RED CELL STRUCTURE AND FUNCTION

HAEMATOLOGY FOUNDATION COURSE
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CONTENTS

BACKGROUND

Red blood cells are produced in the bone marrow (from 6^{th} to 7^{th} months of fetal Life) by a process called erythropoiesis.

The process can be divided into:

a) Early phase – commitment of pluripotent stem cell progeny into erythroid differentiation. This is independent of erythropoietin (glycoprotein hormone).
b) Late phase – erythropoietin dependent.

The tissues and cells that produce red cells are called ‘the erythron’.

BACKGROUND (cont)

The purpose of erythropoiesis is to produce red cells at a rate that maintains a constant red cell mass in the body.

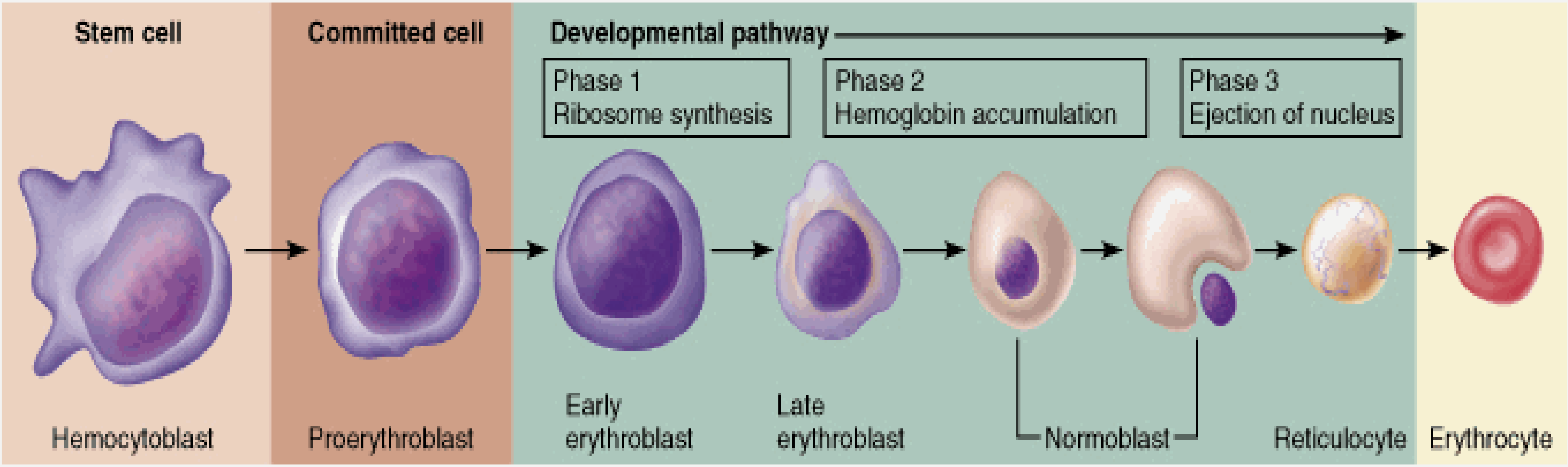
Define anaemia in terms of red cell mass.

What routine FBC parameter can be used to assess red cell mass?

What factors affect automated measurements of this parameter?

BACKGROUND (cont)
ERYTHROPOIESIS

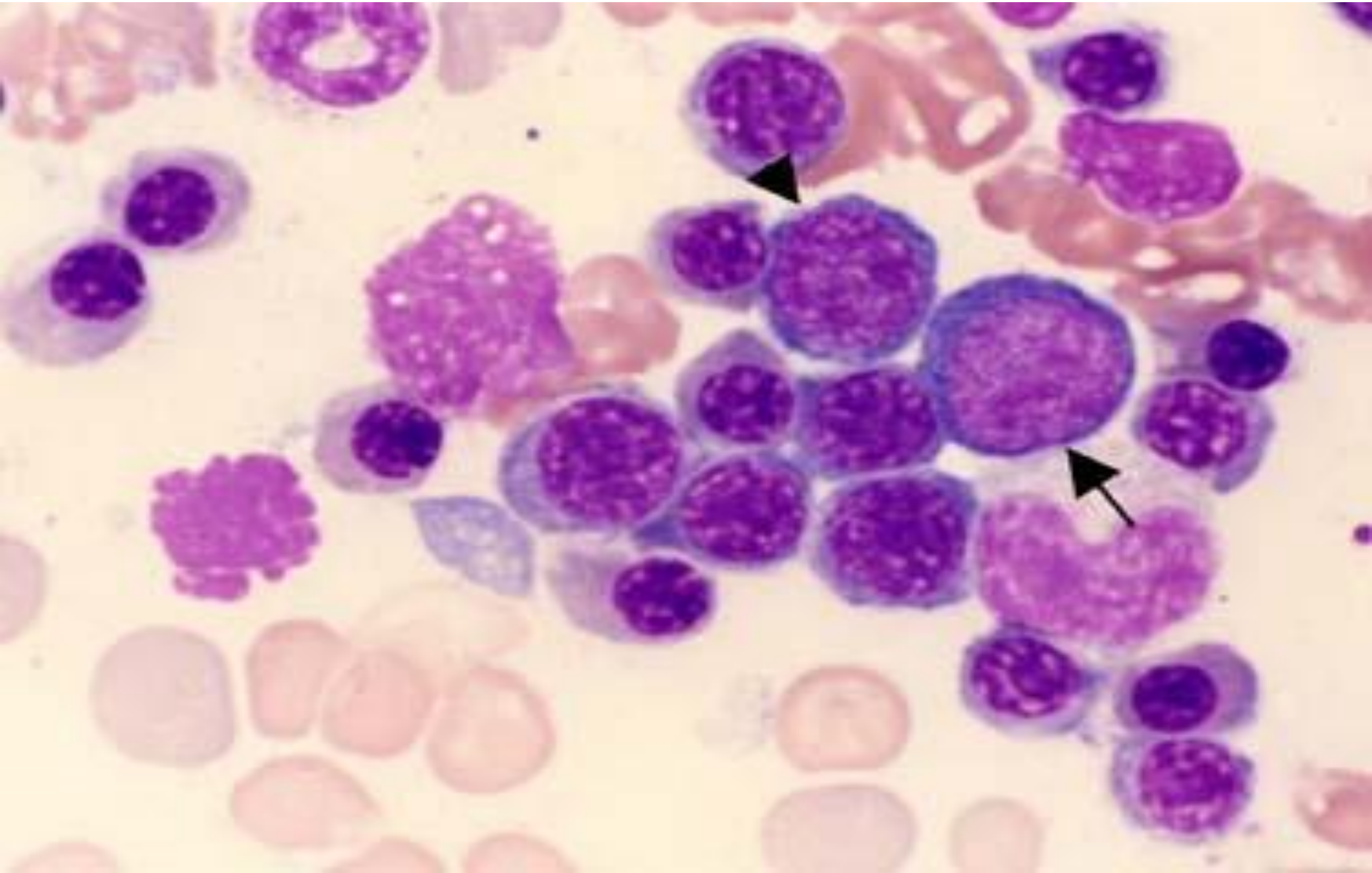
DEVELOPMENT OF RED BLOOD CELLS



Many of the morphologic criteria used in staging the maturation of erythroid precursors are related to haemoglobin production and content.

BACKGROUND (cont)

Bone marrow aspirate showing erythropoiesis



BACKGROUND (cont)

What is the significance of the presence of nucleated RBC in the peripheral blood?

Name a few conditions in which this occurs.

BACKGROUND (cont)

CONTROL OF ERYTHROPOIESIS

A well balanced mechanism maintains the erythron within “normal” limits; and it responds to both normal and Abnormal situations.

Erythropoietin plays a central role in this mechanism.

- It is a glycoprotein that exerts its action via receptors – EPORs on cell surfaces.
- 90% is produced in the kidney and 10% in the liver.
- There are no preformed stores but it is synthesised de novo in response to anaemia.

BACKGROUND (cont)

CONTROL OF ERYTHROPOIESIS

- Alterations in haemoglobin in the blood lead to changes in tissue oxygen tension in the kidney.
- In response to hypoxia, the kidney secretes EPO.
- EPO induces erythroid progenitors to differentiate into pronormoblasts.
- Erythroid marrow expands resulting in increase in red cells.
- The erythron increases leading to an increase in tissue oxygen levels.

Erythropoietin is now believed to be pleiotropic. Which patients may not benefit from treatment with it?

THE MATURE ERYTHROCYTE

- A highly specialised anuclear cell lacking cytoplasmic organelles.
- Unable to synthesize new protein, carry out oxidative reactions or undergo mitosis.
- 95% of the cytoplasmic protein is haemoglobin.
- Expresses a variety of proteins including transport proteins, adhesion molecules, receptors and proteins involved in signalling pathways.
- Normal life is between 100 – 120 days.

SHAPE AND DIMENSIONS

•At rest, the normal red blood cell (RBC) is shaped like a flattened, bilaterally indented sphere - biconcave disc

•In fixed, stained blood smears, the mature RBC appears as a circular cell, 7-8μ in diameter, with a central pallor.

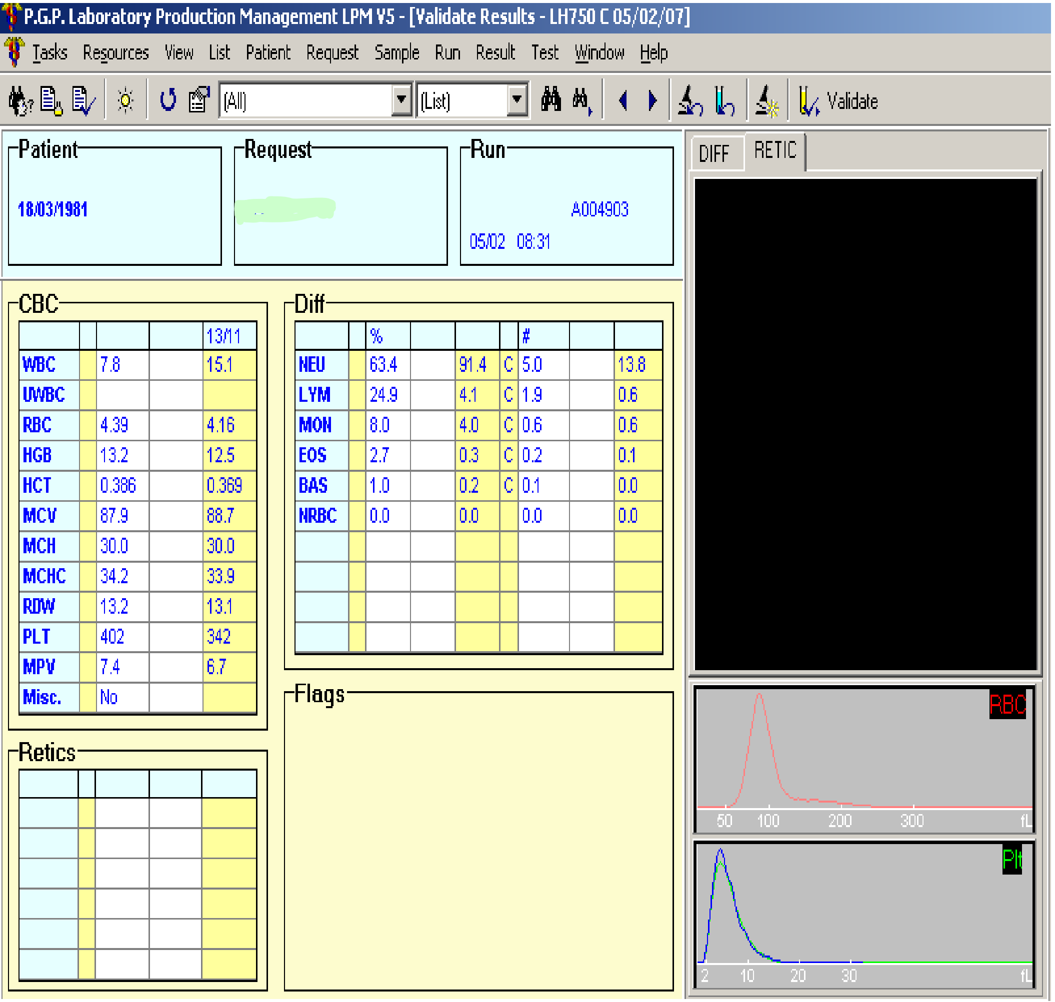
•Dimensions of RBC in the living state are obtained from cells suspended in isotonic solutions, e.g. photomicrographic techniques.

•Average values for mean cellular volume in normal subjects range from 85 – 91 fl.

SHAPE AND DIMENSIONS (cont)

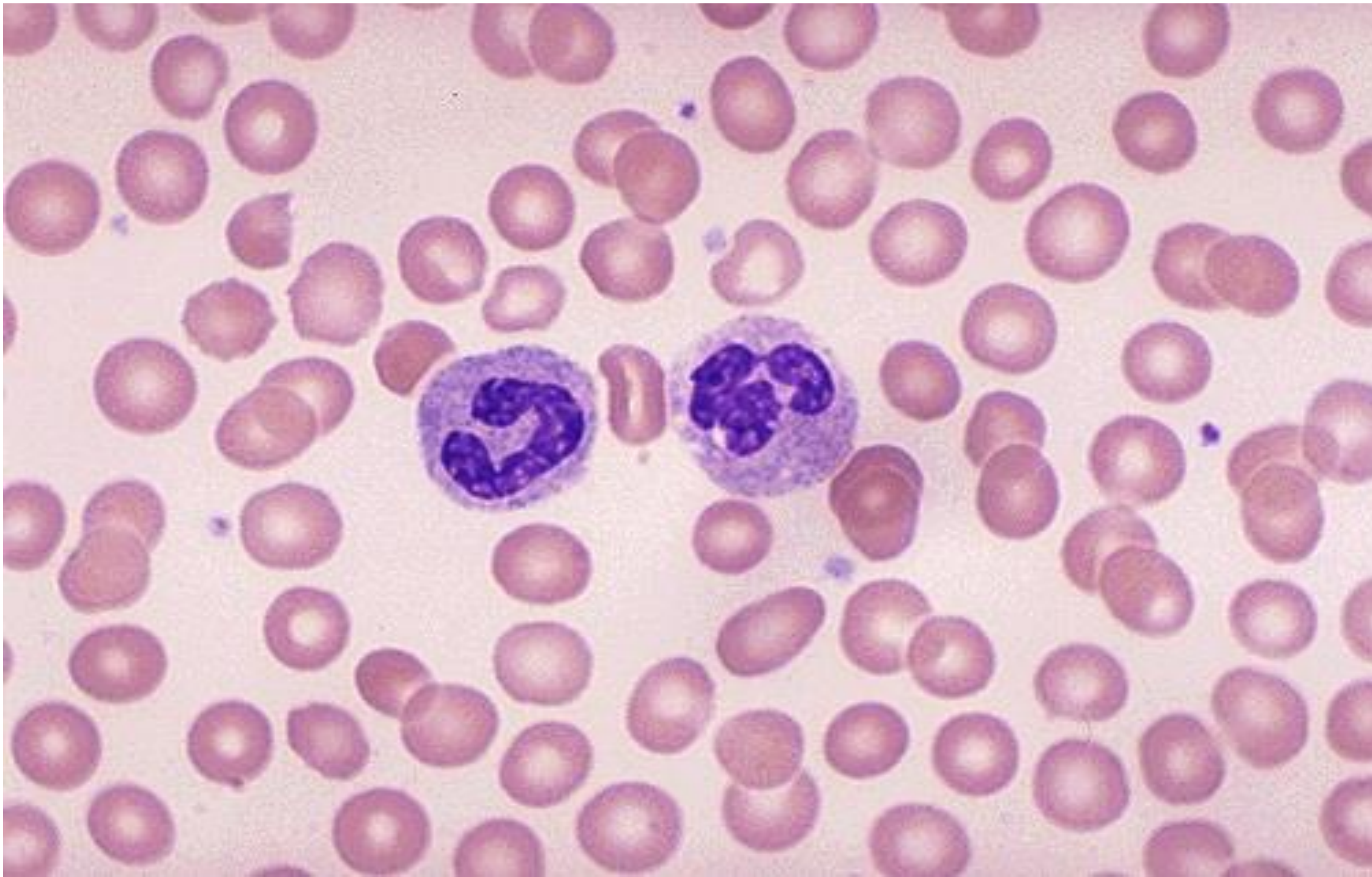
- Variations in cell size can be documented by a frequency distribution curve of red cell volumes generated from the output of a Coulter counter.
- **How do Aperture-Impedance counters measure MCV?**
- **How is MCV calculated from other RBC indices?**
- **What affects automated MCV measurements?**
- **Define anaemia in terms of the MCV.**
- **Give examples.**

SHAPE AND DIMENSIONS (cont)



SHAPE AND DIMENSIONS (cont)

Normal red blood cells



DEFORMABILITY

Defined as the ability to maintain membrane integrity under adverse mechanical stress such as:

- High shear stress.
- Rapid elongation and folding in the microcirculation.
- Passage through small fenestrations of the spleen.
- Repeated fluctuating oxygen exposure.

Cell deformability depends on both the membrane and the cytoplasm – mainly the elasticity and viscosity of the Membrane.

MEMBRANE STRUCTURE

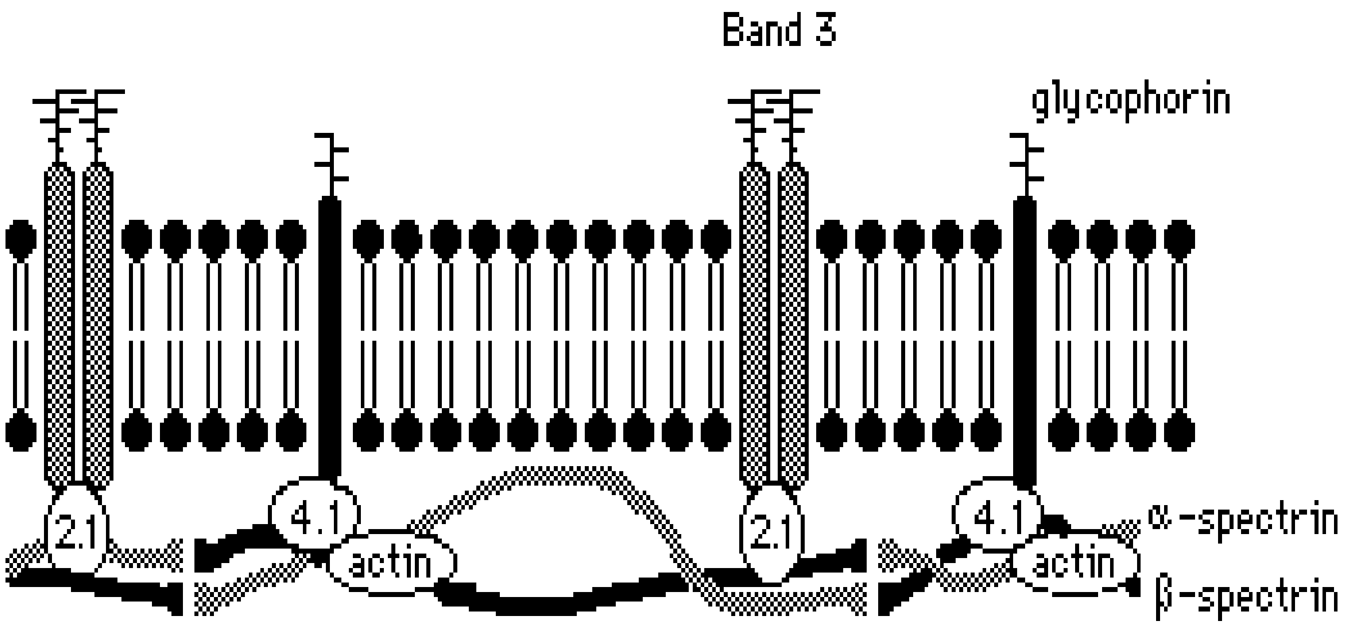
A matrix formed by a double layer of phospholipids, first proposed in 1925 and refined by Danielli and Davson in 1935.

- The lipid molecules are oriented with the nonpolar groups directed toward one another –forming hydrophobic interactions.
- The hydrophobic polar heads groups are directed outward – they interact with the aqueous environment on both the cytoplasmic and plasma surfaces.

The lipid bilayer can be visualised as a two-dimensional viscous solution in which float globular proteins.

A net work of structural proteins lies on the cytoplasmic side of the membrane – the cytoskeleton.

MEMBRANE STRUCTURE



Chemical composition

- 52% protein: structural (cytoskeleton) proteins – α and β spectrin, ankyrin (2.1), protein 4.1and actin (5); PAGE analysis.
- 40% lipid
- 8% carbohydrate

MEMBRANE STRUCTURE (cont)

Abnormalities of cytoskeletal proteins have been linked to abnormal red cell shapes, abnormal membrane stability and haemolytic anaemias. Give an example or two.

What congenital or acquired abnormalities are associated with target cells?

FUNCTION(S) OF THE RED CELL

- to package and transport haemoglobin at high concentration.

Haemoglobins are wide spread and specialized haemoproteins; present in prokaryotes, fungi, plants and animals.

In vertebrates, haemoglobin is constituent of the red cell cytoplasm – approx. 90% of the dry weight.

In most invertebrates, the oxygen-carrying pigment is transported freely in the plasma – a relatively inefficient delivery system.

ONTOGENY OF HAEMOGLOBINS

Haemoglobin expression during ontogeny is characterised by three developmental periods:

- Embryonic
- Fetal
- Adult

Embryonic	Fetal	Adult
Gower 1 zeta(2), epsilon (2) Gower 2 alpha (2), epsilon (2) Portland zeta (2), gamma (2)	Haemoglobin F alpha(2), gamma (2)	Haemoglobin A alpha (2), beta (2) Haemoglobin A_2 alpha (2), delta (2)

ONTOGENY OF HAEMOGLOBINS (cont)

Haemoglobin A is the predominant adult Haemoglobin – 96% of the total.

A minor adult haemoglobin (A2) is produced from 35wks of gestation (< 3.5%) – it has little physiologic relevance, but it is increased in β-thalassemia and other conditions.

Fetal haemoglobin is present in adults at levels of<1%. Levels increase in thalassemias, etc

THE ROLE OF HAEMOGLOBIN

It is the primary medium of exchange of oxygen and carbon dioxide.

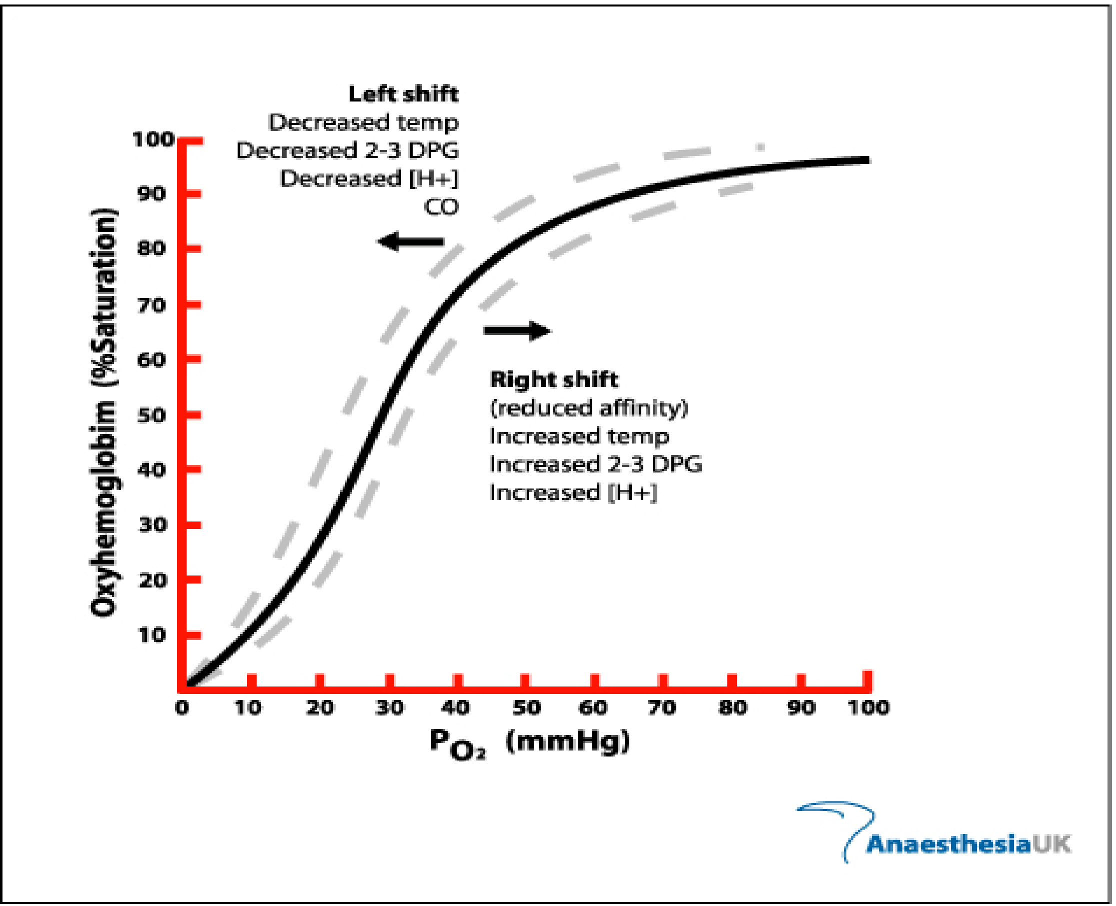
Must fulfil four requirements:

1. Capable of transporting a large quantity of O_2.
2. It must be highly soluble.
3. It must take up and release oxygen at appropriate pressures.
4. It must be a good buffer.

Normal haemoglobin meets these requirements

THE ROLE OF HAEMOGLOBIN (cont)

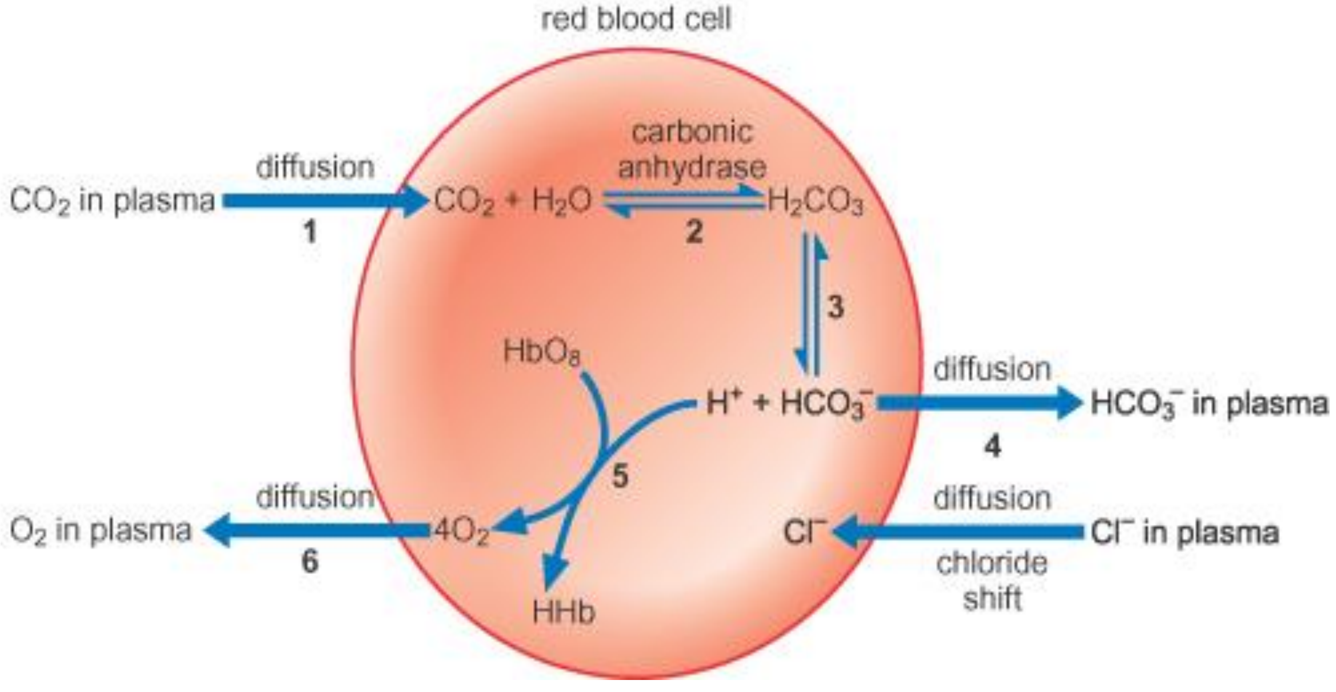
When fully saturated, each gram of Hb binds 1.39 ml of O_2 . The degree of saturation is related to the PO_2 , as described by the oxygen dissociation curve below.



Fetal haemoglobin Binds 2,3-DPG to a lesser extent than adult haemoglobin resulting in increased Oxygen affinity.

THE ROLE OF HAEMOGLOBIN (cont)

Transport of carbon dioxide in the red blood cells



The reactions are reversed in the lungs resulting in CO2 being excreted in expired air.

RED CELL METABOLISM

The red cell possess specialised set of metabolic pathways that preserve its structural and functional integrity.

1. Pathways providing energy and reduced cofactors:
- Embden-Meyerhof glycolytic pathway.
- Pentose phosphate pathway
2. Pathways for reversal of oxidative injury:
- Glutathione synthetic pathway
- Glutathione peroxidase / reductase
- Cytochrome b5 reductase (MetHb reductase)
3. Others
- Enzymes involved in nucleotide degradation and salvage

THE SPLEEN AND AGED/DAMAGED RBC

The adult spleen receives approx. 2L/minute blood – cells are therefore viewed on a regular basis.
The normal removal of aging or damaged RBCs is called culling.
Removal of inclusions within RBCs with release back into circulation is termed pitting (reduces deformability).

Close apposition of RBCs with macrophages during their passage in through the fenestrations of the sinus, enables macrophages to identify poorly deformable cells, aged cells, damaged cells, the presence of antibody/complement and to phagocytize the cell.

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