red marrow which consists of a fine supporting reticulin framework interspersed with vascular channels and developing marrow cells (Orkin and Zon, 2008). A single layer of endothelial cells separates the extravascular marrow compartment from the intravascular compartment. When the hemopoietic marrow cells are mature and ready to circulate in the peripheral blood, the cells leave the marrow parenchyma by passing through fine "windows" in the endothelial cells and emerge into the venous sinuses joining the peripheral circulation (Orkin and Zon, 2008).

1.2.3.2 Hematopoietic Regulatory Factors:-

In general it can be stated that hemopoiesis ismaintained in a steady state in which production of mature cells equals cell loss. Increased demands for cells as a consequence of disease or physiologic change are met by increased cell production (Orkin and Zon, 2008). Several hematopoietic growth factors stimulate differentiation along particular paths and proliferation of certain progenitor cells. Erythropoietin (EPO), a hormone produced mainly by the kidneys and in small amounts by the liver, stimulates proliferation of erythrocytes precursors, and thrombopoietin stimulates formation of

thrombocytes (platelets) (Zhu and Emerson,2002). In addition, there are several different cytokines that regulate hematopoiesis of different blood cell types. Cytokines are small glycoproteins produce by red bone marrow cells, leucocytes, macrophages, and fibroblasts. They act locally as autocrines or paracrines that maintain normal cell functions and stimulate proliferation. Two important families of cytokines that stimulate blood cell formation are called colony stimulating factors (CSFs) and the interleukins (Zhu and Emerson, 2002).

Table (1.2.3.2): Hematopoietic regulatory factors

Factor	**Function**
Stem Cell Growth Factor	Stimulates pluripotent hematopoietic stem cells (hemocytoblasts)
Interleukin-3 (multi-CSF*)	Stimulates pluripotent hematopoietic stem cells and progenitors of eosinophils,neutrophils, basophils, monocytes, andplatelets
Granulocyte-MacrophageCSF (GM-CSF)	Stimulates development of erythrocytes, platelets, granulocytes (eosinophils,neutrophils, and basophiles,), and monocytes.
Macrophage CSF (M-CSF)	Stimulates development of monocytes and macrophages
Granulocyte CSF (G-CSF)	Stimulates development of neutrophils
Interleukin-5	Stimulates development of eosinophils
Interleukin-7	Stimulates development of B-lymphocytes

*CSF= Colony stimulating factors (Zhu and Emerson, 2002)

1.2.4 Formation of Red blood cells (Erythropoiesis):-

Erythropoiesis is the formation of erythrocytes from committed progenitor cells through a process of mitotic growth and maturation. The first recognizable erythyroid cell in the bone marrow is the proerythroblast or pronormoblast, which on Wright or Giemsa stain is a large cell with basophilic cytoplasm and an immature nuclear chromatin pattern. Subsequent cell divisions give rise to basophilic, polychromatophilic, and finally orthochromatophilic normoblasts, which are no longer capable of mitosis (Atkinson *et al*., 2004). During this maturation process aprogressive loss of cytoplasmic RNA occurs as the product of protein synthesis, hemoglobin, accumulates within the cell; as a result the color of the cytoplasm evolves from blue to gray to pink. At the same time the nuclear chromatin pattern becomes more compact than clumped until, at the level of the orthochromatophilic normoblast, there remains only a small dense nucleus, which is finally ejected from the cell. The resulting a nucleate erythrocyte still contains some RNA and is recognizable as a reticulocyte when the RNA is precipitated and stained with dyes such as newmethylene blue. Normally, reticulocytes remain within the bone marrow for approximately 2 days as they continue to accumulate hemoglobin and lose some of their RNA. The reticulocyte then enters the peripheral blood, were, after about one more day, it loses its residual RNA and some of its excessive plasma membrane and becomes indistinguishable from adult erythrocytes. Under normal conditions the transit time from the pronormoblast to the reticulocyte entering the peripheral blood is about 5days (Atkinson *et al*., 2004).

1.2.5 Causes that stimulate red blood cell production:-

Causes include: Hemorrhage, Damage to bone marrow, Exposure to high altitude, Exercise, Hemolytic disease and Low hemoglobin levels (Jain, 2007).

1.2.6 Morphology of the red cells and their precursors and regulation of erythropoiesis:-

1.2.6.1 RBCs precursors morphology:-

A. Pronormoblast (Rubriblast)

Pronormoblast is the earliest morphologically recognizable red cell precursor. Size: 20-25μm in diameter.

Nucleus: large, round to oval and contains 0-2 light bluish, indistinct nucleoli. The chromatin forms a delicate network giving the nucleus a reticular appearance.

Cytoplasm: there is a narrow (about 2μm) rim of dark blue cytoplasm. There may be a perinuclear halo. The nuclear/cytoplasm ratio is about 8:1 (Jain, 2007).

B. Basophilic Normoblast:-

Size: 16-18μm in diameter.

Nucleus: round or oval and smaller than in the previous stage. The chromatin forms delicate clumps so that its pattern appears to be denser and coarser than that seen in the pronormoblast. No nucleoli are seen.

Cytoplasm: slightly wider ring of deep blue cytoplasm than in the pronormoblast and there is a pirenuclear halo. The nuclear/cytoplasm ratio is about 6:1(Jain, 2007).

C. Polychromatophilic Normoblast:-

Size: 12-14μm in diameter

Nucleus: smaller than in the previous cell, has a thick membrane, and contains coarse chromatin masses.

Cytoplasm: as the nucleus is shrinking the band of cytoplasm is widening. It has a lilac (polychromatic) tintbecause of beginning of hemoglobinization. The nuclearcytoplasmic ratio varies from 2:1 to 4:1 (Jain, 2007).

D. Orthochromatic Normoblast:-

Size: 10-12μm in diameter.

Nucleus: small and central or eccentric with condensedhomogeneous structure less chromatin. It is ultimatelylost by extrusion.

Cytoplasm: a wide rim of pink cytoplasm surrounds theshrinking nucleus. The entire cell is somewhat smaller than the polychromatophilic normoblast. The nuclear / cytoplasmic ratio varies from 1:2-1:3 (Jain, 2007).

E. Reticulocyte:-

After the expulsion of the nucleus a large somewhat basophilic anuclear cell remains which when stained with new methylene blue, is seen to contain a network of bluish granules. This network is responsible for the name of the cell and consists of precipitated ribosomes. As the bone marrow reticulocyte matures the network becomes smaller, finer, thinner, and finally within 3 days disappears. About 1% of reticulocytes enter the peripheral circulation.
Size: 8-10μm in diameterNucleus: the reticulocyte does not contain a nucleus.
Cytoplasm: faintly basophilic (blue) (Jain, 2007).

F. Mature erythrocyte:-.

Size: 7-8µm in diameter

Cytoplasm: biconcave, orange-pink with a pale staining center occupying one-third of the cell area (Jain, 2007).

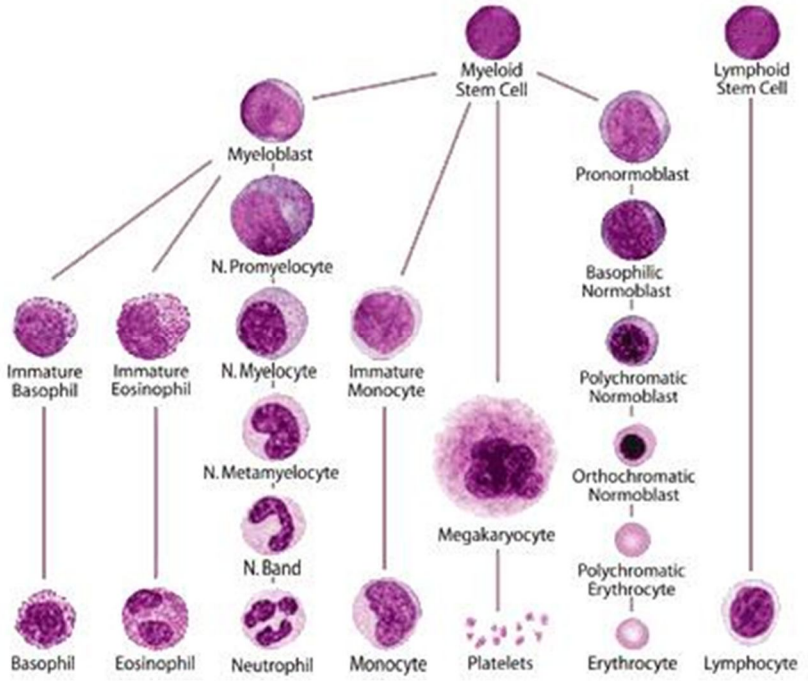

Figure 1.2.6: Blood series morphology (Jain, 2007)

1.2.6.2 Regulation of Erythropoiesis:-

Erythropoietic activity is regulated by the hormoneerythropoietin which in turn is regulated by the level of tissue oxygen. Erythropoietin is a heavily glycosylated hormone (40% carbohydrate) with a polypeptide of 165aminoacids. Normally, 90% of the hormone is produced in the peritubular (juxtaglomerular) complex of thekidneys and 10% in the liver and elsewhere (Palis and Segal, 1998). There are no preformed stores of erythropoietin and the stimulus tothe

production of the hormone is the oxygen tension inthe tissues (including the kidneys).

When there is tissue airhypoxia due to: Low blood hemoglobin levels (e.g., anemia), Imped oxygen release from hemoglobin for somestructural or metabolic, defects (e.g., the hemoglobinopathies), Poor blood flow as in severe circulatory defects and Low atmospheric oxygen (e.g., high altitude) Erythropoietin production increases and this stimulateserythropoiesis by increasing the number of progenitorcells committed to erythropoiesis(Palis and Segal, 1998).

Erythropoietin accelerates nearly every stage of red cell production: It increases the rate at which the committed stem cells divide and differentiate, It increases the rate of cell division, It speeds up the incorporation of iron into the developing red cells, It shortens the time cell maturation, and hastens the entry of reticulocytes into theperipheral circulationSimilarly, increased oxygen supply to the tissues due to: Increased red cell mass (e.g., polycythemia),Ability of hemoglobin to release oxygen to thetissues more readily than normal reduces the Erythropoietin drive (Palis and Segal,1998).

1.2.7 Ineffective erythropoiesis:-

Erythropoiesis is not entirely efficient since 10-15% of eryhtropoiesis in a normal bone marrow is ineffective, i.e., the developing erythroblasts die within the marrow without producing mature cells. Together with their hemoglobin, they are ingested by macrophages. This process is substantially increased in a number of anemias (Palis & Segal, 1998).

1.2.8 Hemoglobin:-

1.2.8.1 Hemoglobin structure:

Hemoglobin is anoligomeric metalloprotein / chromoprotein, It consists of four polypeptide chain, each with its own Heme. I.e. Hemoglobin = 4 Heme + 4 Globin chains. Hemoglobin tetramer is composed of two identical dimers, α1β1 and α2β2.The two globin chains within each dimer are held tightly together by inter chain hydrophobic interactions between α and β subunits, The two dimers are held together primarily by polar bonds and able to move with respect to each other,The weaker interactions between these mobile dimers result in two different relative positions in Deoxyhemoglobin compared to Oxyhemoglobin (Handin *et al*.,2007).

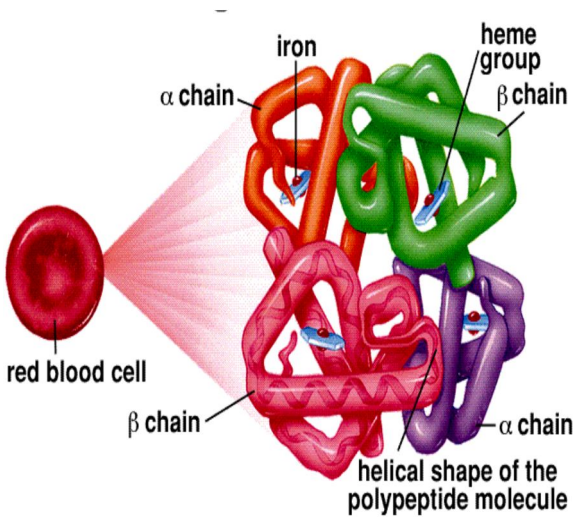

Figure 1.2.8: Hemoglobin (Jain, 2007)

Heme is a cyclic tetrapyrrole i.e. consists of four molecules of pyrrole, this imparts a red color. There are methyl (M), vinyl (V) and proprionate (Pr) groups

attached to it Globin Chains. There are four globin chains in each molecule of adult hemoglobin (Hb-A). These are designated as α and β.

There are two α (α1 α2) and two β (β1 β2) chains, these four chains form two dimers: i.e. α1 β1 and α2 β2

Hb-A (adult hemoglobin) consists of: β1, β2 (two beta chains) and α1, α2 (two alpha chains) (Ferrari & Bianzoni, 1997).

1.2.8.2 Non pathological variants of Hemoglobin:-

In the embryo: Gower 1 (ζ2ε2), Gower 2 (α2ε2) and Hemoglobin Portland (ζ2γ2).In the fetus: Hemoglobin F (α2γ2).In adults: Hemoglobin A (α2β2) The most common with a normal amount over 95%, Hemoglobin A2 (α2δ2) - δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5-3.5% and Hemoglobin F (α2γ2) - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells. However, the level of Hb F can be elevated in persons with sickle-cell disease and beta-thalassemia (Handin *et al.*, 2007).

1.2.8.3 Synthesis of Hemoglobin:-

Synthesis of hemoglobin begins in the proerythroblasts and continues even into the reticulocyte stage of the red blood cells.The process begins in the mitochondrion with the condensation of succinyl-CoA and glycine to form 5-aminolevulinic acid. Succinyl-CoA, then binds with glycine to form a pyrrole molecule. In turn, four pyrroles combine to form protoporphyrin IX, which then combines with iron to form the heme molecule. Finally, each heme molecule combines with a long polypeptide chain, a globin synthesized by ribosomes, forming a subunit of hemoglobin called a hemoglobin chain. Each chain has a

molecular weight of about 16,000; four of these in turn bind together loosely to form the whole hemoglobin molecule (Maton *et al*., 2006).

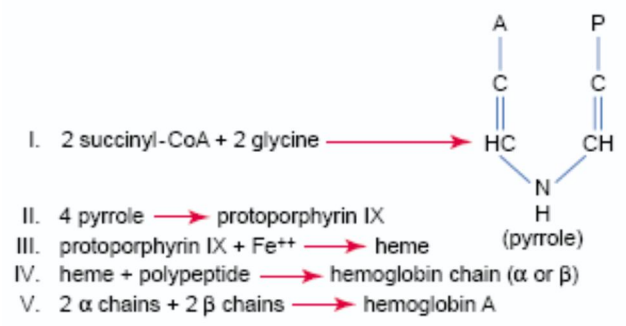

Figure 1.2.8.2 Hemoglobin synthesis (Maton *et al*., 2006)

1.2.8.4 Oxygenation of Hemoglobin:-

Hemoglobin molecule can bind four O2 molecules (one per heme). Hb exhibits cooperative binding kinetics i.e. if O2 is already present, binding of subsequent O2 molecules occurs more easily. This permits to bind maximum quantity of O2 at lungs (PO2 = 100 mm Hg) and to deliver a maximum O2 at peripheral tissues (PO2 = 20 mm Hg). This is shown by a sigmoid curve of oxygen dissociation of hemoglobin. The oxygen binding characteristics of hemoglobin change in response to binding of various allosteric modulators via: The partial pressure of O2, pH of the surrounding medium and Presence of 2,3-diphosphoglycerate (Maton *et al*.,2006).

1.2.8.5 Functions of Hemoglobin:

Transport of O2 from lungs to tissues.Transport of CO2 and H+ from tissues to the lungs for excretion.Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism (Hall, 2010).

1.2.9 Red blood cell metabolism:

RBCs contain nomitochondria, so there is norespiratory chain, nocitric acid cycle, and nooxidation of fatty acids or ketone bodies. The RBC is highly dependent upon glucose as its energy source. Energy in the form of ATP is obtained only from the glycolytic breakdown of glucose with the production of lactate (anaerobic glycolysis).ATP produced being used for keeping the biconcave shape of RBCs & in the regulation of transport of ions & water in and out of RBCs. (Na+–K+-ATPase and the anion exchange protein)(Bianconi and Piovesan,2013).

1.2.9.1Glucose transport through RBC membrane:

Glucose is transported through RBC membrane by facilitated diffusion through glucose transporters (GLUT-1).Glucose transporters (GLUT-1) are independenton insulin i.e. insulin does not promote glucose transport to RBCs.It functions by generating a gated pore in the membrane to permit passage of glucose(Bianconi & Piovesan,2013).

1.2.9.2 Glycolysis:

Glucose is metabolized in RBCs through anaerobic glycolysis (that requires nomitochondria and nooxygen).One molecule of glucose yields 2 molecules of ATP by one anaerobic glycolytic pathway. In addition, 2 molecules of lactate are produced. Lactate is transported to blood & in the liver it converted to glucose (Rodi *et al*., 2008).

1.2.9.3Anaerobic Glycolysis:

Importance of glycolysis in red cells: Energy production: it is the only pathway that supplies the red cells with ATP.Reduction of methemoglobin: glycolysis

provides NADH for reduction of metHbby NADH-cytob5 reductase.In red cells 2,3 bisphosphoglyceratebinds to Hb, decreasing its affinity for O2, and helps its availability to tissues(Erich,2000).

1.2.9.4 Production of 2,3 biphosphoglycerate(2, 3-BPG):

In RBCs, some of glycolysis pathways are modified so that 2, 3 bisphosphoglycerate is formed (by bisphosphoglyceratemutase).2, 3 bisphosphoglycerate decreases affinity of Hbfor O2. So, it helps oxyhemoglobin to unload oxygen.Storing blood results in decrease of 2,3-BPG leading to high oxygen affinity Hb. This leads to oxygen trap (Murray *et al*., 2006).

1.2.9.5 Pentose phosphate pathway (HMP-SHUNT)

RBCs contain an active pentose phosphate pathway (PPP) for glucose that supplies NADPH (PPP is the only source for NADPH in RBCs.NADPH is important in keeping glutathionein the reduced glutathione.Reduced glutathione plays a very important role in the survival of the red blood cells. (prevents oxidation of membrane).The erythrocytes contain carbonic anhydraseCarbon dioxide combines with water only after it enters the red cells where hemoglobin, the most important buffer for carbonic acid, is present.CO2+ H2O □HCO3-+ H+.The red cells also contain rhodanese enzyme responsible for the detoxification of cyanides(Bianconi & piovesan, 2013).

1.2.10 RED CELL MEMBRANE STRUCTURE:

About 50% of membrane is protein, 40% is fat and up to 10% is carbohydrate. RBCs membrane comprises a lipid bilayer (which determine the membrane fluidity), proteins (which is responsible for flexibility) that are either peripheral or integral penetrating the lipid bilayer & carbohydratesthat occur only on the

external surface (Uzoigwe *et al.*, 2006).Major Phospholipids are:Phosphatidylcholine (PC),Phosphatidylethanolamine (PE), and Phosphatidylserine (PS) along with Sphingomyelin (Sph).The choline-containing phospholipids, predominate in the outer leaflet.Phosphatidyl Choline and Sphingomyelin.The amino-containing phospholipids predominate in the inner leaflet.Phosphatidyl Ethanolamine and Phosphatidyl Serine.Glycosphingolipids (GSLs) 5–10%(Neutral GSLs, gangliosides, and ABO blood group substances (Uzoigwe,2006).The membrane skeleton is four structural proteins that include α&βspectrin, ankyrin, protein 4.1 & actin.Spectrinis major protein of the cytoskeleton & its two chains (α&β) are aligned antiparallel manner.α&βchains are loosely interconnected forming a dimer, one dimer interact with another, forming a head to head tetramer.Ankyrinbinds spectrin and in turn binds tightly to band 3securing attachment of spectrinto membrane.Band 3 is anion exchange protein permits exchanges of Cl-for HCO3+.Actinbinds to the tail of spectrin& to protein 4.1 which in turn binds to integral proteins, glycophorinsA, B & C which are transmembraneglycoproteins; Defects of proteins may explain some of the abnormalities of shape of RBCs membrane as hereditary spherocytosis and elliptocytosis(Uzoigwe *et al*., 2006).

Fate of RBC:-

When RBCs reach the end of their lifespan, the globin is degraded to amino acids (which are reutilized in the body), the iron is released from heme and also reutilized, and the tetrapyrrole component of heme is converted to bilirubin, which is mainly excreted into the bowel via the bile (Uzoigwe *et al.*,2006).

1.2.11 Protection of Blood Cells from Oxidative Stress & Damage:-

Reactive oxygen species (ROS): Oxidants produced during metabolism, in blood cells and most other cells of the body include: Superoxide (O2.),.hydrogen peroxide (H2O2), peroxyl radicals (ROO·), and hydroxyl radicals (OH·)Free radicals are atoms or groups of atoms that have an unpaired electron.OH·is a particularly reactive molecule and can react with proteins, nucleic acids, lipids, and other molecules to alter their structure and produce tissue damage(Rodi *et al*.,2008).

1.3 Fuel oil:

Fuel oils are petroleum products that are used in many types of engines, lamps, heaters, furnaces, stoves, and as solvents. It's come from crude petroleum and is refined to meet specifications for each use. Fuel oils are mixtures of aliphatic (open chain and cyclic compounds that are similar to open chain compounds) and aromatic (benzene and compounds similar to benzene) petroleum hydrocarbons (Herny, 2001). In addition, they may contain small amounts of nitrogen, sulfur, and other elements as additives. Fuel oils are distinguished from each other primarily by their boiling point ranges, chemical additives, and uses. All fuel oils are liquids at room temperature, although they can evaporate. The rates at which the various fuel oils will evaporate are dependent on the temperature and the composition of the individual fuel oil. Most fuel oils are yellowish to light brown in color. They generally have a kerosene-like odor, are flammable, and burn at temperatures between 177°C and 329°C (Herny,2001).`

1.3.1Fuel oil and environment:-

Fuel oils are composed of a large number of different chemicals, and each fuel oil is a slightly different mixture of these chemicals. Some of these chemicals

evaporate into the air when fuel oils are spilled onto soils or surface waters (e.g., streams, rivers, lakes, or oceans) or are stored in open containers (Bebarta and Dewitt, 2004). Other chemicals in the fuel oils dissolve in water following spills to surface waters or leaks from underground storage tanks. Some of the chemical constituents of fuel oils may slowly move down through the soil to the groundwater. Another group of chemicals in fuel oils can attach to particles in the soil or water and, in water, may sink down into the sediment. The chemicals that evaporate may break down in air by reacting with sunlight, e.g., photooxidation, or other chemicals in the air. The chemicals that dissolve in water may also be broken down by organisms (primarily bacteria and fungi) in the soil or water. However, this may take up to a year to occur, if ever, depending on the environmental conditions. Chemicals that attach to soil or other matter (e.g., marsh sediment) may remain in the environment for more than a decade (Bebarta and Dewitt, 2004).

1.3.2Exposure:-

The most likely way for you to be exposed to fuel oils in the home is if you use a kerosene heater. If you handle fuel oils or use a fuel oil to clean equipment at your job, or if fuel oils are stored at your workplace, you may also be exposed to them through contact with the skin or in the air. Some workers may be exposed to fuel oils through their skin if they come into contact with them without adequate protection, such as gloves, boots, coveralls, or other protective clothing. There are no data on background levels of fuel oils that may be found in the environment or workplace. (Concawe, 1985).

You may also be exposed to fuel oils if you swim in waters where fuel oils have been spilled. If fuel oils have leaked from underground storage tanks and entered underground water, you may drink contaminated water from well containing fuel oils. The vapor (the gas phase) of fuel oils can also move through the soil and

enter basements of homes or buildings near areas where leaks have occurred. Children may also be exposed by playing in soil contaminated with fuel oils. A major pathway of exposure is washing one's hands with fuel oils to remove paint, grease, etc (Concawe, 1985).

The interested fuel oil is benzene.

1.3.3Benzene:-

Benzene, also known as benzol, is a colorless liquid with a sweet odor. Benzene evaporates into air very quickly and dissolves slightly in water. it is highly flammable. Most people can begin to smell benzene in air at approximately 60 parts of benzene per million parts of air (ppm) and recognize it as benzene at 100 ppm. Most people can begin to taste benzene in water at 0.5–4.5 ppm. One part per million is approximately equal to one drop in 40 gallons. Benzene is found in air, water, and soil. Benzene comes from both industrial and natural sources (McDermott *et al*., 1999).

1.3.4 Chemical structure:-

The chemical formula of benzene is C6H6, so it has six carbon (C) atoms and six hydrogen (H) atoms. Its chemical structure can be described as a hexagon ring with alternating double bonds, as shown in this illustration.

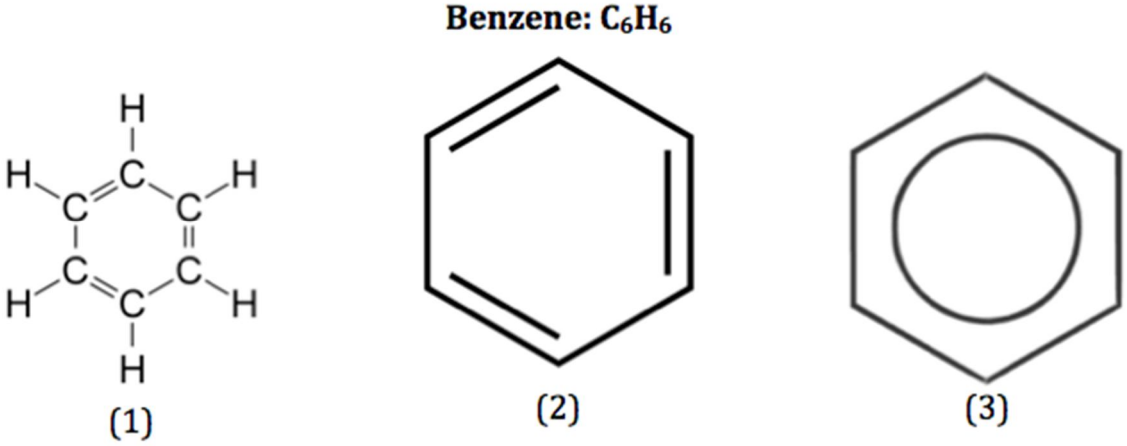

Figure 1.3.4 Benzene structure (Lamma and Grun, 2006)

The chemical structure of benzene shows that for each carbon atom, there is one hydrogen atom. There are three ways to draw the chemical structure of benzene. The illustration on the left (1), shows all the carbon and hydrogen atoms and how they are bonded together. The second illustration, (2), is also another way to draw benzene, where each edge of the hexagon corresponds to each carbon atom in the structure, and the hydrogen bonds are not shown. The third illustration, (3), shows that a circle can be drawn in place of these alternating double bonds. Because of its chemical formula, C6H6, benzene is classified as a hydrocarbon, which is a compound that consists of only carbon and hydrogen atoms. Its structure and formula reveal benzene to be an aromatic hydrocarbon, which is defined as a compound that is composed of hydrogen and carbon that has alternating double bonds forming a ring (Lamma and Grun, 2006).

1.3.5Benzene exposure limits:-

Occupational exposure limits (OEL) are set to protect workers from excessive exposure to toxic chemicals in the workplace. An OEL defines the maximum average concentration of a chemical in the breathing zone acceptable for anormal 8-hour working day for 5 days a week. The OEL is often accompanied by a short-term exposure limit, which is the maximum average concentration to which workers should be exposed for a short period of time (usually 15 minutes). As the hematotoxic and leukemogenic effects have been identified at ever-lower levels, the OEL for benzene has been extensively revised and reduced from 100 parts per million (ppm) in 1946 to values ranging from 0.1 to 1 ppm in 2008 (Capleton and Levy,2005). The American Conference of Governmental Industrial Hygienists set a Threshold Limit Value of 0.5 ppm in 1997, and the European Union has established a legal binding limit value of 1 ppm (Bratveit *et al*., 2007).

1.3.6Sources of exposure to benzene:-

Benzene is highly volatile, and most exposure is through inhalation. Benzene is degraded rapidly in the upper atmosphere. Because of its solubility in water, a minor amount may be removed by rain to contaminate surface waters and soil. However, it is not persistent in surface water or soil, either volatilizing back to air or being degraded by bacteria (Lamma and Grun, 2006).

1.3.6.1 Occupational exposure to benzene:

Industrial processes: As benzene occurs naturally in crude petroleum at levels up to 4 g/l, human activities using petroleum lead to exposure. These activities include processing of petroleum products, coking of coal, production of toluene, xylene and other aromatic compounds, and use in industrial and consumer products, as a chemical intermediate and as a component of petrol (gasoline) and heating oils. The presence of benzene in petrol and as a widely used industrial solvent can result in significant occupational exposure and widespread emissions to the environment. Automobile exhaust accounts for the largest source of benzene in the general environment. Off-gassing from building materials and structural fires lead to increased atmospheric benzene levels. Industrial discharge, landfill leachate and disposal of benzene-containing waste are also sources of exposure (Capleton and Levy, 2005).

1.3.6.2 Non occupational related sources of benzene:

Indoor residential air: Benzene has been detected at high levels in indoor air. Although some of this exposure might be from building materials (paints, adhesives, etc.), most is from cigarette smoke in both homes and public spaces. Levels of benzene are higher in homes with attached garages than in those with detached garages. Levels are increased in homes close to petrol filling

stations.Benzene may be released to indoor air from unfluid oil heating and from the use of benzene-containing consumer products in residences. People spending more time indoors, such as children, are likely to have higher exposure to benzene (Capleton and Levy, 2005)

Inside vehicles: Benzene has been measured in air inside vehicles at levels higher than those in residential air, but substantially lower than those at petrol filling stations.

Food and water: Waterborne and foodborne benzene contributes only a small percentage of the total daily (Capleton and Levy, 2005).

1.3.7 Toxic kinetics of benzene:

1.3.7.1 Absorption:

Inhalation is the most important route of absorption during occupational exposure to benzene. Humans absorb 30– 52% of inhaled benzene, depending on the benzene concentration, length of exposure and pulmonary ventilation (Pekari k *et al*., 1992). Benzene penetrates skin (Mojtahedi and Maibach, 2008). However, dermal absorption of benzene is not extensive, as it evaporatesquickly due to high vapor pressure. Hence, under normalworking conditions, dermal absorption of benzene is probably of minor importance (Mojtahedi and Maibach, 2008).

1.3.7.2 Metabolism:

The liver is the major site of metabolism of benzene (Synder and Hedli, 1996).Benzene is detoxified in two phases. During phase I, benzene is oxidized by cytochrome P450 2E1, forming benzene oxide, an electrophilic reactive intermediate. Subsequently, benzene oxide is metabolized bythree pathways (Synder and Hedli, 1996):

1) Rearrangement non-enzymatically to form phenol;

2) Hydration by epoxide hydrolase to 1,2-benzene dihydrodiol, which in turn can be oxidized by dihydrodiol dehydrogenase to form catechol.

3) Glutathione conjugation with glutathione *S*-transferase to form a premercapturic acid, which is converted to phenylmercapturic acid. Phenol can undergo subsequent hydroxylation to hydroquinone, with the consecutive production of *p*-benzoquinone and 1,2,4-trihydroxybenzene. Alternatively, phenol can be hydroxylated to catechol, which is converted to *o*benzoquinone. The benzene ring can also be opened either at the benzene oxide or oxepin stage, forming muconaldehyde. All these metabolites can then undergo a phase II metabolism, leading to excretion of glucuronide and sulfate conjugates, mercapturic acid ring-opened metabolites and DNA adducts in urine (Synder and Hedli, 1996).

1.3.7.2.1 Production of toxic metabolites in target organs:

Benzene itself is not regarded as a toxic substance. Benzene toxicity is believed to involve biological interactions of multiple reactive benzene intermediates with multiple cellular targets within the bone marrow. Especially hydroquinone, *p*-benzoquinone, catechol and muconaldehyde, alone or in combination, are reported to be the most potent metabolites in producing hematotoxicity (Witz*et al*., 1996). Beside the enzyme CYP 2E1, the bone marrow contains several peroxidases; the most prevalent is myeloperoxidase (Tsuruta *et al*., 1999). Phenol, catechol and hydroquinone are transported to the bone marrow, where myeloperoxidase is responsible for converting these metabolites to several biologically reactive quinones (Tsuruta *et al*., 1999).

1.3.7.2.2. **Nonlinear benzene metabolism:**

The production of the major benzene metabolites, as well as albumin adducts of benzene oxide and benzoquinones, exhibit a nonlinear dose-response relationship

attributable to saturated metabolism of benzene. For the *S*phenylmercapturic acid there was an increasing production along with increasing benzene exposure. However, for all major metabolites competing for the cytochrome P450 2E1system, such as phenol, catechol, hydroquinone and muconic acid, there was in fact a decreasing trend after a transition level around 0.03 ppm (Kim *et al*.,2006). Above this level the production of catechol and phenol dropped by 4.4 and 16-fold already when reaching exposure of 0.27 ppm, while the reduction for hydroquinone and muconic acid was only marginal. Hence, at low doses (below 1 ppm) the metabolism favored the production of hydroquinone and muconic acid. Hydroquinone is the precursor of the toxic 1,4-benzoquinone, whereas muconic acid is derived from the extremely reactive and toxic muconaldehydes. From these results, it was concluded that workers exposed to benzene below 0.1 ppm metabolize benzene about nine times more efficiently and therefore more adversely than do heavily exposed workers (Kim *et al*., 2006).

1.3.8 Benzene effects on health:

Several factorsdetermine whether harmful health effects will occur, as well as the type and severity of suchhealth effects. These factors include the amount ofbenzene to which you are exposed and the length oftime of the exposure. Most information on effectsof long-term exposure to benzene is from studiesof workers employed in industries that make or usebenzene. These workers were exposed to levels ofbenzene in air far greater than the levels normallyencountered by the general population.Brief exposure (5–10 minutes) to very high levels of benzene in air (10,000–20,000 ppm) can result in death. Lower levels (700–3,000 ppm) can cause drowsiness, dizziness, rapid heart rate, headaches, tremors, confusion, and unconsciousness. In most cases, people will stop feeling these effects when they are no longer exposed and begin to breathe

fresh air.Eating foods or drinking liquids containing high levels of benzene can cause vomiting, irritation of the stomach, dizziness, sleepiness, convulsions, rapid heart rate, coma, and death. The health effects that may result from eating foods or drinking liquids containing lower levels of benzene are not known. If you spill benzene on yourskin, it may cause redness and sores. Benzene in your eyes may cause general irritation and damage to your cornea.Benzene causes problems in the blood. People who breathe benzene for long periods may experience harmful effects in the tissues that form blood cells, especially the bone marrow. These effects can disrupt normal blood production and cause a decrease in important blood components. A decrease in red blood cells can lead to anemia. Reduction in other components in the blood can cause excessive bleeding. Blood production may return to normal after exposure to benzene stops. Excessive exposure to benzene can be harmful to the immune system, increasing the chance for infection and perhaps lowering the body's defense against cancer.Long-term exposure to benzene can cause cancer of the blood-forming organs. This condition is called leukemia. Exposure to benzene has been associated with development of a particular type of leukemia called acute myeloid leukemia (AML). The Department of Health and Human Services has determined that benzene is a known carcinogen (can cause cancer).

The International Agency for Cancer Research has determined that benzene is carcinogenic to humans.Exposure to benzene may be harmful to the reproductive organs. Some women workers who breathed high levels of benzene for many months had irregular menstrual periods. When examined, these women showed a decrease in the size of their ovaries. However, exact exposure levels were unknown, and the studies of these women did not prove that benzene caused these effects. It is not known what effects exposure to benzene might have on the

developing fetus in pregnant women or on fertility in men. Studies with pregnant animals show that breathing benzene has harmful effects on the developing fetus. These effects include low birthweight, delayed bone formation, and bone marrow damage. We do not know what human health effects might occur after long-term exposure to food and water contaminated with benzene. In animals, exposure to food or water contaminated with benzene can damage the blood and the immune system and can cause cancer.Children can be affected by benzene exposure in the same ways as adults. Benzene can pass from the mother's blood to a fetus. It is not known if children are more susceptible to benzene poisoning than adults (WWW.atsdr.gov, 1995).

1.3.8.1 Benzene associated heamatotoxicity and growth factor signaling:

Benzene-exposed workers had reduced expression ofvarious cytokines, including the CXC-chemokineCXCL4 (platelet factor 4) and connective tissue–activating peptide (CTAP-III), compared with unexposed workers (Vermeulen*et al*., 2005). These chemokines are mainly released by platelets, but the levels showed no correlation with peripheral blood platelet counts. Thus it was concluded that, the altered levels of these mediators probably reflect a qualitative difference between thrombocytes derived from benzene-exposed and -unexposed individuals. Twenty-nine genes, including the two chemokines CXCL4 (down regulated) and chemokine (C-X-C motif) ligand 16 (unregulated), were likely to be differentially expressed in workers heavily exposed to benzene (mean exposure= 44 ppm) compared with unexposed workers (Vermeulen *et al*.,2005). Thus, alteration in the cytokine network, and especially the Chemokine system, seems to be important in benzene toxicity.

1.3.8.2 Benzene-Associated Aplastic Anemia:

Chronic exposure to high benzene concentrations haslong been associated with aplastic anemia (Smith MT, 1996). Most of these cases have been diagnosed based on pancytopenia in peripheral blood given the recent observation of myelodysplastic syndrome in benzene-exposed individuals (Linet *et al*.,1996).The reported association between benzene exposure and aplastic anemia might at least partly represent an association between benzene exposure and myelodysplasia (Linet et al., 1996)

1.3.8.3 Benzene-Associated Myelodysplastic Syndrome:

Several studies (Linet*et al*., 1996) have described an association betweenbenzene exposure and myelodysplatic syndromes, Patients were referred to hospitals based on initial clinical presentation and/or a medical history of occupationally related benzene intoxication. Their benzene exposurewas independently verified, and estimated full-shift exposure averaged between 50 and 300 ppm, which is very high compared with the OEL of 1 ppm benzene or less in most western countries (Capleton and Levy, 2005). The patients had been exposed for varying periods of time ranging from 6 to 22 years and were removed from exposure on average 2.7 years before evaluation. Thus, these observations are probably not representative for western industry, where the time-weighted average exposure is generally much lower and the high exposure during specific tasks usually lasts for brief periods of the work shift (Capleton and Levy, 2005)

1.3.8.4 Chromosomal abnormalities in benzene exposed individuals:

Monosomy of chromosome 7, trisomy 8 and translocations between chromosomes 8 and 21 (t (8; 21)) are chromosomal changes observed in AML (Rowely JD, 2000). An increased incidence of these aberrations has been reported in workers exposed to benzene (Zhang *et al*., 2002). Studies of

chromosomal abnormalities in blood cells have suggested that benzene metabolites particularly produce monosomy of chromosomes 5 and 7 in human lymphocytes from healthy workers exposed to benzene (Zhang *et al*., 2002) and in human bone marrow cells obtained from healthy volunteers, with bone marrow cells being more susceptible than lymphocytes. The aberrations t (8; 21) and trisomy 8 have also been reported (Smith *et al*., 1998). In a recent pilot study comparing six benzene-exposed workers with five unexposed referents, (Zhang *et al*.,2002) reported that benzene exposure was associated with monosomy of chromosomes 5, 6, 7 and 10 and with trisomy for chromosomes 8, 9,17, 21 and 22. A dose-dependent induction of long-arm deletion of chromosome 6 [del (6q)] and an increased frequency of translocation t (14; 18) among highly exposed workers have been reported (Zhang *et al*., 2002). Both t (14;18) and del(6q) are also frequentlyobserved in lymphoma patients (Taborelli*et al*., 2006). Induction of t(4; 11) and t(6; 11), common in therapy-related leukemia dueto topoisomerase II–inhibiting drugs, was not found. Taken together, these observations suggest that benzene produces selective effects on certain chromosomes. Another study described an association between chromosomal abnormalities in lymphocytes and the frequency of activated T cells in peripheral blood among workers exposed to benzene, styrene, polycyclic aromatic hydrocarbons and/or solvents and unexposed referents (Biro *et al*., 2002), which suggests a link between genotoxicity and immunomodulation an important question then is why the benzene associatedmonosomies and trisomies are not detected in the patients with benzene-associated myelodysplastic syndromes . The present scientific literature cannot answer this question, but possible explanations are: (i) variation in exposure; (ii) certain abnormalities may predispose to the direct development of leukemia without preleukemic

myelodysplasia; or (iii) cells with these abnormalities may not survive long enough to form the basis for disease development(Zhang *et al.,* 2002)

1.3.9 Medical test to determine benzene exposure:-

Several tests can show whether you have been exposed to benzene. Some of these tests may be available at your doctor's office. All of these tests are limited in what they can tell you. The test for measuring benzene in your breath must be done shortly after exposure. This test is not very helpful for detecting very low levels of benzene in your body. Benzene can be measured in your blood. However, because benzene rapidly disappears in the blood, measurements may be useful only for recent exposures. In the body, benzene is converted to products called metabolites. Certain metabolites of benzene, such as phenol, muconic acid, and S-phenylmercapturic acid can be measured in the urine. The amount of phenol in urine has been used to check for benzene exposure in workers. The test is useful only when you are exposed to benzene in air at levels of 10 ppm or greater. However, this test must also be done shortly after exposure, and it is not a reliable indicator of how much benzene you have been exposed to, because phenol is present in the urine from other sources (diet, environment). Measurements of muconic acid or S-phenylmercapturic acid in the urine are more sensitive and reliable indicators of benzene exposure. The measurement of benzene in blood or of metabolites in urine cannot be used for making predictions about whether you will experience any harmful health effects. Blood counts of all components of the blood and examination of bone marrow are used to determine benzene exposure and its health effects. For people exposed to relatively high levels of benzene, complete blood analyses can be used to monitor possible changes related to exposure. However, blood analyses are not useful when exposure levels are low (WWW.atsdr.gov, 1995).

1.4 Previous Studies:

Researchers from the National Cancer Institute and the Chinese Center for Disease Control to study the impacts of long-term exposure to low levels of benzene.

They conducted a cross-sectional study in a region near Tianjin, China including 250 shoe workers exposed to benzene-containing glues and 140 unexposed age- and sex-matched controls who worked in three clothes-manufacturing factories. They conducted extensive exposure assessments for 16 months, testing air samples in the factories as well as at each worker's home. Using blood and urine samples, the researchers linked individual air-monitoring data to end-points including white blood cell and platelet counts, lymphocyte subsets and progenitor cell colony formation.

As expected, workers exposed to benzene at levels of 1 ppm and higher had fewer total white blood cells, granulocytes, lymphocytes, B cells, and platelets than did unexposed workers. The researchers also found that compared to controls, workers exposed to less than 1 ppm benzene had significantly decreased numbers of all types of white blood cells and platelets (Martyn and Mechail, 2005).

Scientists with NIH's National Cancer Institute (NCI) and China's Center for Disease Control have been studying benzene's effects on Chinese factory workers for nearly 30 years. The toxin already had been linked to leukemia when the research project started. Over the years, their studies found that workers exposed to benzene had a greater risk of most blood malignancies; that the risk of non-Hodgkin lymphoma and lung cancer rose as exposure increased; and that

benzene can have a toxic effect on blood even at or below levels generally considered acceptable in workplaces in Western countries (Shang, 2015)

Forouz in 2017 in Iran, who studied to evaluate hematological indices of workers exposed to benzene concluded that only two parameters (MCHC and ESR) were affected by benzene among workers who expended 5 -15 years at benzene station. In the light of these results my study highlight the hypothesis that exposure for more than 10 years induces harmful effects to workers at benzene stations (Forouz *et al*., 2017).

Study on Elobid city,Sudan was conducted to evaluate hematological changes among fuel stations workers, it concluded the exposure to benzene may develop bone marrow depression as indicators for benzene induced hematotoxicity(Ahmed *et al*, 2015).

Other study conducted in USA in 2014 (Frank, 2014).in that study Frank and his workers reported that Hb levels of workers at benzene stations were not differ significantly compare to control.

Studies conducted by Hameed in 2009 concluded the level of Hb, WBCs, and platelets for benzene station workers were gradually decrease as duration became longer. In their study they divided their population into 3 groups of durations at 10 years intervals (1-10), (11-20) and (above 20) years. They observed that the levels of Hb, WBCs and platelets were not affected by exposure to benzene in the first group (1-10), but gradual decrease appeared in other two groups and that the effect of benzene exposure was more prominent in group three in which workers exposed to benzene more than 20 years (Hameed *et al*., 2009)

1.5 Rationale:

Assessments of Hb and RBCs indices which can indicate to some blood abnormalities (eg.anemia) .These abnormalities are relatively common in general practice medicin. This study was to assessment some blood parameters under impact of benzene inhalation among petrol stations workers, and then establishes the prevention.

1.6 Objectives:

1.6.1 General objective:

To evaluate Hb, RBCs count and RBCs indices in petrol stations workers in Khartoum.

1.6.2 Specific objectives:

1-To measure Hb in petrol stations workers.

2- To measure RBCs in petrol stations workers.

3- To measure RBCs indices in petrol stations workers.

4- To relate the duration of working in petroleum station and hematological parameters.

Chapter two

2-Material and method

2-Materials and methods

2:1 Study design and duration:

This case control study was conducted from (May 2017 - July 2017) to evaluate Hb and RBCs indices among benzene stations workers then the effect of exposure to benzene and non- exposed individual (control).

2:2 Study area and population:

Study area was Petroleum stations in Khartoum state with sample size of 60 venous blood samples were collected from workers and 60 samples were collected from non -exposed individuals as control.

2:3 Sampling:

Individuals who exposed to benzene were selected and data collected using self-administrated pre coded questionnaire which was specifically designed to obtain information.

2:4 Samples:

Venous blood collected using sterile plastic syringes after cleaning the venipuncture area with 70% ethanol, blood was added to EDTA anticoagulant.

2:5 Inclusion criteria:

- Workers exposure to benzene for long time during day.
- Workers who are smokers and non-smokers.
- Non- exposed individuals as control for comparing.

2:6 Exclusion criteria:

- Workers who were employed less than one year.
- Workers who had no direct contact with benzene.

.2:7 Methods:

2:7:1Complete Blood Count (CBC) (automated) (Sysmex):

2:7:1:1 Principle of hematologyanalyzer:

A- Blood cells which arenon-conductors of electricity are dilutedin a buffered electrolyte solution.

B-Allowed passing through the orificeof an aperture tube between two electrodes.

C- Interruption of the current by the non-conducting cells alters the electric charge and a pulse is produced.

D- The amplitude of each pulse is proportional to the volume of the cell and the cell count is determined from the total number of pulses obtained from a measured volume of blood.

2.7.1.2 Red blood cell count:

RBC is determined from the number of pulses generated in a specific volume of blood. The advantage of this approach is that end-user calibration is not required. Analyzersutilizing relative counting principles determine the RBC from the number of pulses generated in a fixed time period.

2:7:1:3Photometric method hemoglobin:

The photometer system consists of a photodiode, a cuvette with a length of 15 mm and a filter at a wavelength of 535nm (bandwidth 20 nm).

2.7.1.4 Hematocrit:

HCT is a parameter that is a measure of the total or cumulative volume of red blood cells relative to the total volume of whole blood. This is also commonly referred to as the packed cell volume (PCV) and is expressed as a percentage value or as a fraction (unit L/L).The measurement of HCT on automated hematologyanalyzers has little to do with the actual packing of red cells. It is obtained using impedance technology whereby the passage of each individual cell through the aperture generates an electrical pulse that is assumed to be proportional to the volume of the cell. The HCT on a Sysmex analyzer is obtained using the cumulative pulse height method.

2.7.1.5 Mean cell volume:

The mean volume of red cells is calculated from the RBC and HCT using the following formula:

$$\text{MCV (FL)} = \frac{\text{HCT}}{RBCs}$$

The normal reference range for MCV is age dependent. The words normocytic, microcytic and macrocytic are used to describe red cell populations with normal, low and high MCVs respectively.

2.7.1.6 Mean cell hemoglobin:

The average amount of hemoglobin per red cell is calculated from the RBC and HGB using the following formula:

$$\text{MCH (pg)} = \frac{\text{HGB}}{RBCs}$$

The normal reference range for MCH is age dependent. The MCH value tends to be proportional to MCV. The size of a cell is largely determined by the

hemoglobin content. Cells that have a normal MCH are referred to as normo-chromic whereas those with low values are termed hypo-chromic

2.7.1.7 Mean cell hemoglobin concentration:

The MCHC is calculated from HCT and HGB using the following formula:

$$\text{MCHC (g/dl)} = \frac{\text{HCT}}{HBG}$$

The MCHC normal reference range is remarkably constant throughout life and generally has a very tight range with minimal variation expected. The MCHC, especially in older references, is also used to define normochromic and hypo-chromic red cell populations.

Chapter three

3.Data analysis and Results

3.Results

Table(3.1): Demographic of study participants:

Variables		Samples Frequency		Percent%
		Case	Control	
Age Years	20-40	42	42	70
	41-55	18	18	30
Smoking	Yes	27	27	45
	No	33	33	55
Employment Duration (Years)	>5	37	—	61.7
	5-10	18	—	30
	< 10	5	—	8.3

60 samples collected from petrol stations workers (males) and 60 samples collected as control from other healthy individuals (males). Study populations were divided into 2 age groups, 20-40 years 42 individuals (70%), 41-55 year 18 individuals (30%). Frequency of smokers and nonsmokers is 27 (45%) and 33 (55%) respectively. Frequency of employment duration group (< 5) years 37 (61.7%), (5-10) years 18 (30%), (> 10) years 5 (8.3%).

Table(3.2):Comparison between mean of Hb and RBCs indices of petrol stations workers and controls:

Table (3.2) shows no significant differences in means of parameters and (p. values > 0.05).

Parameters	Samples		P.value
	Case Mean±SD	Control Mean±SD	
Hb g/dl	13.9±0.96	14.7±1.0	0.070
RBCs /L	5.16±0.42	5.04±0.46	0.067
HCT %	44±4.6	44±5.9	0.60
MCV fl	85±6.3	86±6.6	0.090
MCH pg	27±2.9	29±1.8	0.083
MCHC g/dl	33±2.4	33±3.4	0.095

Table (3.3) The effect of smoking on parameters:

Table (3.3) show there is no significant differences in means of parameters among smoking and nonsmoking workers. (p.values > 0.05).

Parameters	Smoking		P.value
	Yes Mean±SD	No Mean±SD	
Hb g/dl	14.0±1.0	13.7±0.9	0.199
RBCs /L	5.14±0.4	5.18±0.4	0.761
HCT%	46±3.8	46±5.2	0.998
MCV fl	88±6.8	89±5.9	0.598
MCH pg	27±2.7	27±3.1	0.676
MCg/dl	30±2.3	30±2.5	0.480

Table(3.4) :Effect of Employment duration on parameters:

Table (3.4) show there is no significant differences in mean of parameters and (p. value > 0.05).

Parameters	Control	Employment Duration (years)			P.value
		<5	5-10	>10	
Hb g/dl	14.7	14.o	13.5	14.1	0.752
RBCs /L	5.04×10^{12}	5.20	5.20	5.10	0.309
HCT %	44	47	47	48	0.325
MCV fl	86	90	88	90	0.376
MCH pg	29	27	27	27	0.117
MCHC g/dl	34	30	30	30	0.341

Chapter Four

4-Discussion, Conclusion and Recommendation

4-Discussion, Conclusion and Recommendation

4.1 Discussion:

The result of present work showed seem not to be affected which agreed with the result of a study conducted in USA in 2014 (Frank, 2014).in that study Frank and his workers reported that Hb levels of workers at benzene stations were not differ significantly compare to control.

Other studies conducted by Hameed in 2009 concluded the level of Hb, WBCs, and platelets for benzene station workers were gradually decrease as duration became longer. In their study they divided their population into 3 groups of durations at 10 years intervals (1-10), (11-20) and (above 20) years. They observed that the levels of Hb, WBCs and platelets were not affected by exposure to benzene in the first group (1-10), but gradual decrease appeared in other two groups and that the effect of benzene exposure was more prominent in group three in which workers exposed to benzene more than 20 years. In the present study, one worker had working duration of 12 years, while the rest of study populations have duration below 10 years. In this respect the present study agreed with study of Hameed (Hameed *et al*., 2009). This study agreed with another study conducted by Forouz in 2017 who concluded that only two parameters (MCHC and ESR) were affected by benzene among workers who expended 5 -15 years at benzene station. In the light of these results my study highlight the hypothesis that exposure for more than 10 years induces harmful effects to workers at benzene stations (Forouz *et al*., 2017). Although one study was contradicted the results of my study in the way that exposure to benzene reduced HCT and the RBC indices (Ahmed *et al*, 2015). They conclude the exposure to benzene may develop bone marrow depression as indicators for benzene induced hematotoxicity.

4.2 Conclusion:

Hb, RBCs and RBCs indices were seem not to be affected among benzene station works.

4.3 Recommendations:

- Recommended to set a limit of Employment durations.
- Furthers studies to investigate all blood parameters, immunity and chemistry for work duration more than 10 years are highly recommended.
- Regular checkup of Hb, RBCs and RBCs indices among petroleum stations workers.
- It is highly recommended to instruct mandatory wearing of PPE (Personal Protection Equipment) such as muzzles, gloves and safety shoes for benzene station workers.

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Appendices

Appendix (I)

Questionnaire

Sudan University of Science and technology

College of post graduate

Questionnaire of petrol stations employees

No.	Name	Age	Residence Location	Known Illnesses	Career Duration	Smoking	
						Yes	No
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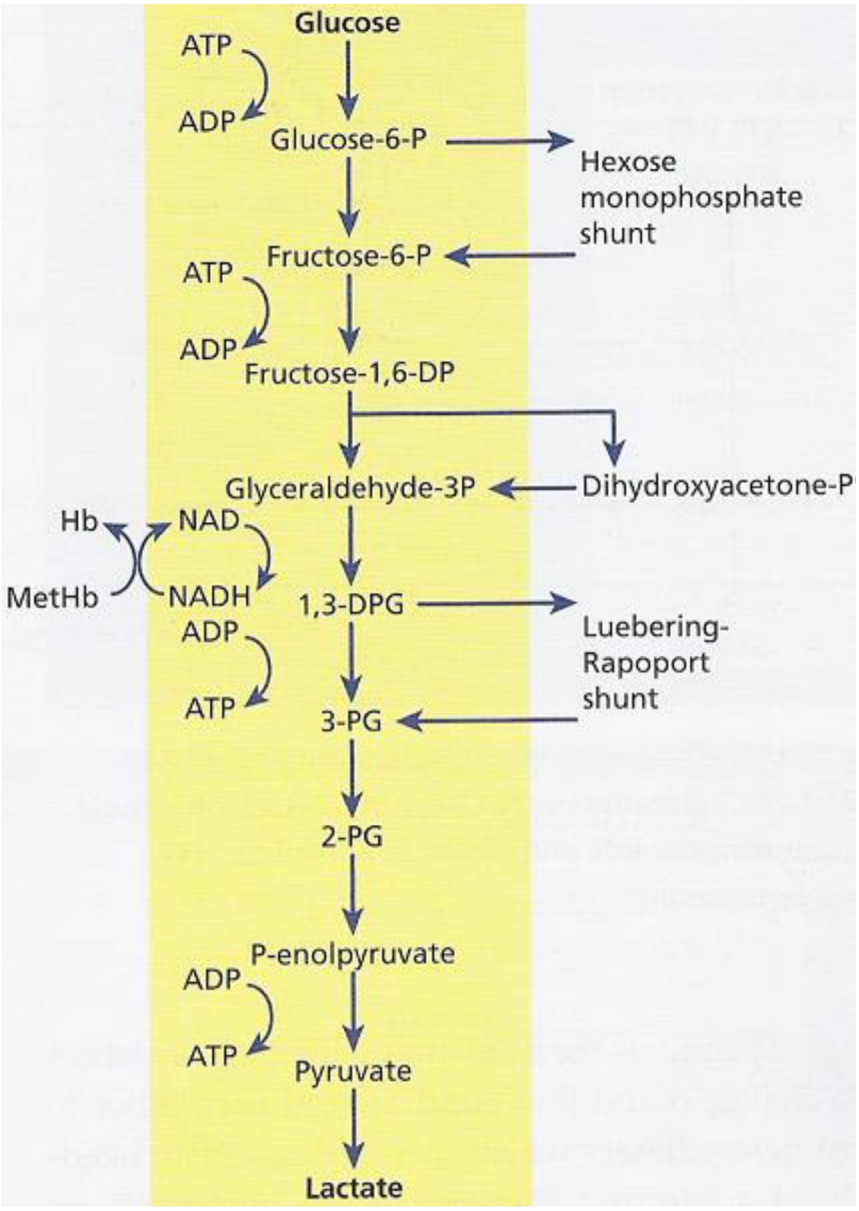
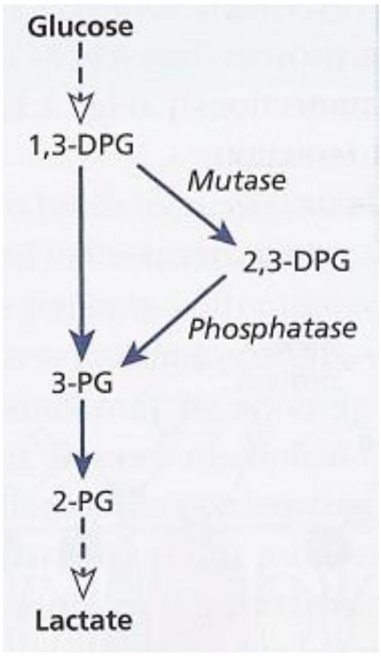
Appendix (II)

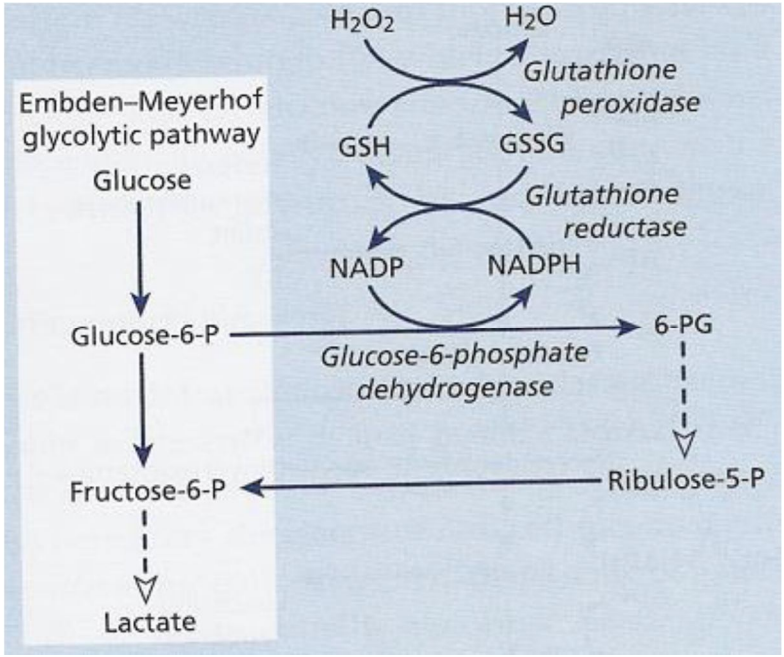
Hexose-Monophosphate Shunt



Embden-Meyerhof Glycolytic pathway



Luebering-Rapoport Shunt



Hexose-Monophosphate Shunt