Dr. Khaled Al-Qaoud

Chapter 6

Hemolytic Anemias
Normocytic Normochromic Anemias

Hemolytic Anemias

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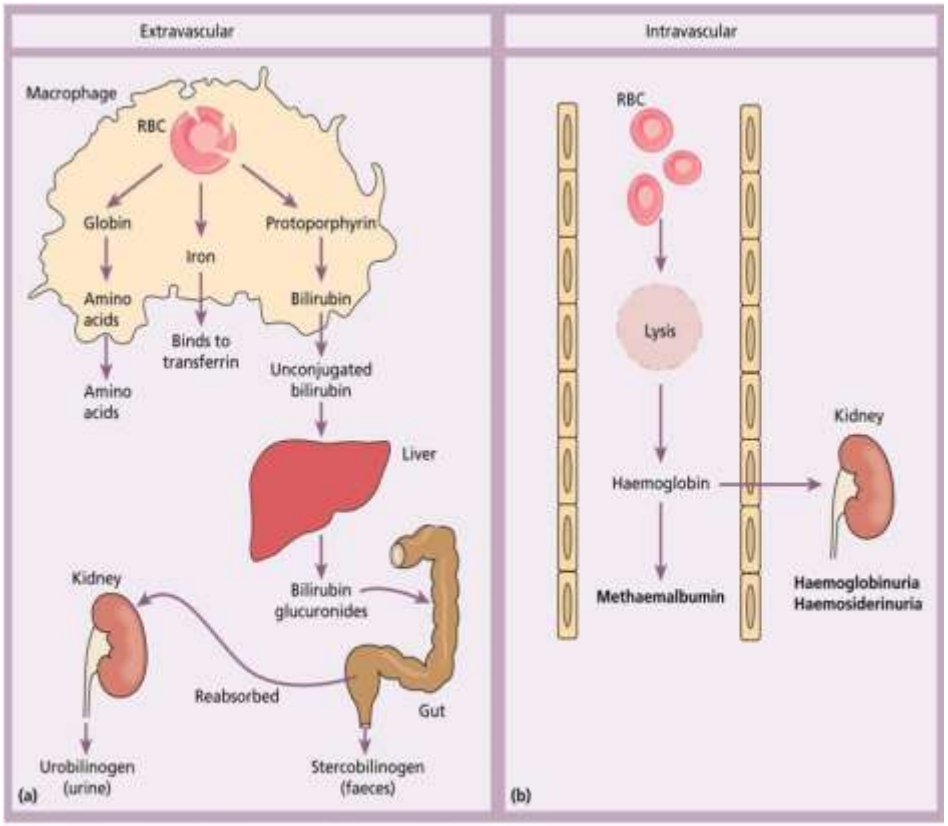

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Causes for Hemolytic Anemias

- Increase in the Rate of RBC destruction

Could Happen either:

- Intravascular: inside blood vessels
- Extravascular: in the reticuloendothelial system (RES): liver, spleen and bone marrow

All hemolytic Anemias are normocytic Normochromic except:

Thalassemia and paroxysmal nocturnal haemoglobinuria (PNH)

Normal RBC destruction

RBC destruction occurs after a mean life span of 120 days

Normal RBC destruction occurs through 2 mechanisms:

- Extravascular (major): RBC are removed from circulation via the macrophages of the RES
- Intravascular: plays minor or no part in RBC destruction.

Classification of Hemolytic Anemias

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Table 6.1 Classification of haemolytic anaemias.

Hereditary	Acquired
Membrane Hereditary spherocytosis, hereditary elliptocytosis	**Immune** *Autoimmune* Warm antibody type Cold antibody type
Metabolism G6PD deficiency, pyruvate kinase deficiency	*Alloimmune* Haemolytic transfusion reactions Haemolytic disease of the newborn Allografts, especially stem cell transplantation
Haemoglobin Genetic abnormalities (Hb S, Hb C, unstable); see Chapter 7	*Drug associated*
	Red cell fragmentation syndromes See Table 6.6
	March haemoglobinuria
	Infections Malaria, clostridia
	Chemical and physical agents Especially drugs, industrial/domestic substances, burns
	Secondary Liver and renal disease
	Paroxysmal nocturnal haemoglobinuria

G6PD, glucose-6-phosphate dehydrogenase; Hb, haemoglobin.

According to underlying causes hemolytic anemias can be classified to:

1. ***Hereditary*: usually results from intrinsic *RBC defect***
2. ***Acquired:* usually results from an extracorpuscular or environmental changes except PNH in which there is an intrinsic factor**

CLINICAL FEATURES

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- Patients with hemolytic anemias shows:

1. Pallor of the mucus membrane
2. Mild fluctuating jaundice
3. Splenomegaly
4. Pigmented (bilirubin) gallstones
5. Ulcers around the ankle especially in those who suffer from sickle cell disease

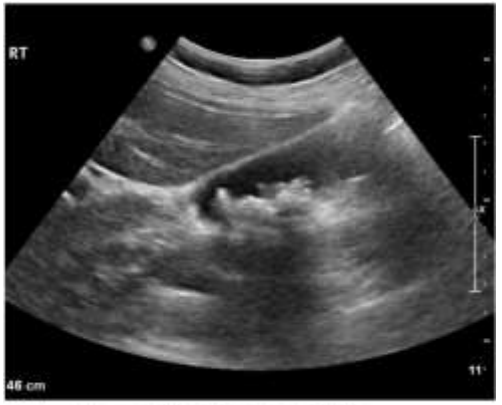

Figure 6.2 Ultrasound of multiple small pigment gallstones typical of those associated with hereditary spherocytosis. (Courtesy of Dr P. Wylie.)

LABORATORY FINDINGS

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Can be divided into 3 major groups:

1. Increase RBC breakdown
    1. Raised serum bilirubin, both unconjugated and bound
    2. Increased urine uribilinogen
    3. Increased fecal stercobilinogen
    4. Absence of serum hepatoglobins because it becomes saturated with hemoglobin and the complexes are removed by RES cells.
2. Features of increased RBC production:
    1. Reticulocytosis
    2. Bone marrow erythroid hyperplasia
    3. Damaged RBCs
    4. Osmic fragility (autohemolysis)

RBC destruction in Hemolytic Anemias

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- **<u>In extravascular destruction,</u>** there is excessive removal of RBC by the cells of RES (Mac)
- **<u>In intravascular destruction</u>** , free Hb is filtered by the glomerulus, if high free Hb will enter the urine.
- **Laboratory findings of the Intravascular hemolysis:**
  - **Hemoglobinemia**
  - **Haemosiderinuria**
  - **methhemalbumenaemia**

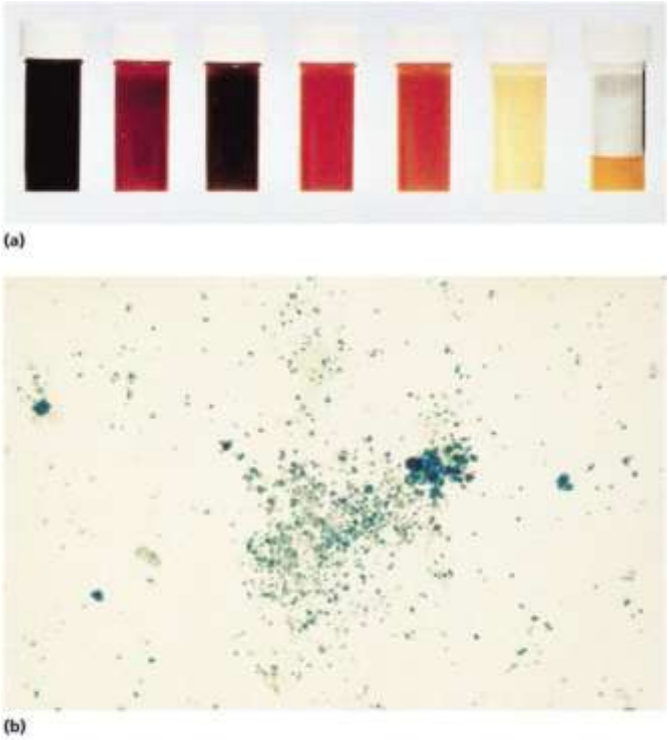

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Figure 6.3 **(a)** Progressive urine samples in an acute episode of intravascular haemolysis showing haemoglobinuria of decreasing severity. **(b)** Prussian blue-positive deposits of haemosiderin in a urine spun deposit (Perls' stain).

What is Methemoglobin: Hb where the iron in the heme group is in the Fe^{3+} (ferric) state, not the Fe^{2+} (ferrous) of normal hemoglobin. Methemoglobin cannot bind oxygen

Causes of intravascular Hemolysis

1. Mismatched Blood Transfusion
2. G6PD deficiency with oxidant stress
3. Some drugs and infections
4. PNH, unstable HB and RBC fragmentation.

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Hereditary Hemolytic Anemias

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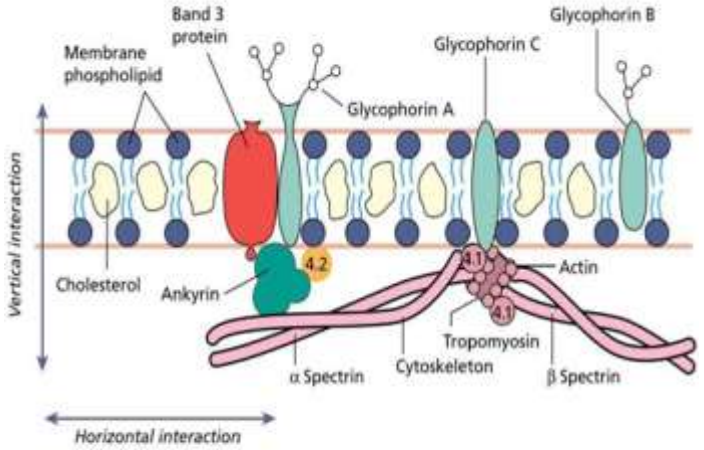
1. Membrane Defects:

a) Hereditary Spherocytosis (HS):

- Usually caused by defects in the proteins involved in the vertical interactions between the membrane skeleton and the lipid bilayer of RBC
- The defect results in the release of parts of the lipid bilayer that are not supported by the skeleton.
- This leads to the decrease of the surface area to volume ratio and the cells became spherical to maintain their contents.



Hematological Findings

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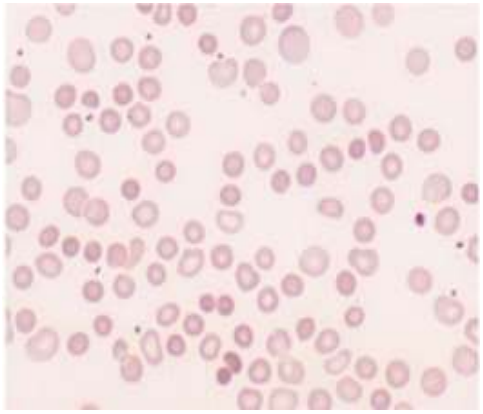
1. Anemia is variable
2. Normal Blood indices
3. Reticulocytes (5-20%)
4. High retics during hemolytic anemia and low retics during aplastic crisis



Blood Film:

1. Microspherocytes (densely stained

Lab test: osmotic fragility test

Hereditary Hemolytic Anemias

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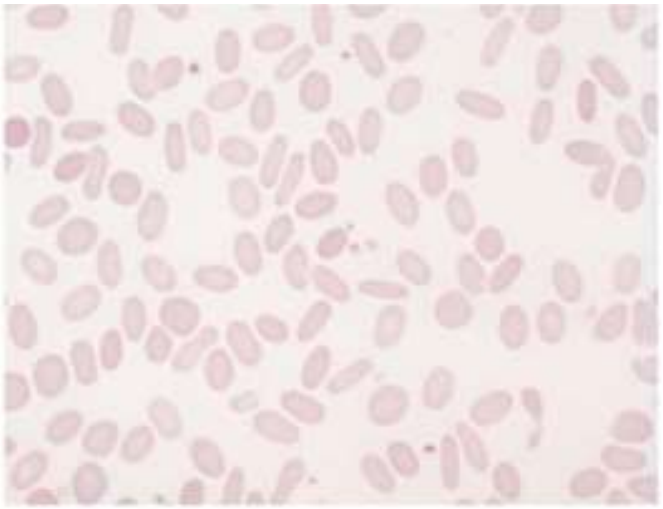
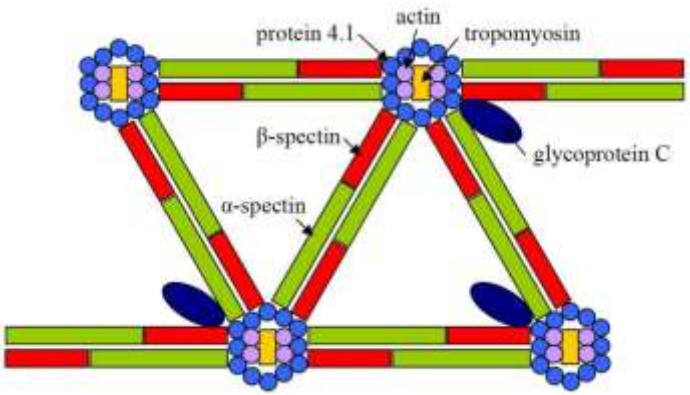
a) Hereditary Elliptocytosis (ovalocytosis)

- Elliptocytes appear in Blood instead of spherocytes
- The Anemia is milder than HS
- The basic defect is the failure of spectrin heterodimers to self associate into heterotetramers
- Mutations in ankyrin genes and other proteins
- Patients may require splenectomy as treatment





A schematic diagram representing the relationships between cytoskeletal molecules as relevant to hereditary elliptocytosis

Hereditary Hemolytic Anemias

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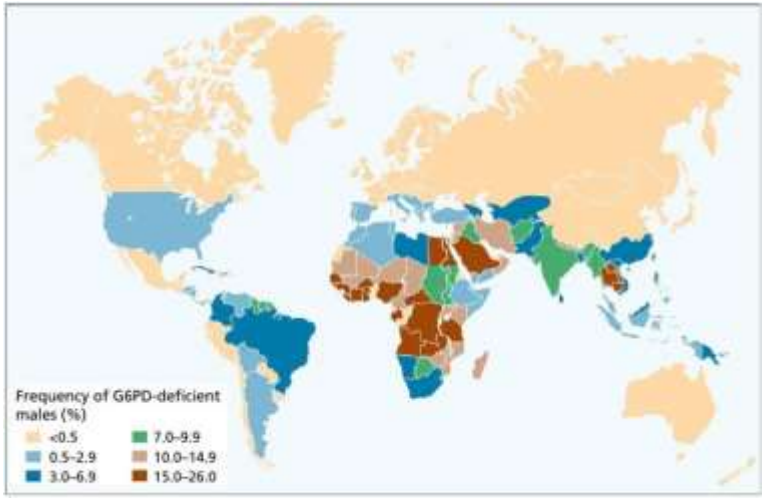
Metabolic Defects

- Glucose-6-Phosphate dehydrogenase deficiency
- Inherited as sex linked and affect males only and females are carriers
- Degree of deficiency varies either mild (10-15%) of normal activity in black Africans or more sever in Orientals and more sever in Mediterranean
- Sever deficiency occurs occasionally in white people.



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Hereditary Hemolytic Anemias

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- Acute hemolysis occurs in response to oxidant stress: Drugs, Fava Beans, infections
- Hb and RBC are protected from oxidants by reduced glutathione
- In G6PD deficiency, there is a decease in the glutathione and as a consequence oxidants are free to damage RBC and oxidize HB
- Oxidation of Hb leads to the formation of Heinze Bodies.

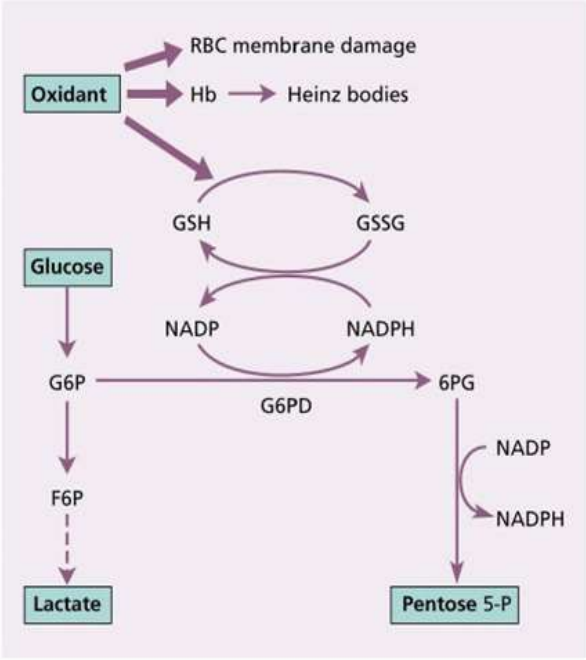

Figure 6.6 Haemoglobin and red blood cell (RBC) membranes are usually protected from oxidant stress by reduced glutathione (GSH). In G6PD deficiency, NADPH and GSH synthesis is impaired. F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GSSG, glutathione (oxidized form); NADP, nicotinamide adenine dinucleotide phosphate.

Hematological Findings

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1. Between crises the blood count is normal
2. During hemolysis crises:
   - Pallor, Jaundice, hemoglobinurea, due to intravascular hemolysis
   - Anemia is Normocytic Normochromic
   - Reticulocytosis
3. Diagnosis by enzyme assay after crisis. During crisis there is reticulocytosis and retic has high enzyme activity

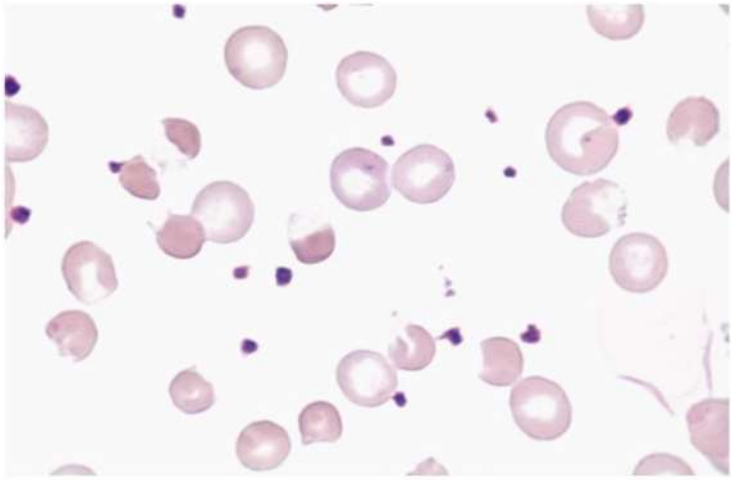

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Figure 6.8 Blood film in G6PD deficiency with acute haemolysis after an oxidant stress. Some of the cells show loss of cytoplasm with separation of remaining haemoglobin from the cell membrane ('blister' cells). There are also numerous contracted and deeply staining cells. Supravital staining (as for reticulocytes) showed the presence of Heinz bodies (see Fig. 2.17).

Acquired Hemolytic Anemias

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- Immune hemolytic anemia

Divided into:

1. Autoimme hemolytic anemia
2. Alloimmune hemolytic anemia
3. Drug induced hemolytic anemia

- March Hemoglobinuria
- Hemolytic anemia due to infections
- Hemolytic anemia due to chemical and physical agents
- RBC fragmentation syndrome
- PNH

Autoimmune Hemolytic Anemia (AIHA)

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- Caused by antibody production by the body against its own RBC
- Characterized by positive direct agglutination test (DAT) coombs test
- Divided into 2 main types:
  1. Warm Autoimme hemolytic anemia at which the reaction of Antibody with RBC occurs strongly at 37C
  2. Cold Autoimme hemolytic anemia at which the reaction of Antibody with RBC occurs strongly at 4 C

Warm Autoimmune Hemolytic Anemia

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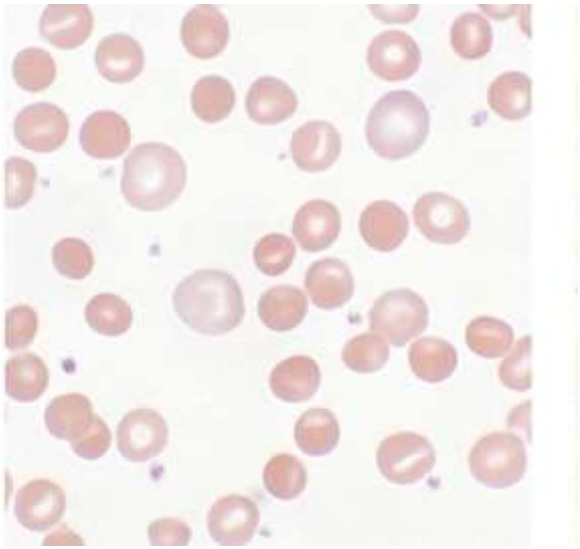
- RBC coated with IgG or IgG and complement
- Coated RBC taken by Macrophages in RES
- Part of membrane lost and cells become spherical
- Coated cells destroyed in the spleen
- The spleen is often enlarged
- Occurs at any age or sex



Blood film in warm autoimmune hemolytic anemia

Cold Autoimmune Hemolytic Anemia

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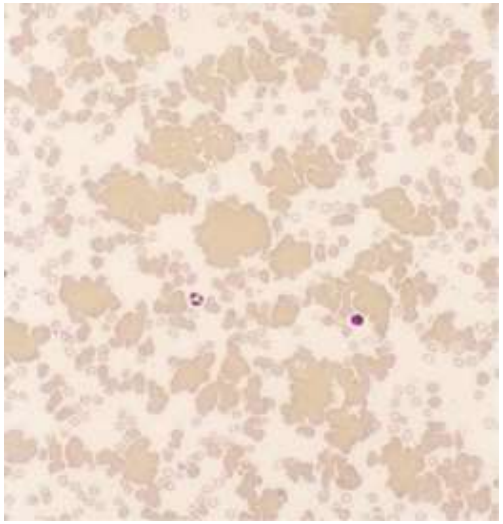
- Antibody attached to RBC mainly in the peripheral circulation where the blood T is cooled
- The antibody is usually IgM associated with intravascular hemolysis
- IgM efficient in complement fixing so RBC damage may occur intra and extravasclar



Blood film in cold autoimmune hemolytic anemia
Shows RBC agglutination

Alloimmune Hemolytic Anemias

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- In this anemia, Antibody produced by one individual reacts with others RBC
- Two important situations:
  1. Incompatible blood transfusion
  2. Rhesus disease of the neoborn
- Increases in cases of allogeneinic transplantation

Drug Induced Hemolytic Anemia

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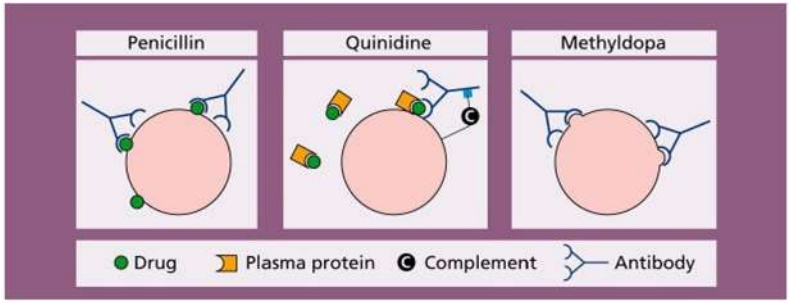
- Drug may induce hemolytic anemias via three different mechanisms:
  - Antibody directed a drug-RBC membrane complex (e.g penicillin etc)
  - Deposition of complement via drug-protein-antibody complex onto RBC surface
  - True autoimmune HA at which the role of drug is not clear
- In each case the anemias disappears when the drug is discontinued



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Mechanisms of drug induced hemolytic anemia

Paroxysmal nocturnal hemoglobinuria (PNH)

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- Rare acquired syndrome
- Clonal disorder of stem cells in which there is deficient synthesis of the glycosylphosphatydlinistol (GPI) anchor
- GPI anchor attaches several surface proteins to the RBC membrane
- The net result In GPI deficiency is that GPI linked proteins such as CD55 and CD59 are absent from the RBC surface of all cells derived from the abnormal stem cells
- Lack of CD55 and CD59 renders cells sensitive to lysis by complement
- Intravascular hemolysis

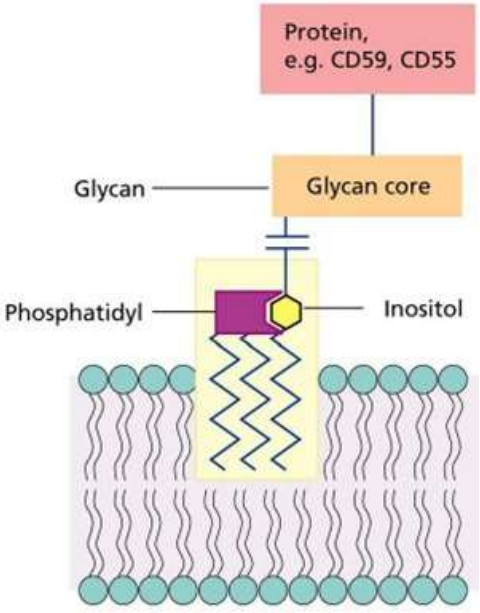

Figure 6.12 Schematic representation of the phosphatidylinositol glycan which anchors many different proteins to the cell membrane (e.g. CD59).

Paroxysmal nocturnal hemoglobinuria (PNH)

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Special tests:

1. Hams test:

- RBC lysis in the serum at low pH. Low pH activates the alternative pathway of the complement system which leads to RBC lysis

2. Flow cytometry:

looking for loss of expression of GPI-linked proteins such as CD55 and CD59

sensitive test