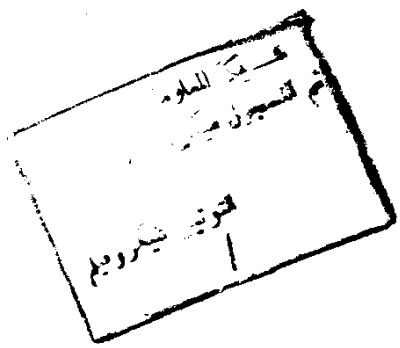
RED CELL MEMBRANE

Essay

Submitted for Partial Fulfillment of

The Master Degree in Clinical Pathology

By



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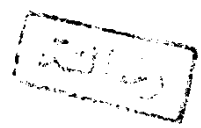

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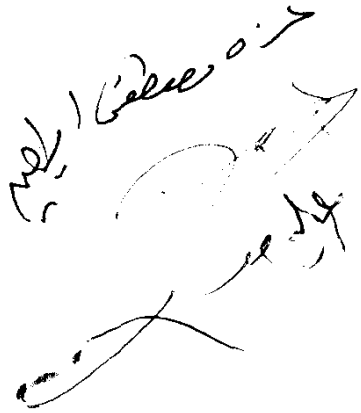

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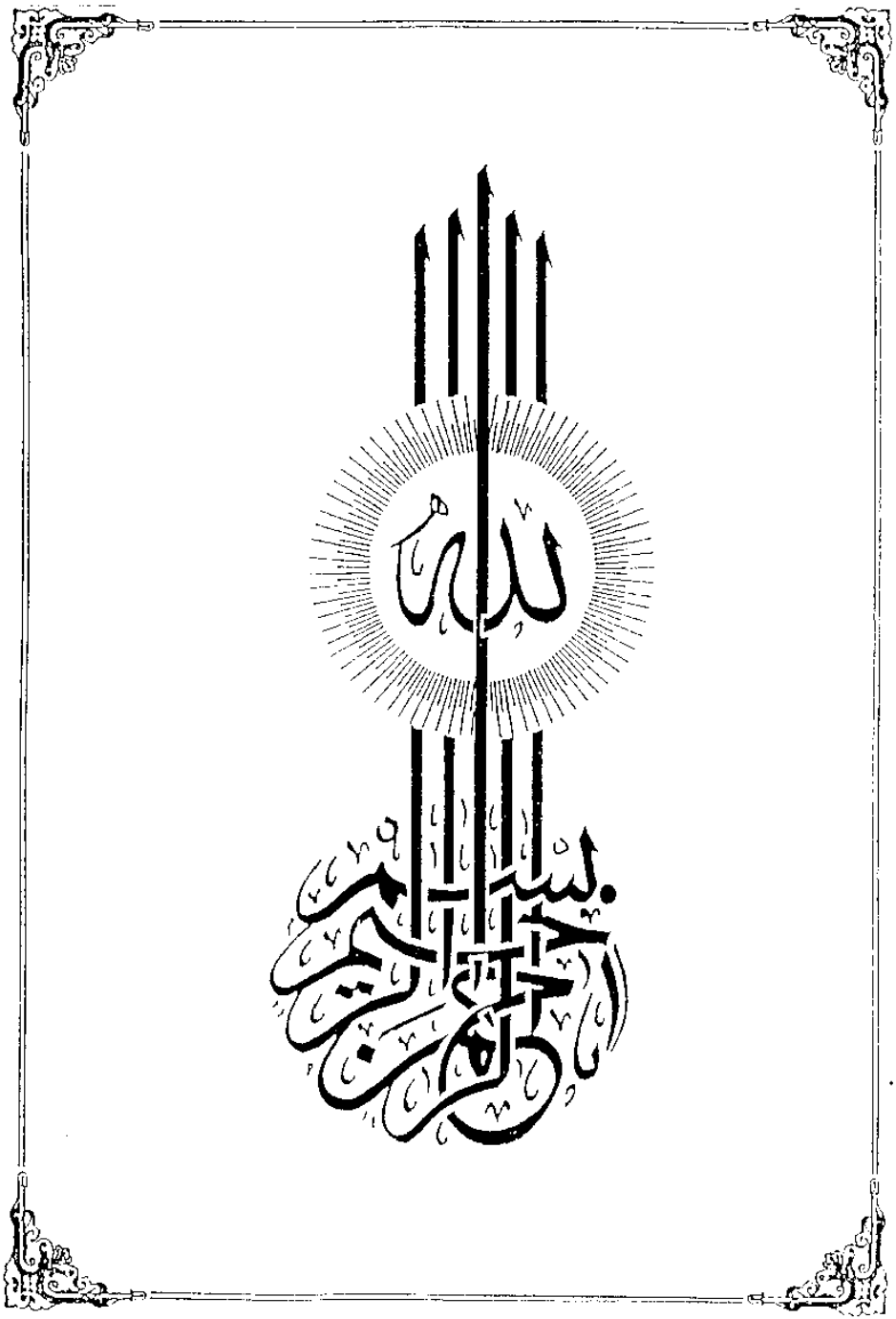

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ABBREVIATIONS

BFU	Burst forming units
BPA	Burst promoting action
Ca M	Calmodulin
CFU	Colony forming units
Epo	Erythropoietin
ERC	Erythropoietin responsive cell
GAPD	Glyceraldehyde phosphate dehydrogenase
GM-CSF	Granulocyte macrophage-colony stimulating factor
HE	Hereditary elliptocytosis
HPP	Hereditary pyropoikilocytosis
HS	Hereditary spherocytosis
IL-3	Interleukin - 3
IMP	Intramembrane particles
LCAT	Lecithin - cholesterol acyltransferase
PAS	Periodic - acid - schiff
PC	Phosphatidylcholine or lecithin
PE	Phosphatidylethanolamine
PNH	Paroxysmal nocturnal haemoglobinuria
PS	Phosphatidylserine
RSC	Reversibly cells
SDS-PAGE	Sodium dodecyl sulfite polyacrylamide gel electrophoresis

INTRODUCTION

The actual biochemical structure and organization of red cell membrane still remains to be elucidated.
The red cell membrane viewed by transmission electron microscopy appears as trilaminar structure consisting of a dark-light-dark band arrangement of layers.

An outer hydrophilic portion chemically composed of glycolipid, glycoprotein and protein, a central hydrophobic layer containing protein, cholesterol and phospholipid and an inner hydrophilic layer containing protein.

The red cell membrane is a semipermeable lipid layer supported by a protein mesh-like cytoskeleton structure.
The fluid lipid matrix contains equal amounts of cholesterol and phospholipids with a mosaic of proteins interspersed throughout at various intervals which consists of integral membrane protein as glycoprotein and peripheral protein as spectrin.

Partial deficiencies of spectrin, deficiency of cholesterol and phospholipid, reduction in membrane surface area are directly related and are major features determining the heterogenous clinical manifestations of hereditary spherocytosis.

The specific defect of spectrin mainly affecting its interaction with protein bands as abnormalities of spectrin dimer - dimer association, defect in binding of spectrin to ankyrin occurs in hereditary elliptocytosis.

On the other hand an elevated cholesterol / phospholipid ratio in the red cell membrane where there is an increased cholesterol

(25-65%) and normal phospholipids was found in sickle cell anaemia.

Aim of the work

The aim of this work is to review the changes and abnormalities of the red cell membrane which interfere with different status of disease.

Normal Structure of Red Blood Cell

NORMAL STRUCTURE OF RED BLOOD CELL

Structural Features

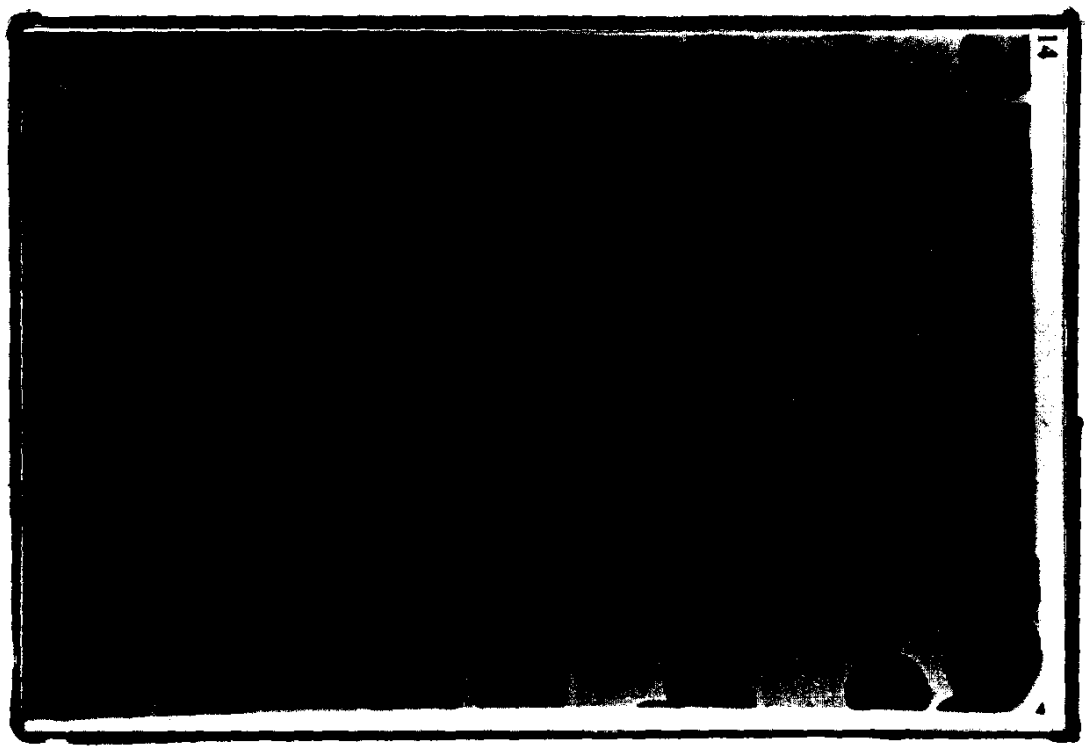
The mature mammalian erythrocyte is one of the most highly specialized cells because of lacking such cytoplasmic organelles as nucleus, mitochondria, or ribosomes so the red cell is unable to synthesize new protein, carry out the oxidative reactions associated with mitochondria, or undergo mitosis.

The erythrocyte consists of a membrane surrounding a solution of protein and electrolytes. More than 95% of the protein is the oxygen-transport protein, hemoglobin. The remainder includes those enzymes required for energy production and for the maintenance of hemoglobin in a functional, reduced state **(Richard et al, 1981).**

Dimensions

The mature adult human RBCs has a diameter of 7.2 um, a surface area of 140 um^2 and a volume of 90 fl. A sphere with a volume of 90fl would have a diameter of 5.5 um and a surface area of 95 um^2 **(Bull et al, 1985).**

The difference between the actual figures of RBC surface area and those of a sphere of identical volume reflects the fact that the red cell is a discocyte with an excess surface area (i.e. an amount exceeding that of a sphere of equal volume) of about 45 um^2 and surface area/volume ratio of 145/90=1.5 . Along with considerations of cellular viscosity and membrane deformability, this excess surface area is an important factor in allowing this 7-8 um disc to traverse 3 um capillaries and slits in the walls of sinusoids **(Hui et al, 1980; Allan et al, 1980).**



Normal mature red cells

Shape

At rest, the normal human erythrocyte is shaped like a flattened, bilaterally indented sphere, a shape often referred to as a "biconcave disc".In fixed, stained blood smears, only the flattened surfaces are observed; hence, the appearance is circular with an area of central pallor corresponding to the indented regions **(Richard et al, 1981).**

A number of hypotheses have been advanced to explain how the cell produces and maintains the resting biconcave shape **(Fung Y.C et al, 1968).**

The possible forces that interact to produce the shape include:

1- Elastic force within the membrane.
2- Surface tension.
3- Electrical forces on the membrane surface and
4- Osmotic or Hydrostatic pressures.

Some investigators have proposed that additional factors such as stearic interference or even polymeric protein strands operate to draw the dimple region together.
The maintenance of erythrocyte shape is dependant on factors within the cell as well as in the external environment as:

1- Osmotic (Hypotonic) swelling **(Evans E. et al, 1972)**
2- Discocyte - echinocyte transformation **(Brecher G. et al, 1972)**
3- Discocyte - stomatocyte transformation **(Weed R. I. et al, 1973)**

So shape, deformability and surface features of the erythrocyte are determined by 3 interacting components: the membrane, haemoglobin, and the non haemoglobin components. i.e: salts, water and the substrates, cofactors and enzymes mainly of glycolysis.

Membrane function is effected by the kind and the amount of haemoglobin present ,by cell water and cation content, and by the energy level of the cell **(Schrier et al, 1982).**

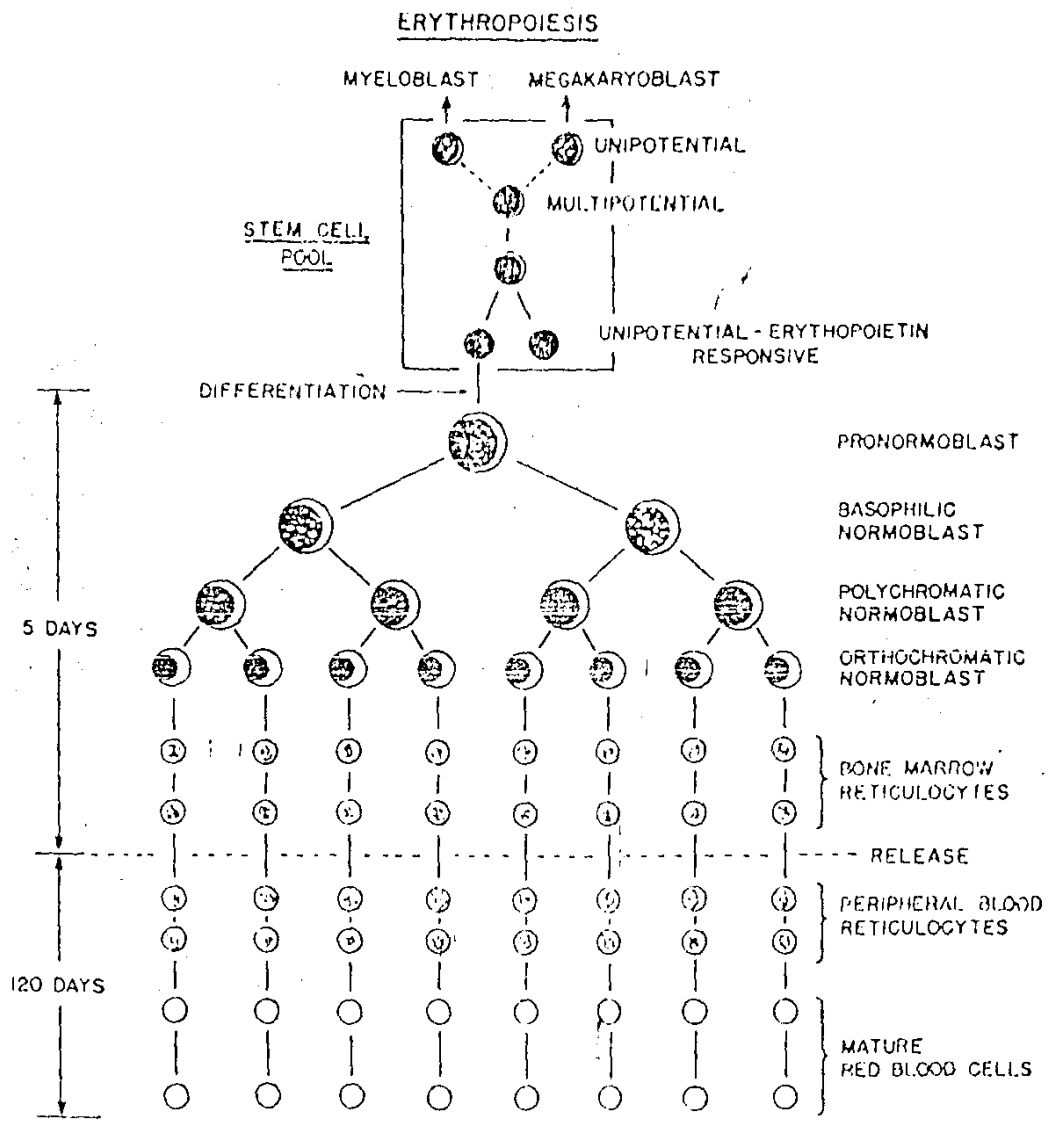
Red Cell Parameters

The three basic parameters which can be measured in relation to the red cell population are:

1- The concentration of haemoglobin per unit volume of blood after lysis of the red cells. (haemoglobin concentration)
2- The number of red cells per unit volume of blood. (red cell count).
3- The haematocrit. **(Dacie et al, 1990).**

Red Cell Life Span

As red cells do not contain ribosomes, they can not synthesize new protein to replace the essential molecules (eg: enzymes, structural proteins)which become denatured in the course of time. Red cells therefore have a limited life span of 110-120 days at the end of which they are ingested and degraded by the phagocytic cells of the marrow, spleen, liver, and other organs **(Wickramasinghe et al, 1986).**



(Fig.2) **Processes of differentiation, proliferation and maturation of the red cell series.**

Williams 1977

Erythropoiesis

The haemopoietic cells can be divided into two categories:

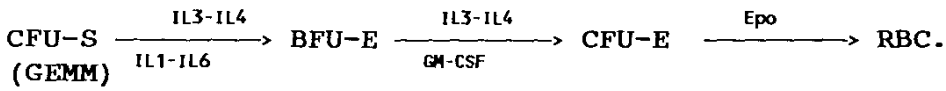
1- The early precursors which have not yet been recognized morphologically with certainly but which can be studied by functional tests (described as the morphologically unrecognized precursors).
2- The morphologically recognizable precursors which consist of the series of proerythroblast till the mature red blood cell.

The morphologically unrecognized precursors also consist of two categories: Haemopoietic stem cells and cells which do not have a capacity for self renewal **(Ogawa et al, 1983)**.
Studies in experimental animals and in humans have shown the presence of a multipotent myeloid stem cell whose develop into granulocytes, erythrocytes, monocytes, and megakaryocytes (GEMM) colonies. This stem cell is frequently referred to as the "colony-forming unit in spleen"or CFU-S **(Lord et al, 1983)**.

Two separate classes of erythroid progenitor cells were recognized using the plasma-clot culture technique. A progenitor cell with extensive proliferative potential requiring high levels of erythropoietin and a long culture period was termed a burst forming unit-erythroid (BFU-E). A more differentiated progenitor cell termed colony forming unit-erythroid (CFU-E) which give rise to small clusters of erythroid cells after several days of culture and need small amount of erythropoietin **(Monette et al, 1980)**.

The CFU-E is a mature descendant of the more primitive BFU-E and depends upon erythropoietin in vivo for its existence.The BFU-E

respond to erythropoietin by differentiating into the CFU-E but they are not maintained by erythropoietin. The BFU-E needs both erythropoietin and additional factors, termed burst promoting activities,for its optimal in vitro growth. Burst promoting activities include IL3, GM-CSF and IL-4 **(Iscove et al, 1977).**



(William 1990)

The CFU-E form the recognizable lineage of nucleated red cells in the marrow. The earliest of these is the proerythroblast or pronormoblast which is a large cell measuring some 15 to 20 um in diameter. There is a large rounded nucleolated nucleus and the cytoplasm is deeply basophilic.The deep blue colour of the more immature cells is due to the presence of large amounts of ribonucleic acid which is associated with the active protein synthesis, an extreme example of which is the dense basophilia of plasma cells. As the cells mature the nuclei become smaller and denser, losing their nucleoli and eventually the nuclei become pyknotic and structureless before being extruded from the cell after which they are phagocytosed. Any remaining nuclear material is very effectively removed by a process of "pitting" during passage through the splenic sinus, so that it is very rare to find particulate material in normal circulating erythrocytes. The cytoplasm also matures, the dense blue colour being gradually replaced by pink staining haemoglobin so that by the time the nucleus is lost and the erythrocyte is discharged into the peripheral circulation but a faint bluish tinge remains. The successive stages of maturation are shown diagrammatically in fig 2., which also indicates that loss of the ability to divide