Hemolytic anemia

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Topics

- Normal red cell destruction.
- Definition of hemolytic anemia.
- Clinical features.
- Laboratory findings.
- Classification.
- Hereditary spherocytosis.
- G6PD deficiency.
- Immune hemolytic anemia.
- Red cell fragmentation syndromes.
- March hemoglobinuria.
- Role of infections in hemolytic anemia.

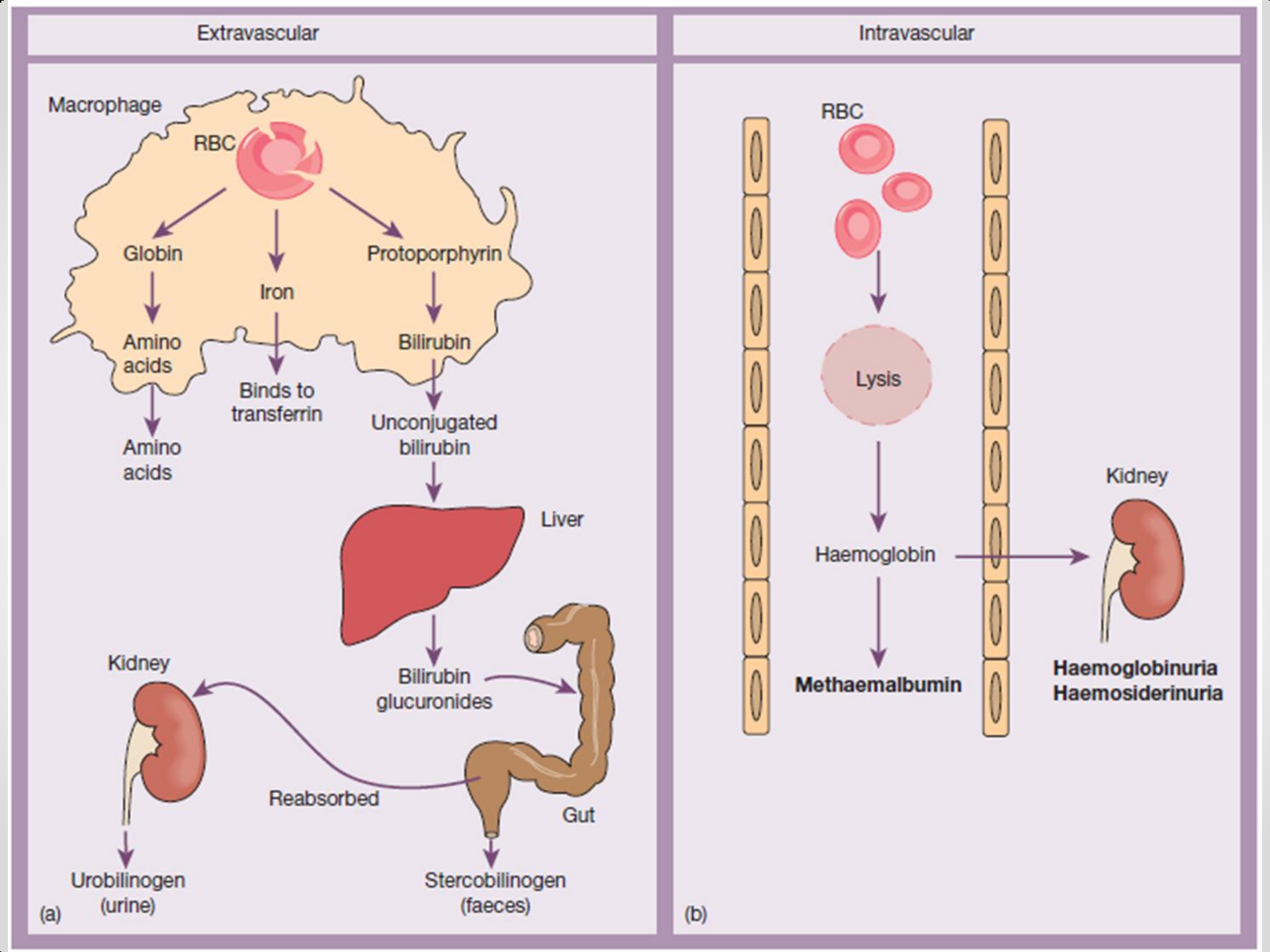
Objectives

➢ **At the end of the lecture, you will be able to:**

- Enumerate and describe the types of hereditary hemolytic anemia.
- Enumerate and describe the types of acquired hemolytic anemia.

Normal red cell destruction

- Red blood cell destruction usually occurs after a mean lifespan of 120 days, as the cells have no nucleus, red cell metabolism gradually deteriorates as enzymes are degraded and the cells become non-viable.
- The aged red cells are removed extravascularly by the macrophages of the reticuloendothelial (RE) system, especially in the bone marrow but also in the liver and spleen.
- Intravascular hemolysis plays little or no part in normal red cell destruction.


Extravascular
Intravascular
Macrophage
RBC
Globin
Iron
Protoporphyrin
Amino acids
Binds to transferrin
Bilirubin
Amino acids
Unconjugated bilirubin
Liver
Kidney
Bilirubin glucuronides
Reabsorbed
Gut
Urobilinogen (urine)
Stercobilinogen (faeces)
(a)
RBC
Lysis
Haemoglobin
Methaemalbumin
Kidney
Haemoglobinuria
Haemosiderinuria
(b)

Hemolytic anemia

- Defined as anemia that result from an increased or accelerated rate of red cell destruction (premature destruction of RBC).
- The normal bone marrow, is able to produce red cells at 6–8 times the normal rate. So, red cell destruction may be increased several-fold before the patient becomes anemic (compensated hemolysis).
- Anemia due to hemolysis may not be seen until the red cell lifespan is less than 30 days (decompensated hemolytic anemia).

Clinical features

- Pallor.
- Jaundice.
- Splenomegaly.
- Dark urine.
- Pigment (bilirubin) gallstones.

Laboratory findings

The laboratory findings are divided into three groups:

1- Features of increased red cell breakdown:

- serum bilirubin raised.
- urine urinobilinogen increased.
- serum haptoglobins absent because the haptoglobins become saturated with hemoglobin.

2- Features of increased red cell production:

- reticulocytosis.
- bone marrow erythroid hyperplasia.

3- Damaged red cells:

- morphology (e.g. microspherocytes, fragments).
- osmotic fragility.
- specific enzyme, protein or DNA tests.

Classification

Many classifications were adopted.

❖ Classifications according to the site of destruction of the red cells:

❑ Extravascular hemolysis (removal of red cells by cells of the RE system).

❑ Intravascular hemolysis (destruction directly in the circulation).

- Which mechanism dominates will depend on the pathology involved.

❖ Classifications according to underlying pathology:

❑ Hereditary hemolytic anemia are the result of ‘intrinsic’ red cell defects.

❑ Acquired hemolytic anemia are usually the result of an ‘extracorpuscular’ change.

Table Classification of haemolytic anaemias.

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

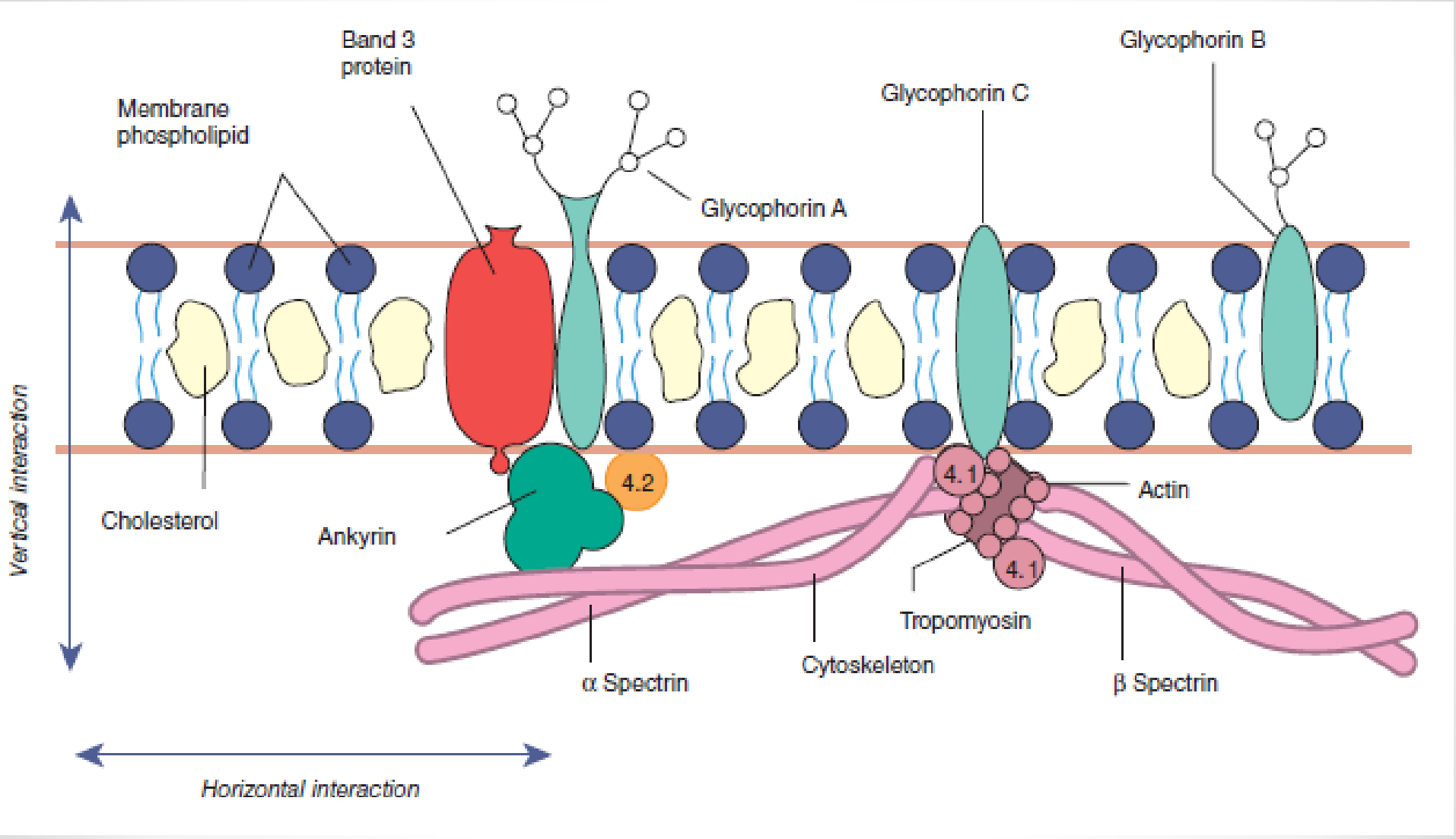
Hereditary hemolytic anemia

- **Membrane defects**

Hereditary spherocytosis

- Is the most common hereditary hemolytic anemia.
- HS is usually caused by defects in the proteins:
  - Ankyrin
  - α- or β-spectrin
  - Band 3
  - Pallidin (protein 4.2)

involved in the vertical interactions between cell membrane skeleton and lipid bilayer of the red cell.


Band 3
protein
Membrane
phospholipid
Glycophorin C
Glycophorin B
Glycophorin A
Cholesterol
Ankyrin
4.2
4.1
Actin
4.1
Tropomyosin
α Spectrin
Cytoskeleton
β Spectrin
Vertical interaction
Horizontal interaction

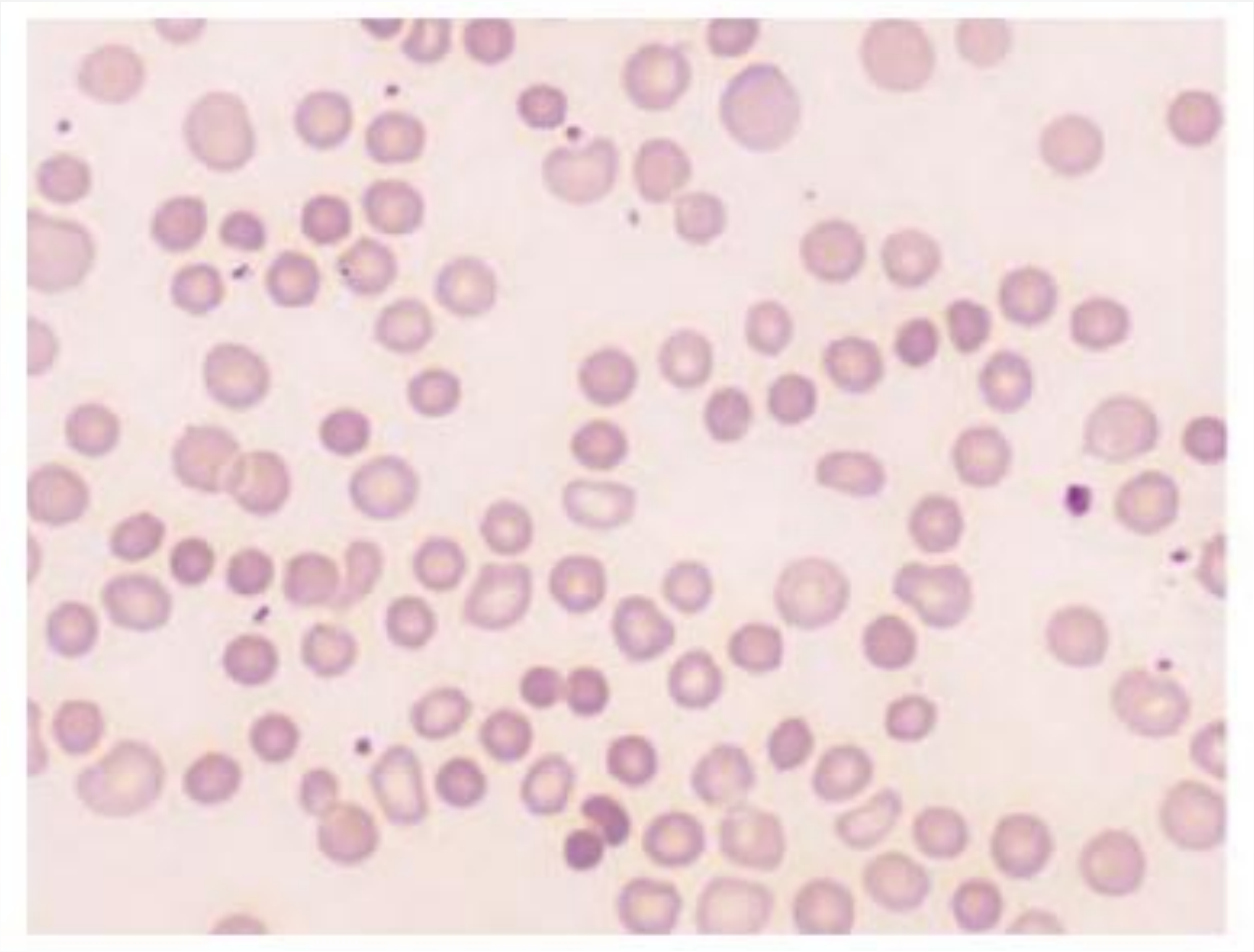
- The bone marrow produces red cells of normal biconcave shape but these lose membrane and become spherical (loss of surface area relative to volume).
- The loss of membrane caused by the release of parts of the lipid bilayer that are not supported by the skeleton.
- the spherocytes are unable to pass through the microcirculation, especially in the spleen, and die prematurely.

Inheritance

- autosomal dominant; rarely autosomal recessive.

Investigation

- Anemia.
- Reticulocytosis.
- The blood film shows microspherocytes, which are densely staining with smaller diameters than normal red cells.
- Osmotic fragility test.
- EMA test.



Blood film in hereditary spherocytosis

The spherocytes are deeply staining and of small diameter, larger polychromatic cells are reticulocytes (confirmed by special stain).

- **Metabolism defects**

G6PD deficiency

- Glucose-6-phosphate dehydrogenase (G6PD) is reducing agent, which is needed for the production of reduced glutathione; a deficiency renders the red cell susceptible to oxidant stress.
- There is a wide variety of normal genetic variants of the enzyme G6PD, the most common being type B in Western and type A in Africans. In addition, more than 400 variants caused by point mutations or deletions, producing G6PD enzyme with less activity than normal result in G6PD deficiency.

Inheritance:

- Is sex-linked, affecting males, and carried by females who show approximately half the normal red cell G6PD values. The female heterozygotes have an advantage of resistance to Falciparum malaria.

Clinical features:

1. Acute hemolytic anemia (intravascular) in response to oxidant stress e.g. drugs, fava beans or infections. With a normal blood count between attacks of hemolysis.
2. Neonatal jaundice.
3. Rarely, a congenital non-spherocytic hemolytic anemia.

Table Agents that may cause haemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PD) deficiency.
Infections and other acute illnesses (e.g. diabetic ketoacidosis)
Drugs
Antimalarials (e.g. primaquine, pamaquine, chloroquine, Fansidar, Maloprim)
Sulphonamides and sulphones (e.g. co-trimoxazole, sulfanilamide, dapsone, sufasalazine)
Other antibacterial agents (e.g. nitrofurans, chloramphenicol)
Analgesics (e.g. aspirin), moderate doses are safe
Antihelminths (e.g. β-naphthol, stibophen)
Miscellaneous (e.g. vitamin K analogues, naphthalene (mothballs), probenecid)
Fava beans (possibly other vegetables)
N.B. Many common drugs have been reported to precipitate haemolysis in G6PD deficiency in some patients (e.g. aspirin, quinine and penicillin) but not at conventional dosage.

Diagnosis

❖ **Between crises:** the blood count is normal. The enzyme deficiency is detected by G6PD enzyme assay.

❖ **During a crisis:**

- The blood film may show contracted and fragmented cells, bite cells and blister cells with reticulocytosis.
- There are also features of intravascular hemolysis.
- Because of the higher enzyme level in young red cells (reticulocyte), enzyme assay may give a ‘false’ normal level in the phase of acute hemolysis. Subsequent assay after the acute phase reveals the low G6PD level when the red cell population is of normal age distribution.

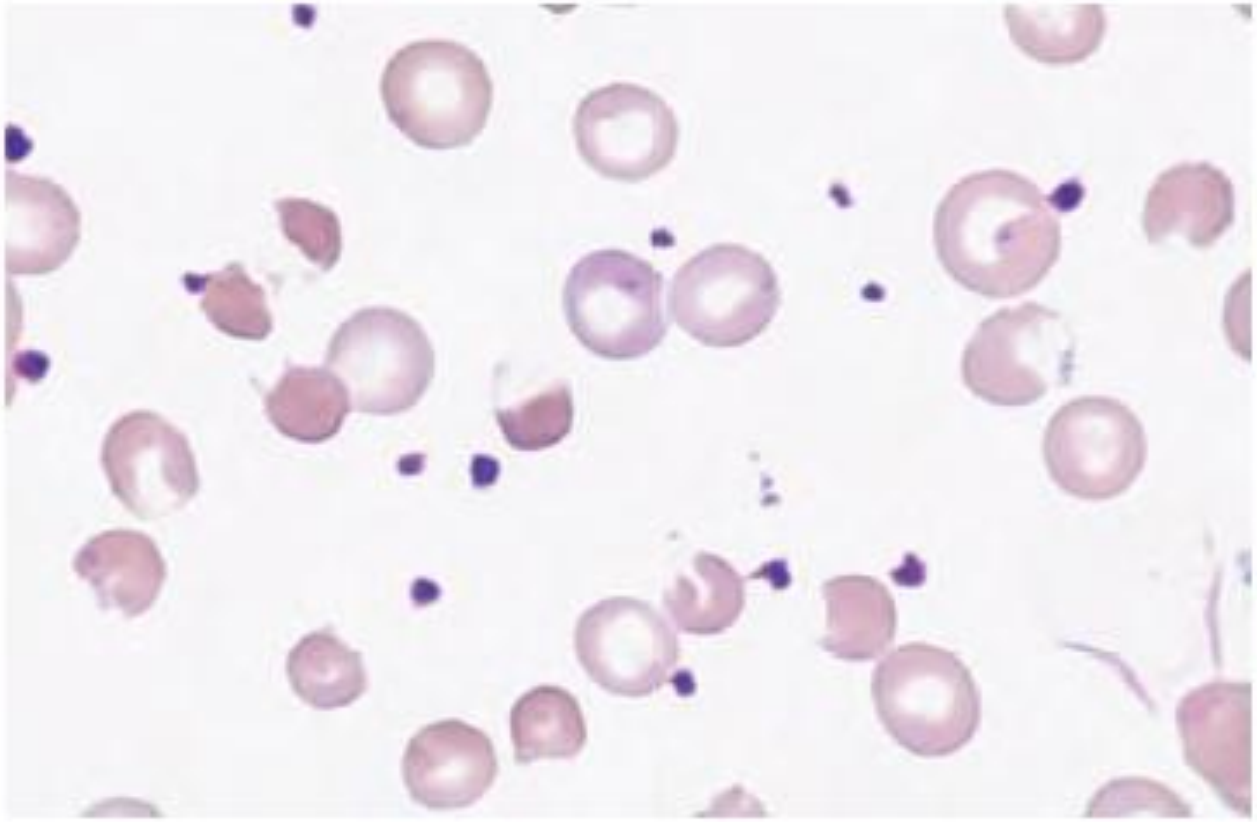


Figure Blood film in G6PD deficiency with acute haemolysis after an oxidant stress. Some of the cells show loss of cytoplasm with separation of remaining haemoglobin from the cell membrane ('blister' cells). There are also numerous contracted and deeply staining cells.

Acquired hemolytic anemia

- **Immune hemolytic anemia**

Autoimmune hemolytic anemia

- Autoimmune hemolytic anemia (AIHA) are caused by autoantibody produced by the body against its own red cells.
- They are characterized by a positive direct antiglobulin test (DAT), also known as the Coombs' test, and divided into 'warm' and 'cold' types according to whether the antibody reacts more strongly with red cells at 37°C or 4°C.

Warm type

Autoimmune
Idiopathic
Secondary
SLE, other 'autoimmune' diseases
CLL, lymphomas
Drugs (e.g. methyldopa)

Cold type

Idiopathic
Secondary
Infections – *Mycoplasma* pneumonia, infectious mononucleosis
Lymphoma
Paroxysmal cold haemoglobinuria (rare, sometimes associated with infections, e.g. syphilis)

Alloimmune hemolytic anemia

- In these anemia, antibody produced by one individual reacts with red cells of another.

Example:

- Transfusion of ABO-incompatible blood (hemolytic transfusion reaction).
- Rh hemolytic disease of the newborn.
- Allogeneic transplantation for renal, hepatic, cardiac or bone marrow grafts.

Drug-induced immune hemolytic anemia

- Drugs may cause immune hemolytic anemia via three mechanisms:

1. Antibody directed against a drug–red cell membrane complex (e.g. penicillin, ampicillin); this only occurs with massive doses of the antibiotic.
2. Deposition of complement via a drug–protein (antigen) – antibody complex onto the red cell surface (e.g. quinidine, rifampicin).
3. A true autoimmune hemolytic anemia in which the role of the drug is unclear (e.g. methyldopa).

❖ In each case, the hemolytic anemia gradually disappears when the drug is discontinued.

Red cell fragmentation syndromes

- These arise through physical damage to red cells either on abnormal surfaces (e.g. artificial heart valves or arterial grafts), arteriovenous malformations or as red cells passing through abnormal small vessels (microangiopathic hemolytic anemia), such as in DIC, TTP or vasculitis.
- The blood film contains many deeply staining red cell fragments.

Table	Red cell fragmentation syndromes.
Cardiac haemolysis	Prosthetic heart valves Patches, grafts Perivalvular leaks
Arteriovenous malformations	
Microangiopathic	TTP-HUS Disseminated intravascular coagulation Malignant disease Vasculitis (e.g. polyarteritis nodosa) Malignant hypertension Pre-eclampsia/HELLP Renal vascular disorders/HELLP syndrome Ciclosporin Homograft rejection

HELLP, haemolysis with elevated liver function tests and low platelets; HUS, haemolytic uraemic syndrome; TTP, thrombotic thrombocytopenic purpura.

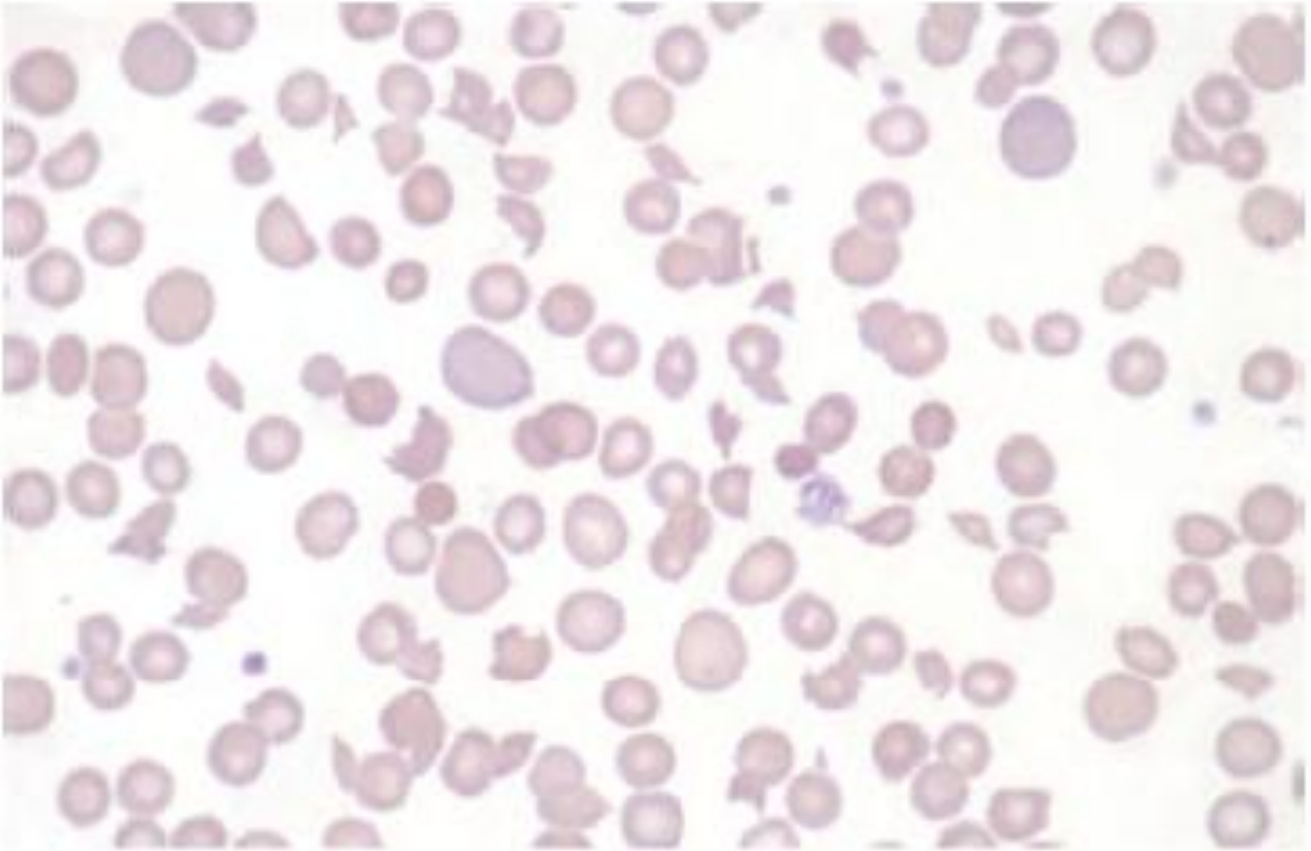


Figure Blood film in microangiopathic haemolytic anaemia (in this patient Gram-negative septicaemia). Numerous contracted and deeply staining cells and cell fragments are present.

March hemoglobinuria

- This is caused by damage to red cells between the small bones of the feet, usually during prolonged marching or running.
- The blood film does not show fragments.

Infections

- **Infections can cause hemolysis in a variety of ways:**

1. Precipitate an acute hemolytic crisis in G6PD deficiency.
2. Microangiopathic hemolytic anemia (e.g. meningococcal or pneumococcal septicemia).
3. Malaria causes hemolysis by extravascular destruction of parasitized red cells as well as by direct intravascular lysis.
4. Clostridium perfringens septicemia can cause intravascular hemolysis with marked microspherocytosis.

Any Question?

Home work:

Enumerate three types of membranopathy and enzymopathy.

THE END

THANK YOU FOR LISTENING