Red Cell Membrane

THE RED BLOOD CELL

Three aspects of red cell metabolism are crucial for normal erythrocyte survival and function:

1-The red blood cell **membrane**

2-**Haemoglobin** structure and function

3-Active red cell **metabolic pathways**

THE RED CELL MEMBRANE

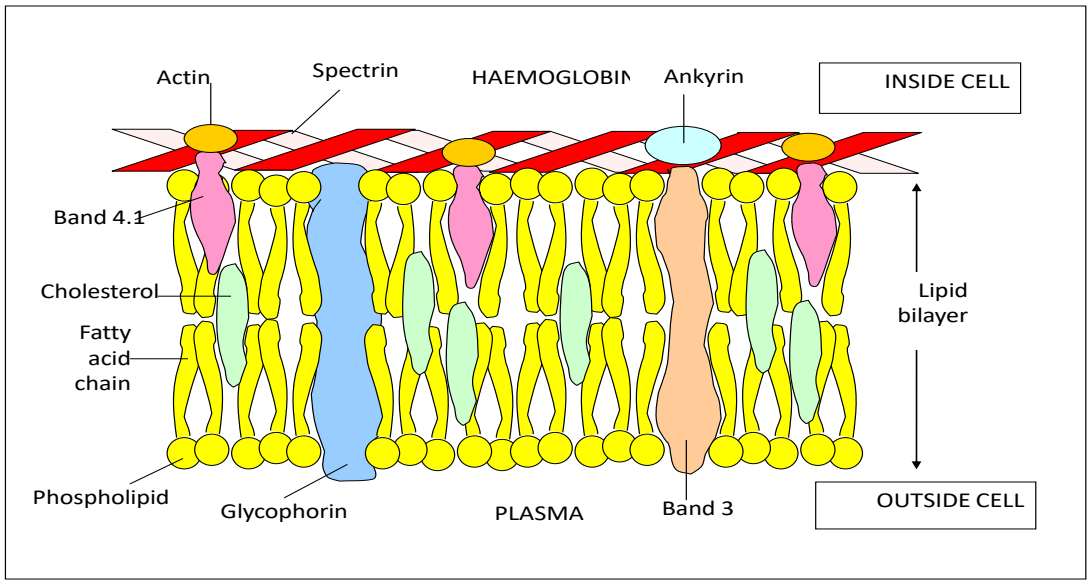
The red cell membrane consists of a bipolar lipid layer supported by structural proteins.

50% of the membrane is protein (inner layer).

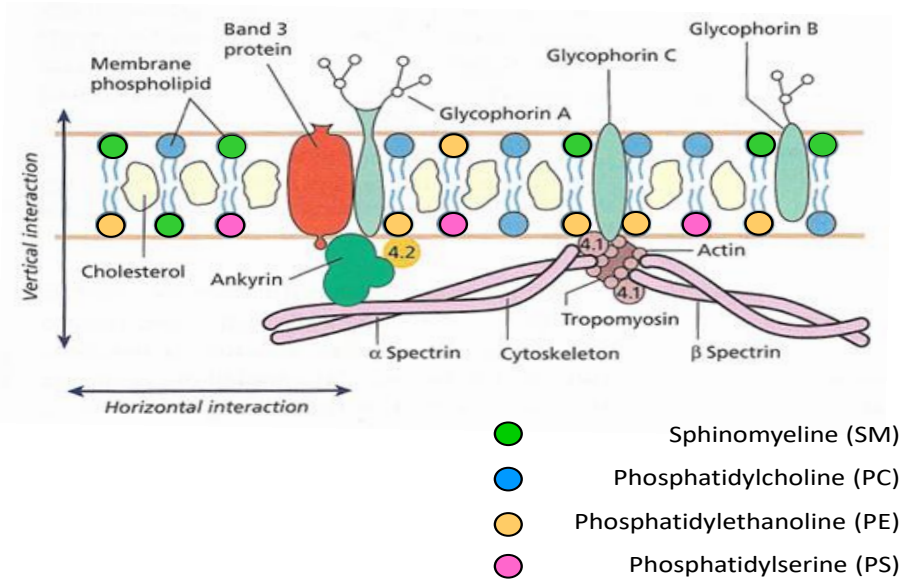
40% is lipid (middle layer)

10% is carbohydrate.(outer layer).

Structure of the red cell membrane.



A schematic representation of the red cell membrane structure



1-Middle Lipid bilayer (40%)

A- Hydrophilic:

60% phospholipid

10% glycolipids.

B- Hydrophobic:

30% neutral lipids (mainly cholesterol)

The phospho- and glycolipids are structural with polar groups **(hydrophilic)** on the external and internal surfaces of the cell.

Non-polar groups **(hydrophobic)** form a barrier at the centre of the membrane.

Phospholipids:

Phosphatidyl ethanolamine → Inner leaflet

,, serine →

,, choline → Outer leaflet

,, inositol

Sphingomyelin

-Their non polar fatty acids at the centre of the membrane, facing each other & r attracted by hydrophobic forces (**so can't permit H20 to leak in-between lipid bilayer).**

-Polar F.A. (hydrophilic) on the external & internal surfaces interacting e' aqueous phase of environment (plasma, Hb).

Cholesterol:

- It is arranged between phospholipid molecules.
- Ratio between cholesterol & phospholipids determine fluidity & deformability of cell membrane (↑cholesterol, ↑membrane rigidity).
- It is present in non esterified form w' is exchangeable e' that in the plasma:
- So in starvation: ↓plasma cholesterol, & so RBCs cholesterol ↓
- In liver diseases ,or e'↓ LCAT,↑non estrified cholesterol , ↑free cholesterol in plasma & RBCs → ↑surface area → ↑target cells.

N.B:

- Retics r the last stage capable of lipid synthesis, so mature RBCs depend on lipid exchange e' plasma for their fluidity.
- Fluidity of lipid bilayer depends on:

1-Cholesterol content: if ↑ → ↓ fluidity.

2-F.A: unsaturated → ↑ fluidity

saturated → ↓ ,,

3- Temperature

4-lipid bilayer is incompressible:

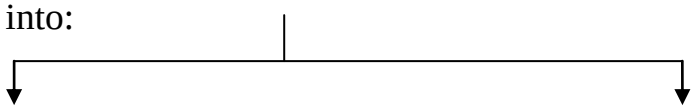
↑ cholesterol → ↑surface area → ↑ target cells.

2-Outer CHO layer (10%):

Consists of glycoproteins or glycolipids w' ve their protein base in the lipid bilayer carrying blood group antigens e.g: P, ABH & lewis Ag.

3-The inner protein layer: (50%):

Divided into:



Skeletal protein Integral (functional) protein

A- Skeletal protein:

- **Spectrin**
- **Actin**
- **Band 4.1**
- **Spectrin:**
- Is the most abundant protein of skeletal proteins

- Formed of diamers (2 α, 2β chains) wound around each other in a heterodiamer connecting at their heads (head to head connection) & ends in a tetramer linked to actin & 4.1 & integral protein (adducin) **{ Horizontal interaction}.**
  - Spectrin is arranged in 2 webs linked to inner surface of lipid layer.
  - Spectrin-ankyrin-band 3 complex : links skeletal to non skeletal proteins inside the lipid bilayer.

B- Non skeletal proteins (Integral):

- They r functional ptn.

1- Band 3:

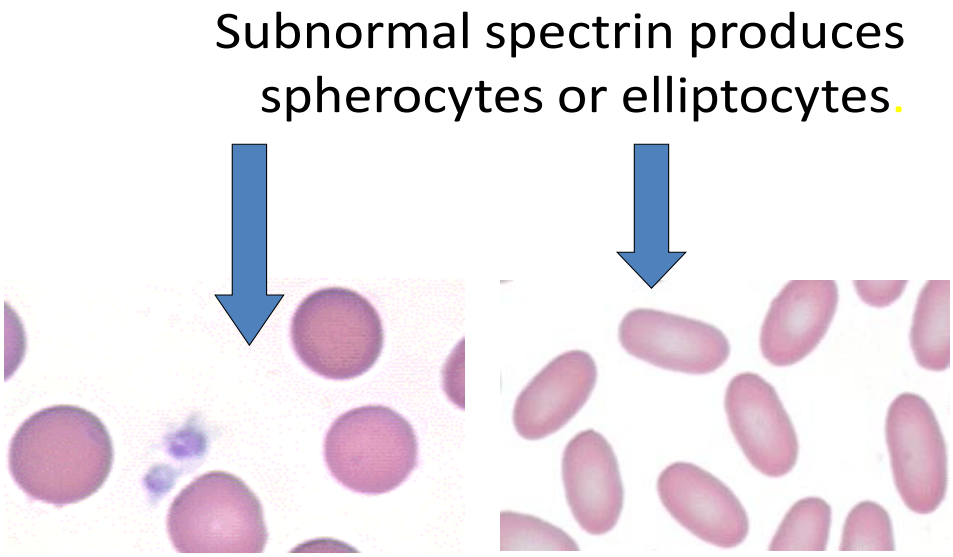
- Transport anions Cl-, HC03
- Carry ABH , Ii blood gp Ags

2-Glycophorins:

- A: carry MNS Ags
- B: carry Ss Ags
- C: attachment site to cytoskeleton

Its ↓ → elliptocytosis

- **3-Band 4.5:** Transports glucose.



Macro-Molecular assembly of membrane proteins

A-Horizontal Interaction

elation between skeletal protein & peripheral protein (1 of the functional ptn) → Adducin ⟶

This is responsible for membrane stability & structural integrity: any defect → **Loss of structural integrity** ⟶ membrane destabilization:

mild: **Elliptocytosis**

severe: **fragmentation**

B-Vertical Interaction:

Interaction between skeletal ptn & integral ptn & phospholipids responsible for stabilization of lipid bilayer & prevention of its loss from membrane

↓ Spectrin & Ankyrin

↓

loss of lipid bilayer

↓

↓ **surface area**

↓

Spherocytosis

Abnormalities of red cell membrane

1- Congenital:

- Spherocytosis
- Elliptocytosis
- Stomatocytosis

2-Acquired:

- PNH
- Liver disease

1-Spherocytosis

Hereditary spherocytosis: due to membrane defect

Acquired spherocytosis: AIHA (warm Ab) (detected by coomb's test).

Hereditary Spherocytosis

It is characterized by:

- Familial disorder (mainly AD , but rarely AR)
- NNA
- H.A
- Spherocytes in P.B
- ↑ O.F
- Splenomegaly
- Anaemia is corrected by splenectomy

Pathogenesis:

- **Intrinsic membrane defect**
- **Selective retention of these cells by N spleen**
- **2ry membrane changes.**

1- Intrinsic membrane defect:

A- membrane protein:

Four abnormalities in red cell membrane proteins have been identified and include

(1) spectrin deficiency alone,

(2) combined spectrin and ankyrin deficiency,

(3) band 3 deficiency, and

(4) protein 4.2 defects.

Spectrin deficiency is the most common defect.

Spectrin deficiency due to ↓synthesis → unstable spectrin

defective spectrin binding to ankyrin → disturbance in the vertical interaction between:

Spectrin, ankyrin & band 3 → loss of lipid bilayer in the form of microvescicles → ↓surface area →spherocytes.

B-↓membrane lipids:

2- 2ry membrane changes :

↓ K+ & H2O → dehydrated cells

↑ Intracellular Na+ → lysis

Na+/K ATPase → ↓ATP

3- Role of Spleen:

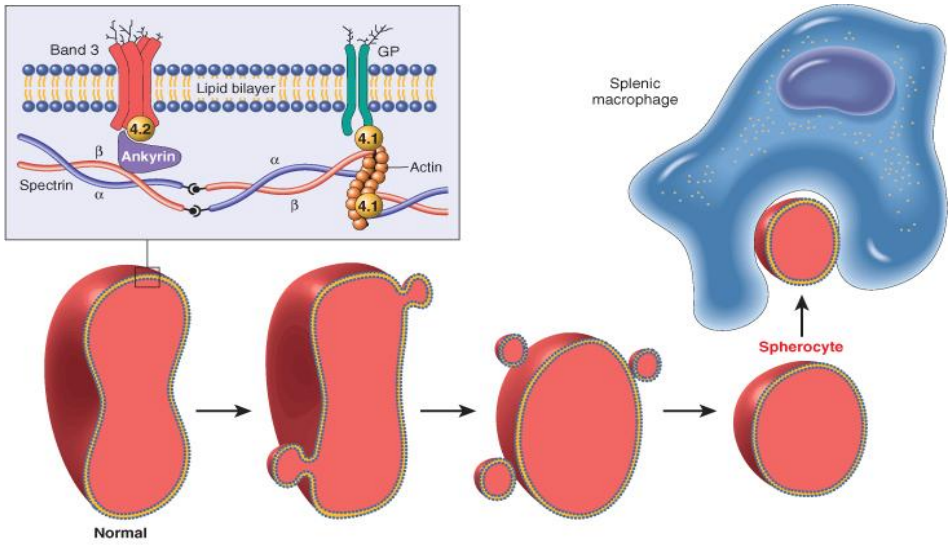
Spherocytes r less deformable than N cells e'↑ in their viscosity & ↓ in their content of ATP

So they can't tolerate their passage through sinuses & cords , ↓ O2 & ↓ glucose in media of spleen.

This will result in their partial phagocytosis by MQ of spleen → more ↓ in surface area → 2ry spherocytes (smaller than original spherocytes).

Clinical Picture:

Of H.A (see before).

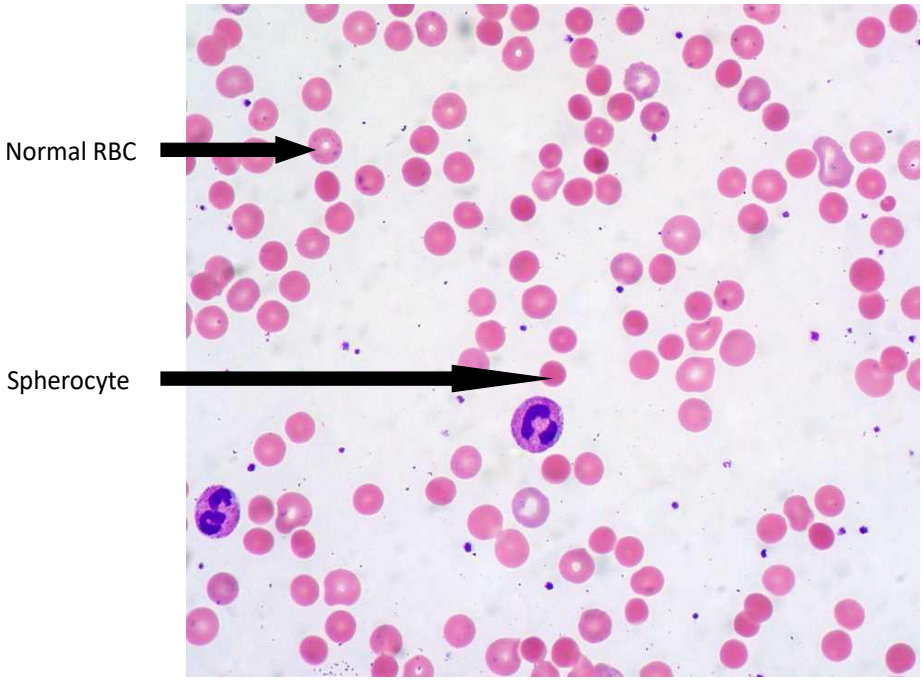


Diagnosis:

A- 3 Evidence : ↑destruction,
↑ production,
↓life span of RBCs.

B- CBC:

- ↓Hb
- ↑ Retics : 5-20%
- Microspherocytes (densely stained, no central pallor, smaller in diameter).
- ↑ MCHC



C-Special tests:

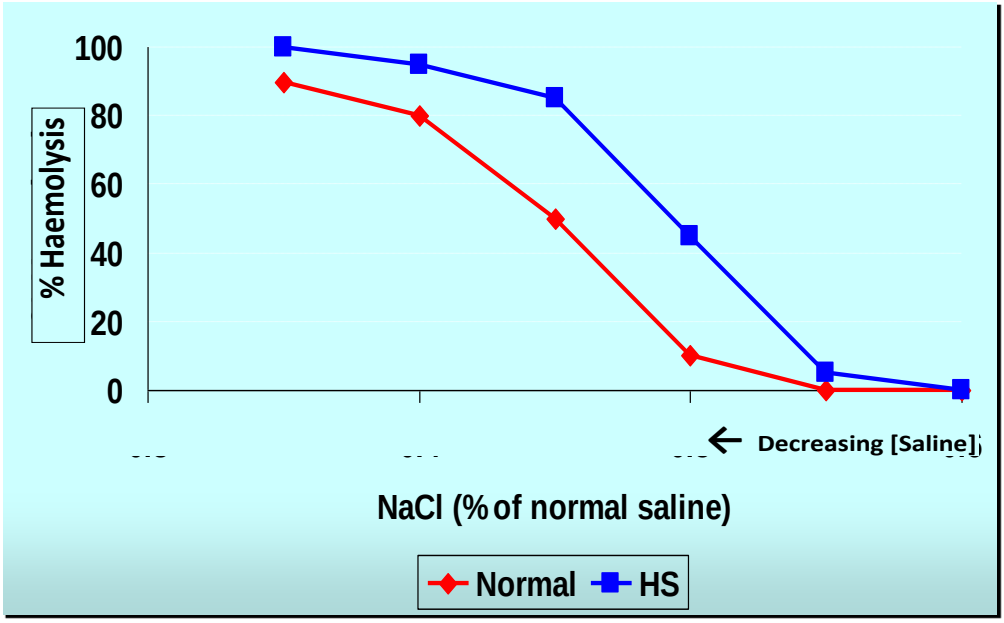
1- Osmotic fragility test:

These cells r osmotically fragile where haemolysis starts earlier than Normal cells e' double population of cells resulting in the presence of tail & O.F ended by tailing due to ↑ retics (resist haemolysis).

2-Autohaemolysis test:

Cells incubated in their own plasma e' & e'out glucose for 48 hs , degree of haemolysis is measured.

If the haemolysis ↑ & corrects by glucose means there was ↓ ATP



3- Glycerol lysis test:

The same principal as O.F. test.

4- Direct coomb's test:

D.D from AIHA coomb's +ve, H.S : coomb's –ve

Ttt:

Blood transfusion in severe cases.

Splenectomy: cures anaemia, as RBCs life span returns N.

2-Hereditary Elliptocytosis (Ovalocytosis)

It is a hereditary disorder of RBC membrane ch by the presence of elliptical (elongated) cells in P.B.

H.E is divided into :

- Common HE
- Spherocytic HE e' OF
- Stomatocytic HE

Common HE:

Pathogenesis:

Defect in RBCs membrane protein skeleton (mainly spectrin), evidenced by :

- ↑ thermal instability (normal cells can withstand temp. up to 50%).
- Disintegration of skeleton by shear stress.
- Defect in self association of spectrin diamers to form tetramers.
- Altered susceptibility of spectrin to trypsin digestion.
- ↓ 4.1 protein.

It is always ass. e' mutation in glycophorin C.

Defect in skeletal protein (Horizontal interaction: actin, spectrin, band 4.1 or adducin) ⟶ **Loss of structural integrity** → membrane destabilization:

mild: **Elliptocytosis**

severe: **fragmented cells**

Inheritance: AD

C/P:

1-Asymptomatic carrier state: diagnosed by biochemical detection of membrane defect (during research).

2-Minimal & mild HE: diagnosed by:

Presence of elliptical cells in CBC

No clinical evidence of haemolysis

Compensated H.A.

3-HE e' transient haemolysis:

Haemolysis usually ppt. by viral, bact,protozoal infections, pregnancy.

4- Chronic H.A: (C/P of H.A).

Lab diagnosis:

1- 3 evidence

2-CBC: mild NNA

elliptical cells : 15-100%

3-Special tests:

i-O.F:

in spherocytic HE

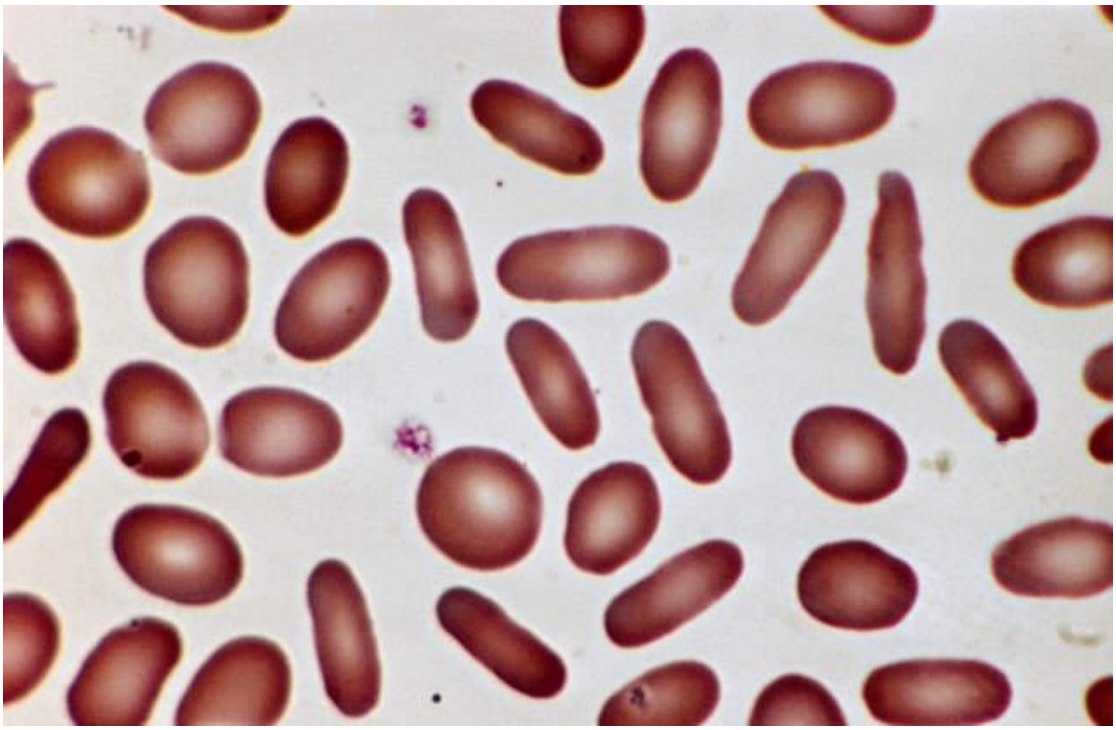
in severe HE due to: small & fragmented cells

ii-Thermal instability

Denaturation of spectrin at 45-46°C (N at 50°C).

Ttt:

Splenectomy if there is haemolysis.



3-Other membrane abnormalities

A-Acantocytosis

Red cells e' irregular projections vary in: length, width & distribution.

RBCs develop N in BM, then acquire that shape in plasma.

Causes:

Due to ↑ lipids in outer leaflet > inner leaflet in the lipid bilayer.

1-Liver diseases:

Due to ↑ cholesterol in the outer leaflet → expansion of its surface area in relation to inner leaflet → projections → severe H.A e' acantocytosis& target cells in P.B.

2-Abetalipoproteinaemia: due to ↑ sphingomylein.

3- Chorea: due to ↑ saturated FA → ↓fluidity (Acantocytosis syndrome).

4-Mc Load phenotype:

RBCs lack Kx antigen, it s sex linked.

Acantocytosis

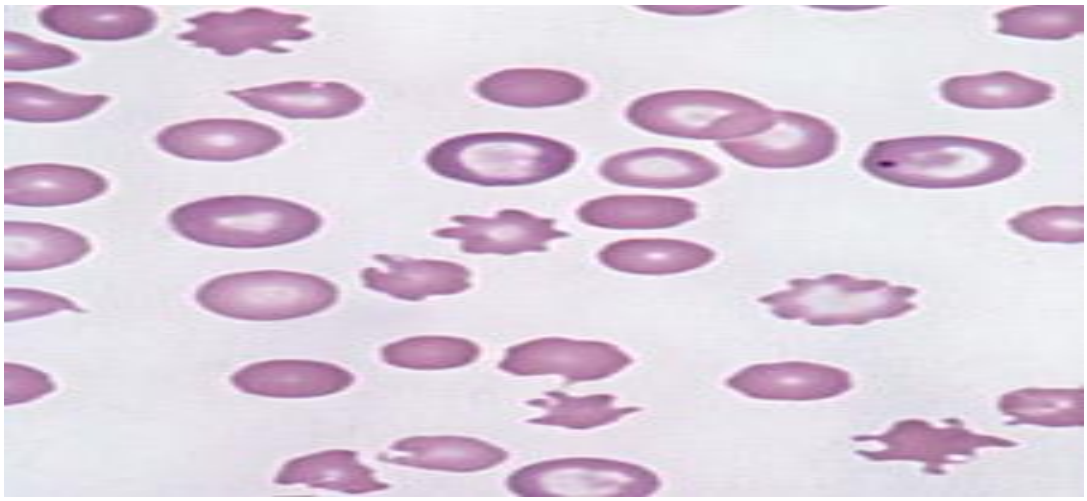


Table: causes of acantocytosis:

Congenital	Acquired
Abetalipoproteinaemia McLoad phenotype PK deficiency	Severe liver diseases MDS Neonatal vit E def.

B- ↓Lecithin Cholesterol Acetyl Transferase (L-CAT)

L-CAT transfers FA from phosphatidyl choline to cholestrol ⟶ esterified cholesterol.

↓ L-CAT → ↑unesterified cholesterol

If in both leaflets : target cells

If in outer leaflet only: Acantocytosis → mild compensated H.A.

C-Xerocytosis

Loss of K+ e'out Na+ gain → ↓H2O ⟶ dehydrated cells ⟶

↓ MCV, ↑MCHC.

D- Stomatocytosis

RBCs contain a wide transverse slit or stroma

Pathogenesis:

↑ Na+ & H2O ↑MCV, ↓ MCHC → mod. to severe anaemia.

Causes:

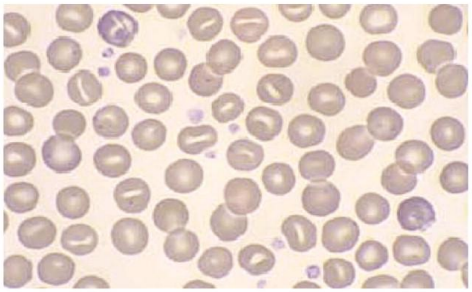
Congenital:

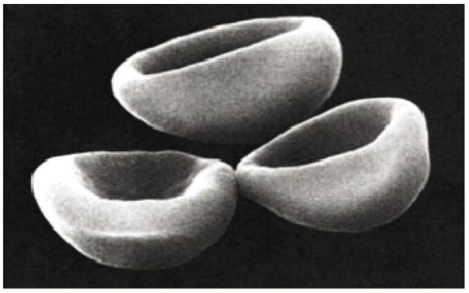
Due to ineffective Na+/K+ pump mechanism.

Acquired: malignant neoplasms, CVS disease, alcohol & drugs.

C/P: mod to severe H.A, 10-30% stomatocytes in P.B, present e' Rh null & McLoad phenotype.

Hereditary stomatocytosis red cell slide.





Flatt J F , Bruce L J Haematologica 2009;94:1039-1041

haematologica
the hematology journal