Heamolytic Anemias

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HAEMOLYTIC ANAEMIAS

- Haemolytic anaemias are caused by increased destruction of red cells.
- The red cell normally survives about 120 days, but in haemolytic anaemias the red cell survival times are considerably shortened.
- Breakdown of normal red cells occurs in the macrophages of the bone marrow, liver and spleen (reticuloendothelial system).
- *Reticulocytes* are immature red cells released prematurely (RETICULOCYTOSIS).

Sites of haemolysis

- Extravascular haemolysis

  In most haemolytic conditions red cell destruction is extravascular. The red cells are removed from the circulation by macrophages in the reticuloendothelial system, particularly the spleen.

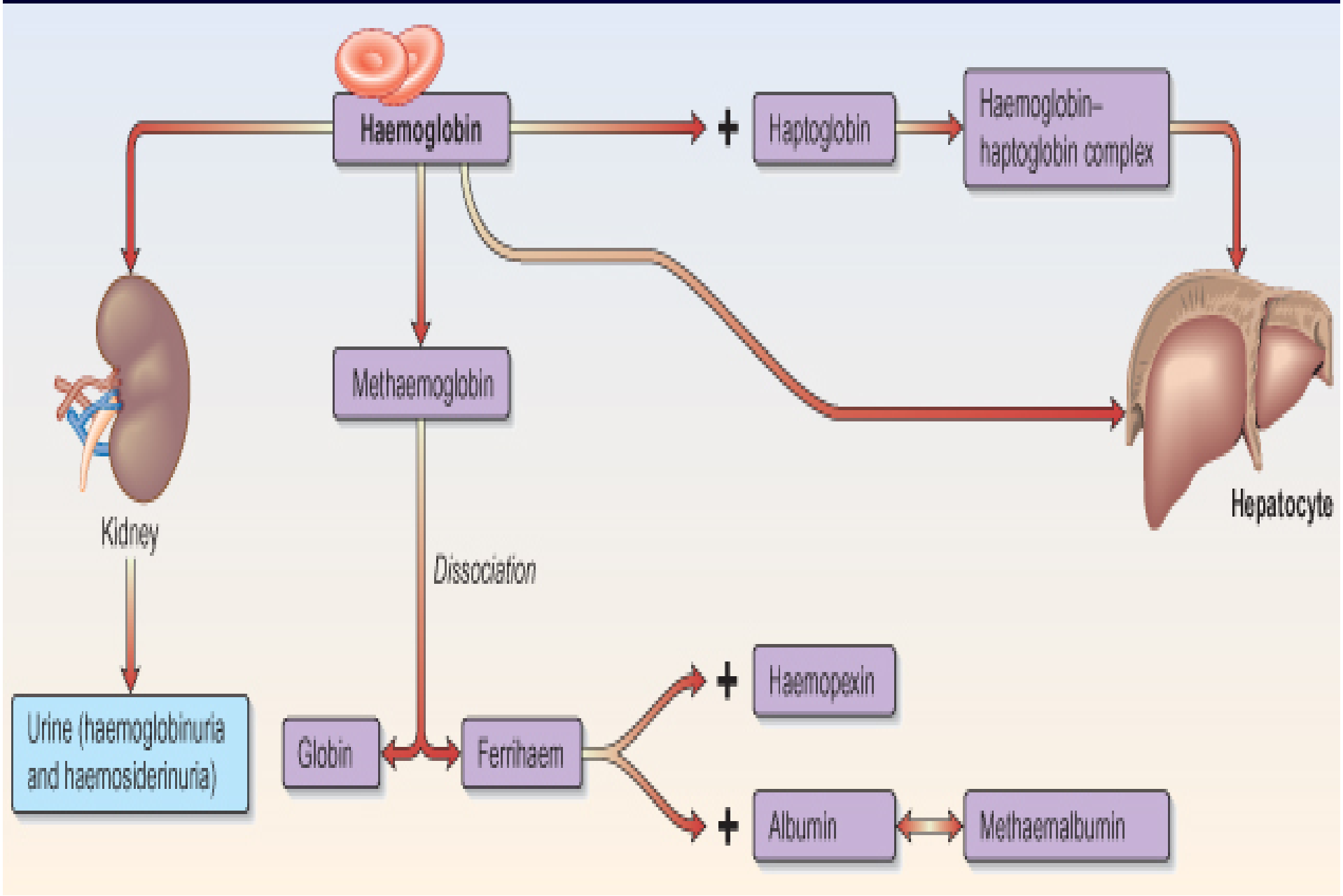
- Intravascular haemolysis

  Red cells are rapidly destroyed within the circulation and haemoglobin is liberated in plasma.

Fate of heamoglobin in intravascular hamolysis

- Heamoglobin is initially **bound** to plasma protein **haptoglobins** but these soon become saturated.
- Excess free plasma Hb is **filtered** by the renal glomerulus and enters the urine **(heamoglobinuria).**
- small amounts are reabsorbed by the renal tubules where it is broken down and becomes deposited in the cells as **haemosideri*n***. This can be detected in the sediment of urine.
- Some of the free plasma Hb is oxidized to **methaemoglobin**, which dissociates into **ferrihaem** and globin, ferrihaem becomes attached to albumin, forming **methaemalbumin**

Fate of heamoglobin in intravascular hamolysis



Evidence for haemolysis

- *Increased red cell breakdown leads to:*

1. Jaundice➡ elevated serum bilirubin (unconjugated)
2. excess urinary urobilinogen.
3. raised serum lactic dehydrogenase (LDH).
4. splenomegaly

- ***Increased red cell production** leads to:*

1. reticulocytosis
2. erythroid hyperplasia of the bone marrow.

Evidence for haemolysis

- ***Evidence of abnormal red cells in some haemolytic anaemias***:

  As spherocytes, sickle cells or red cell fragments.

- *Evidence for Intravascular haemolysis*

  - Raised levels of haemosiderinuria

  - very low or absent haptoglobins

  - presence of methaemalbumin.

Causes of haemolytic anemia

A- Inherited causes

- *Red cell membrane defect*

1. Hereditary spherocytosis
2. Hereditary elliptocytosis

- *Haemoglobin abnormalities*

1. Thalassaemia
2. Sickle cell disease

- *Metabolic defects*

1. Glucose-6-phosphate dehydrogenase deficiency

B- Aquired causes

I- Immune

1. **Autoimmune**
   - Warm
   - Cold
2. **Alloimmune**
   - Haemolytic transfusion reactions
   - Haemolytic disease of the newborn
   - After allogeneic bone marrow or organ transplantation

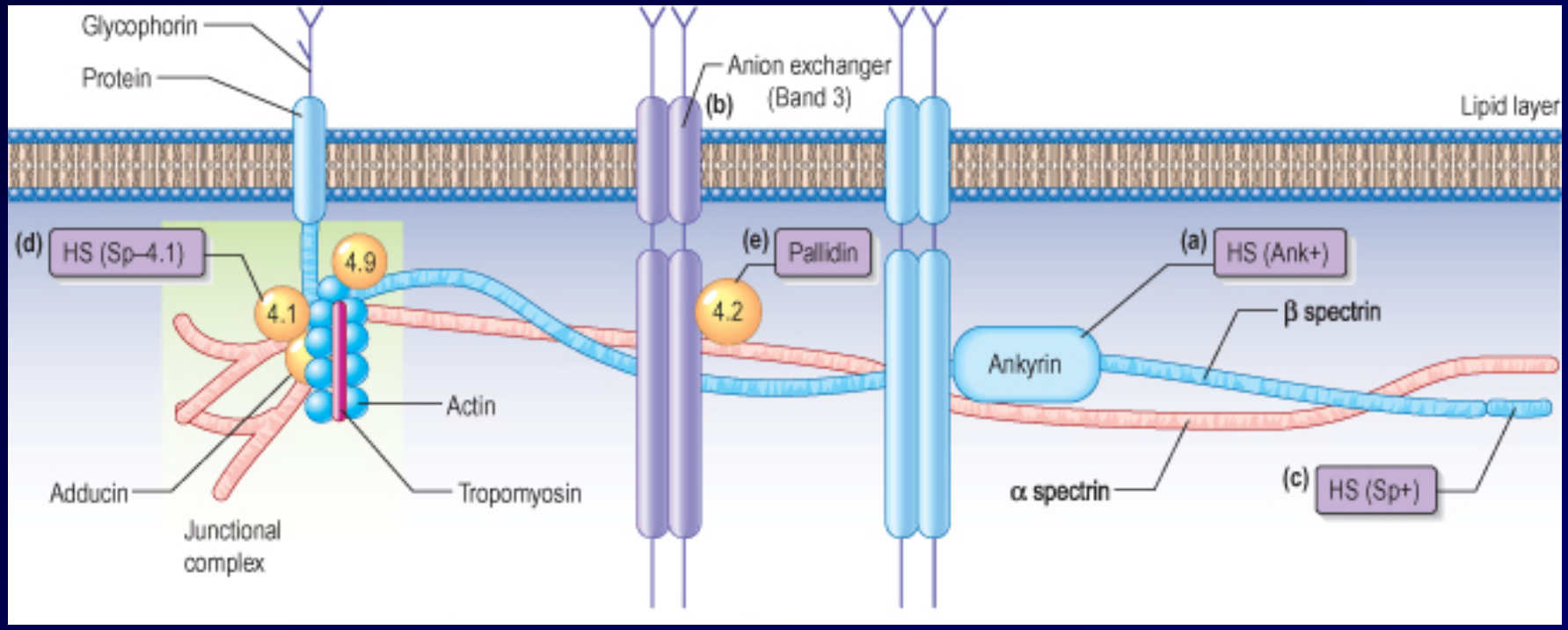
Aquired causes
II- Non-immune

1. **Acquired membrane defects**
   - Paroxysmal nocturnal haemoglobinuria
2. **Mechanical**
   - Microangiopathic haemolytic anaemia
   - Valve prosthesis
   - March haemoglobinuria
3. **Secondary to systemic disease**
   - Renal and liver failure
4. **Miscellaneous**
   - Infections, e.g. malaria, mycoplasma, generalized sepsis.
   - Drugs and chemicals causing damage to the red cell
   - Hypersplenism
   - Burns

Inherited causes

1) Red cell membrane defects

- The normal red cell membrane consists of a lipid bilayer crossed by integral proteins with an underlying cytoskeleton of proteins.



1- Red cell membrane defects

1. HEREDITARY SPHEROCYTOSIS

- HS is the most common inherited haemolytic anaemia.
- inherited in an autosomal dominant manner, but in 25% of patients spontaneous mutation occur.
- HS is due to defects in the red cell membrane, possibly because the lipid bilayer is inadequately supported by the membrane skeleton. The abnormal red cell membrane in HS is associated functionally with an increased permeability to sodium and the cells become spherocytic.
- Spherocytes are more rigid and less deformable than normal red cells. They are unable to pass through the splenic microcirculation and they die.

Hereditary spherocytosis

Clinical features

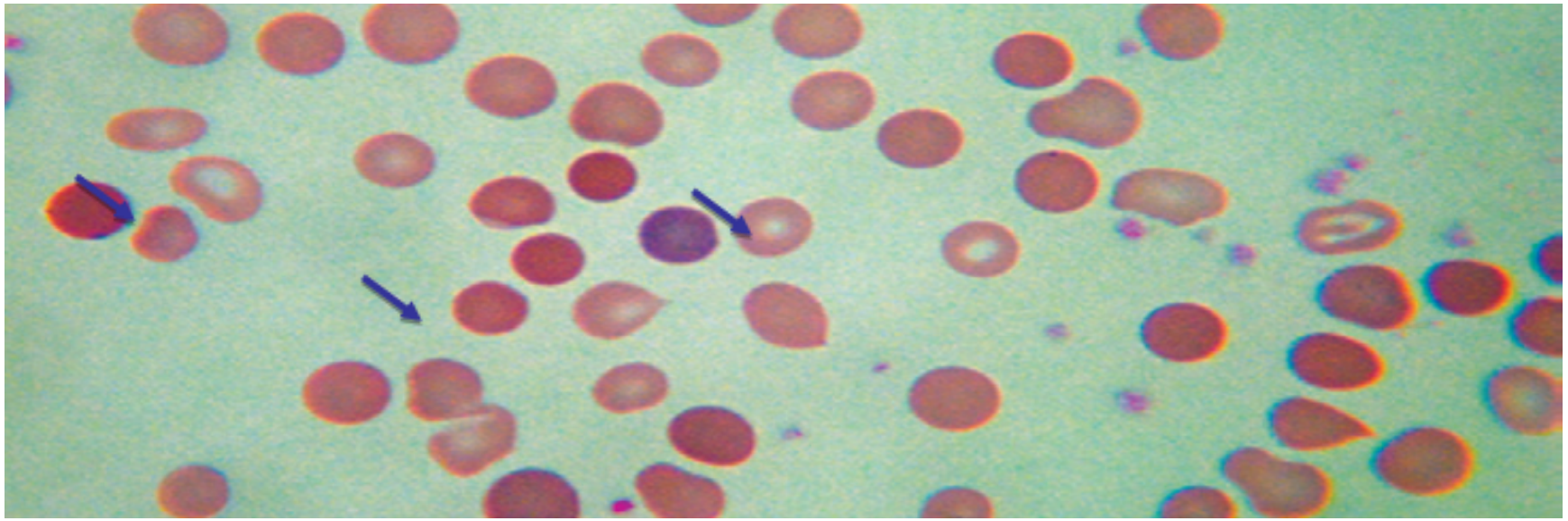
1. The patient could be completely asymptomatic
2. Jaundice at birth. can be delayed for many years.
3. anaemia, splenomegaly and leg ulcers.
4. Chronic haemolysis leads to the formation of pigment gallstones.
5. As in many haemolytic anaemias, the course of the disease may be interrupted by:➢➢➢➢
   a) *Aplastic anaemia; usually occurs after infections, particularly with parvovirus.*
   b) *Megaloblastic anaemia; is the result of folate depletion owing to the hyperactivity of the bone marrow.*

Investigations

1. CBC.... **Anaemia** with variable severity and **reticulocytosis**
2. Blood film shows **spherocytes**.
3. Haemolysis is evident (Erythroid hyperplasia of bone marrow, ↑unconjugated bilirubin, ↑urobilinogen, ↑LDH.
4. **Osmotic fragility test**:

When normal red cells are placed in hypotonic solutions➢➢ they absorb water, swell, and lyse. Spherocytes tolerate hypotonic solutions less well than normal biconcave red cells.

1. **Direct antiglobulin** (Coombs') test is negative in spherocytosis, ruling out autoimmune haemolytic anaemia where spherocytes are commonly present.



Hereditary spherocytosis. A *spherocyte* is a red cell that lacks central pallor because of its spherical shape. In hereditary spherocytosis, there are usually cells in

which the central pallor is reduced rather than absent.

erythrocyte with the normal shape of a biconcave disc (×1000) with central pallor about 1/3 0f diameter.

Treatment of Hereditary spherocytosis

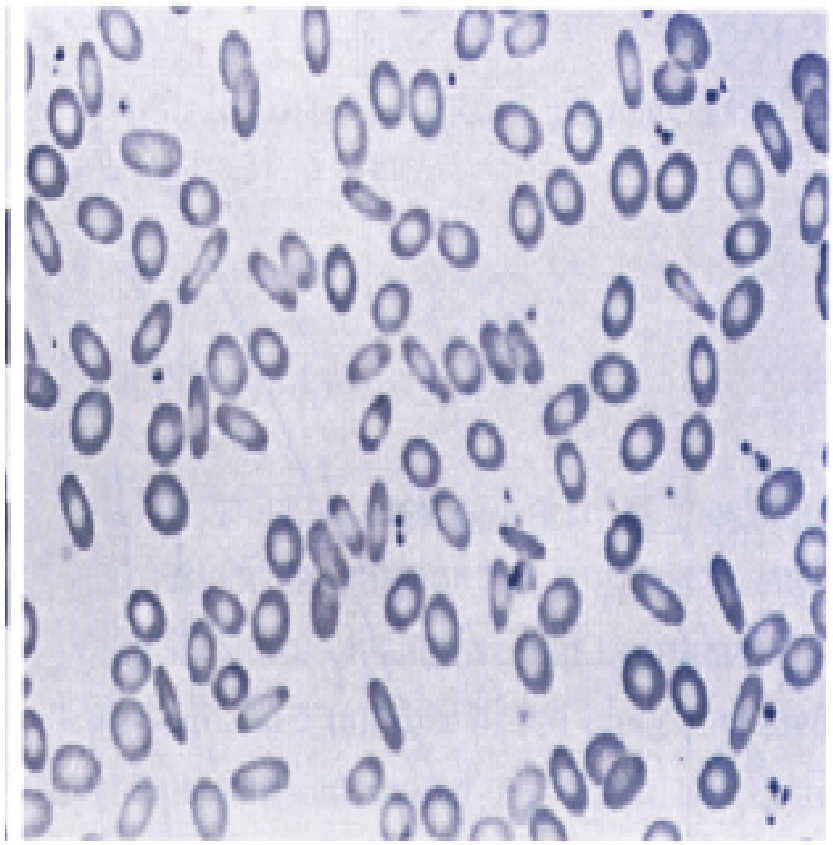
1. **Splenectomy**:

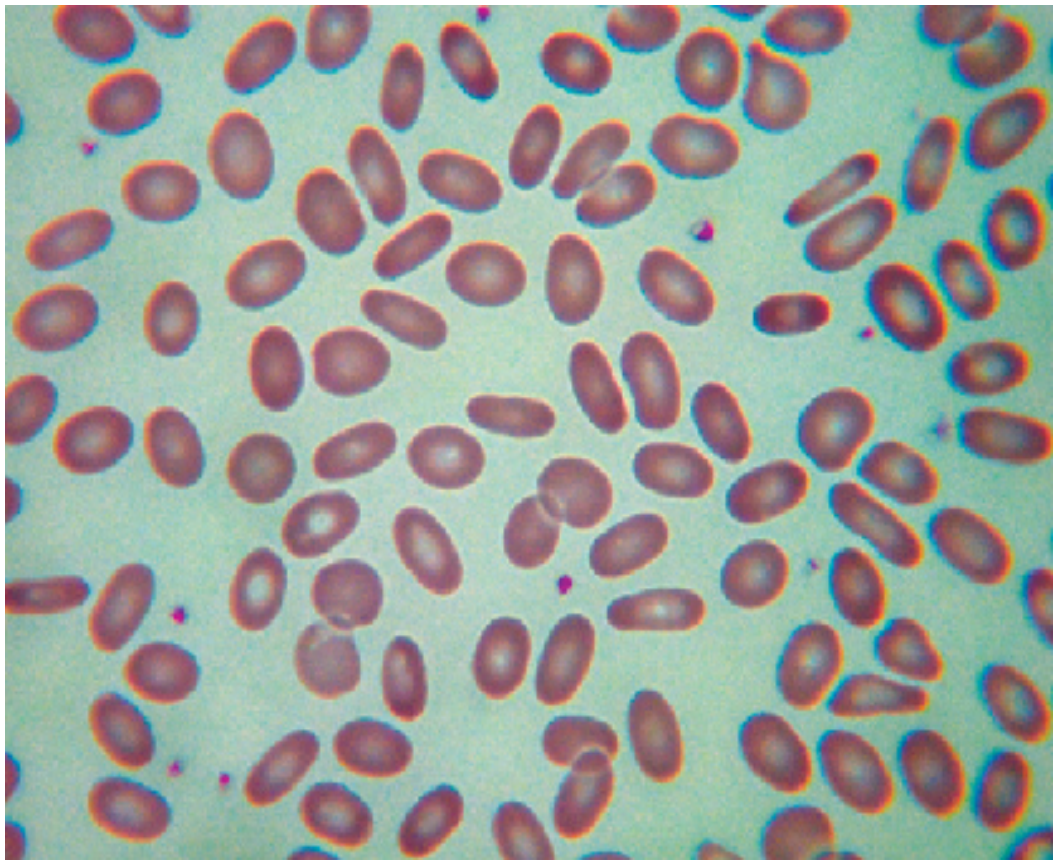
- Should be done in all cases except the mildest cases.
- It is best to postpone splenectomy until after childhood, as sudden overwhelming fatal infections, usually due to encapsulated organisms such as pneumococci, may occur.
- Splenectomy should be preceded by appropriate immunization and followed by lifelong penicillin prophylaxis.

2. Folate deficiency often occurs in chronic haemolysis with rapid cell turnover. Folate levels should be monitored, or **folic acid** can be given prophylactically.

2. Hereditary elliptocytosis

1. Disorder of the red cell membrane inherited in an autosomal dominant manner.
2. The red cells are elliptical due to deficiencies of protein 4.1 or the spectrin/actin/4.1 complex which leads to membrane defect.
3. Clinically it is a similar condition to HS but milder. Only a minority of patients have anaemia and only occasional patients require splenectomy.





Hereditary elliptocytosis. Smooth, cigar-shaped elliptocytes are seen.

2) Heamoglobin abnormalities.

- *Adult HB:*

  - Each normal adult Hb molecule (Hb A) consists of two α and two β globin polypeptide chains (α2β2).
  - HbA comprises about 97% of the Hb in adults.
  - Two other types, Hb A2 (α2δ2) and Hb F (α2γ2), are found in adults in small amounts.

- *Fetal HB*

- Fetal HB (Hb F), which has two α and two γ chains.
- There is increasing synthesis of β chains from 13 weeks of gestation and at term there is 80% Hb F and 20% Hb A.

- The switch from Hb F to Hb A occurs after birth. The exact mechanism responsible for the switch remains unknown.
- There is little Hb F produced (normally less than 1%) from 6 months after birth.
- Hb A2 ($\alpha_2\delta_2$) remains at a level of about 2% throughout adult life.
- The globin genes are arranged on chromosomes 16 and 11 in the order in which they are expressed and combine to give different haemoglobins.

2) Heamoglobin abnormalities. Heamoglobin types.

Normal	A	α2β2	Comprises 97% of adult Hb
	A2	α2δ2	Comprises 2% of adult Hb Elevated in β-thalassaemia
	F	α2γ2	Normal Hb in fetus from 3rd to 9th month Elevated in β-thalassaemia
Abnormal chain poduction	H	β4	Found in α-thalassaemia, biologically useless
	Barts	γ4	Comprises 100% of Hb in homozygous α-thalassaemia, biologically useless
Abnormal chain structure	S	$\alpha_2\beta_2^S$	Substitution of valine for glutamine acid in position 6 of β chain
	C	$\alpha_2\beta_2^C$	Substitution of lysine for glutamic acid in position 6 of β chain

2) Heamoglobin abnormalities.

1. Globin chain production (e.g. thalassaemia)
2. Structure of the globin chain (e.g. sickle cell disease)
3. Combined defects of globin chain production and structure, e.g. sickle cell β-thalassaemia.

- Genetic defects in haemoglobin are the most common of all genetic disorders.

1. Thalassemias

- The defective synthesis of globin genes in thalassaemia leads to 'imbalanced' globin chain production, leading to:

1. Precipitation of globin chains within the red cell precursors resulting in ineffective erythropoiesis.
2. Precipitation of globin chains in mature red cells leads to haemolysis.

Thalassaemia ➢➢Microcytic hypochromic anaemia.

1) β-thalassaemia

- The most common cause of haemolytic anaemia in Egypt.
- Autosomal recessive disorder.
- Synthesis of β chain is controlled by 2 genes
- 1 gene deletion ☞ β thalassemia minor (heterozygous)☞ slightly reduced HbA (80%) and slightly increased Hb A2 & Hb F (10%).
- 2 genes deletions ☞ β thalassemia major (homozygous) & intermedia ☞ markedly reduced HbA (10-30%) and markedly increased Hb F (70-90%).

1) β-thalassaemia

- Molecular genetics

- over 200 genetic defects leading to β-thalassaemia have been characterized.
- The defects are mainly point mutations.

- Clinical classification of β-thalassaemia

1. Thalassaemia minor (or trait), the symptomless heterozygous carrier state
2. Thalassaemia intermedia, with moderate anaemia, rarely requiring transfusions
3. Thalassaemia major, with severe anaemia requiring regular transfusions.

I. Thalassaemia minor (or trait)

- asymptomatic, chatracterized by

1. Anaemia is mild or absent.
2. The red cells are hypochromic and microcytic with a low MCV and MCH.

- Differential diagnosis

- It may be confused with iron deficiency. However, in thalassaemia trait the serum ferritin and the iron stores are normal.
- Hb electrophoresis usually shows a raised Hb A2 and often a raised Hb F

II. Thalassaemia intermedia

- Includes patients who are symptomatic with moderate anaemia (Hb 7-10 g/dL) and not require regular transfusions.
- Thalassaemia intermedia may be due to a combination of homozygous mild β+- and α-thalassaemia, where there is reduced α-chain precipitation and less ineffective erythropoiesis and haemolysis.
- Patients may have:

1) Splenomegaly
2) Bone deformities.
3) Recurrent leg ulcers.
4) Gallstones.

III. Thalassaemia Major (Cooley's anemia)

- Most children affected by homozygous β-thalassaemia present during the first year of life with:

1. failure to thrive and recurrent bacterial infections.
2. severe anaemia from 3-6 months when the switch from γ- to β-chain production should normally occur.
3. General features of haemolytic anaemias.
4. extramedullary haemopoiesis ➢hepatosplenomegaly.
5. Exess medullary erythropoiesis➢expansion of medullary spaces in face and skull ➢ classical thalassaemic facies➢ Enlargrd head, frontal and parietal bossing, promonent malar eminence, protrusion of maxilla, prominent upper teeth.

- Manifestations of complications➢

1- complication of blood transfusion (volume overload, infections).

2- Iron overload ➢ hemosiderosis.

3- 2ry hypersplenism➢ increased transfusions requirments and pancytopenia.

4- Pathological fractures, gall stones ad leg ulcers.

5- Crises➢ ▪ Acute haemolytic crises.

▪ Aplastic crises; caused by infections (human porovirus).

▪ Megaloblastic crises due to increased folic acid requirments.

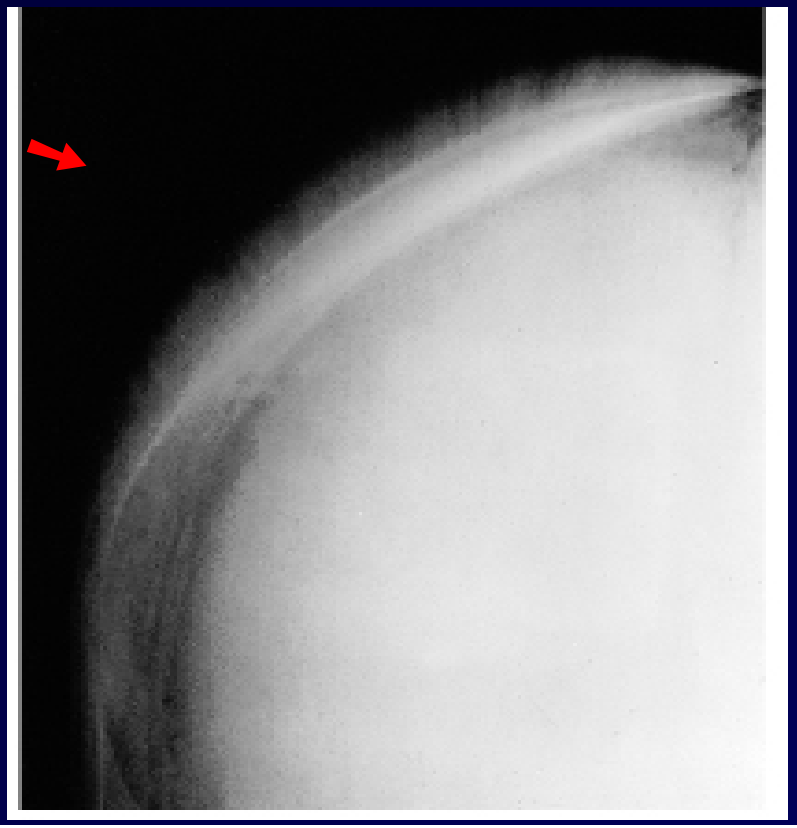
Investigations

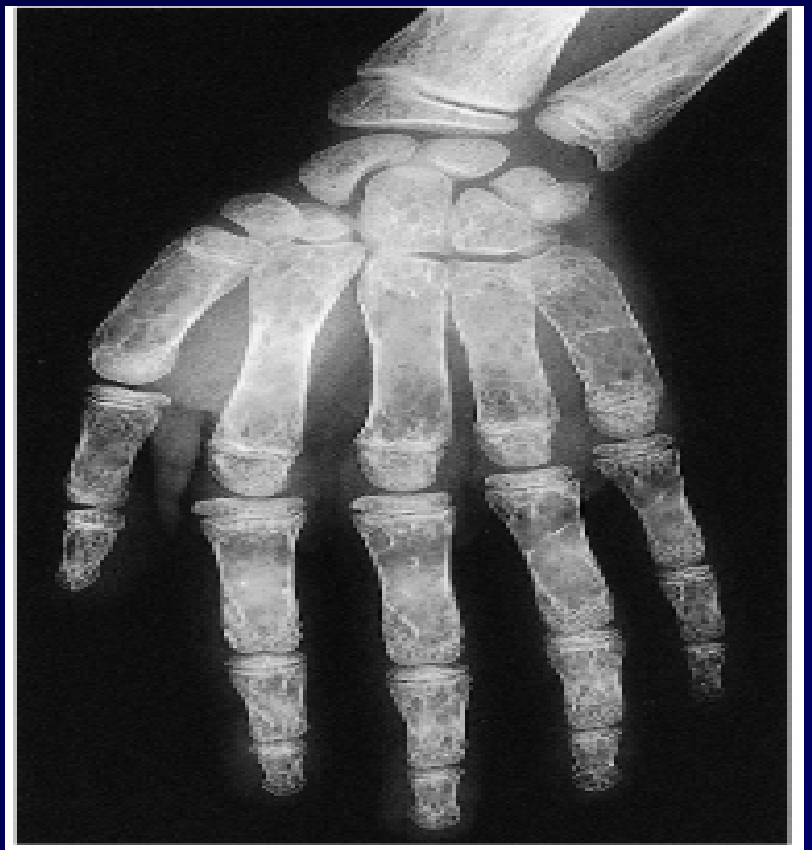
- general investigations to diagnose haemolysis.
- CBC ➢ microcytic hypochromic anaemia & may be target cells.
- Hb electrophoresis ➢increased Hb F (70-90%).

- Radiological features

-Skull X-rays show the characteristic 'hair on end' appearance as a result of expansion of the bone marrow into cortical bone.

-X-ray of the hands shows expansion of the bone marrow.





Management

- The aims of treatment

1. Suppress ineffective erythropoiesis.
2. prevent bony deformities.
3. allow normal activity and development.

- Lines of treatment

1. Regular packed RBCs transfusions to keep the Hb above 10 g/dL. transfusions may be required every 4-6 weeks
2. Long-term folic acid supplements.
3. HBV vaccination.
4. Splenectomy If transfusion requirements increase (hypersplenism), although this is usually delayed until after the age of 6 years because of the risk of infection.

5- Prenatal diagnosis and gene therapy by insertion of normal globin gene in stem cells.

- Problems with therapy

1. Iron overload caused by repeated transfusions (transfusion haemosiderosis) may lead to damage to the endocrine glands, liver, pancreas, lungs, kidneys and the myocardium by the time patients reach adolescence.

- The iron-chelating agent of choice remains desferrioxamine, (form complexes with iron) given as an overnight subcutaneous infusion on 5-7 nights each week.

2) α-Thalassaemia

- α-thalassaemia is often caused by gene deletions, although mutations also occur

1. Four-gene deletions (deletion of both genes on both chromosomes); there is no α-chain synthesis and only Hb Barts (γ4) is present. Hb Barts cannot carry oxygen and is incompatible with life (hydrops foetalis).
2. Three-gene deletion; there is moderate anaemia (Hb 7-10 g/dL) and splenomegaly (Hb H disease). Hb A, Hb Barts and Hb H (β4) are present. Hb A2 is normal or reduced.
3. Two-gene deletion (α-thalassaemia trait); there is microcytosis with or without mild anaemia.
4. One-gene deletion; Silent carrier, the blood picture is usually normal.

SICKLE SYNDROMES

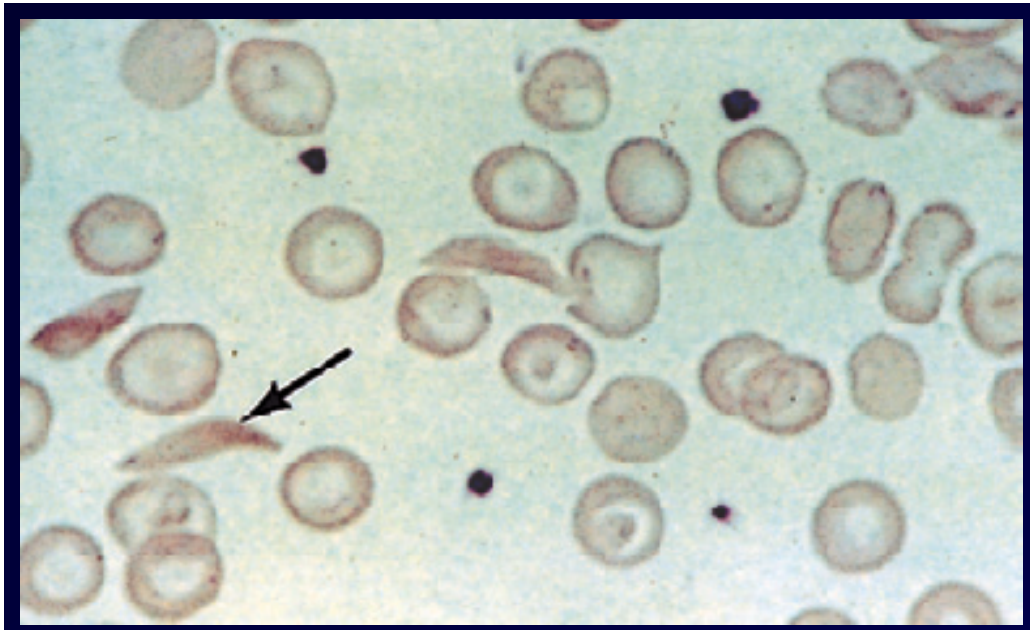
- Sickle cell haemoglobin (Hb S) results from a single-base mutation of adenine to thymine which produces a substitution of valine for glutamine at the 6th codon of the β-globin chain.
- In the homozygous state (*sickle cell anaemia*) both genes are abnormal (Hb SS), whereas in the heterozygous state (*sickle cell trait*, Hb AS) only one chromosome carries the gene (symptomless).
- The disease occurs mainly in Africans (25% carry the gene) but is also found in India, the Middle East and southern Europe.

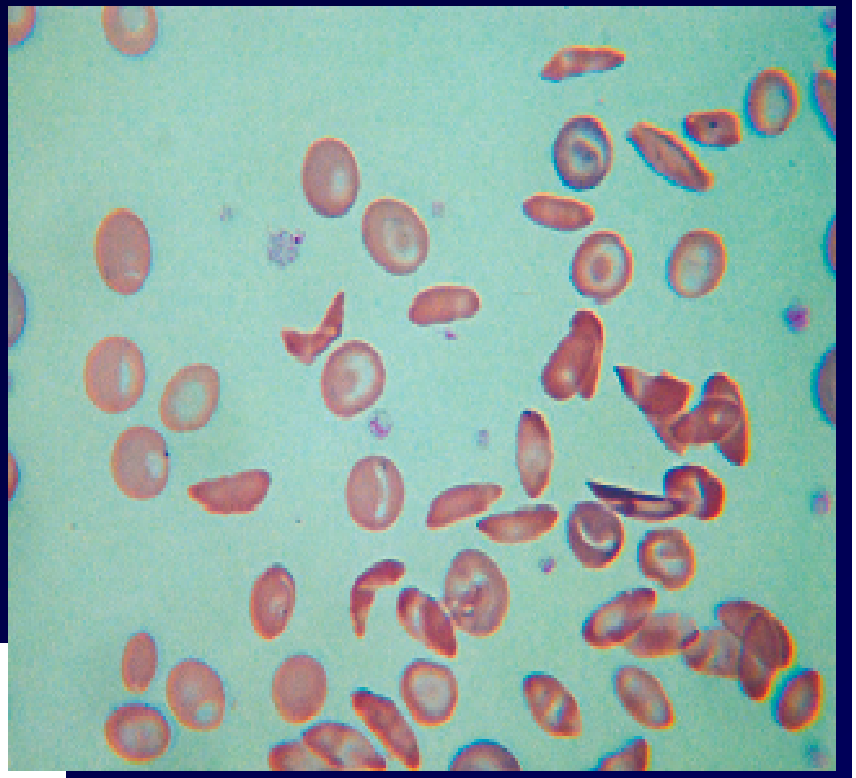
SICKLE SYNDROMES (*Pathogenesis)*

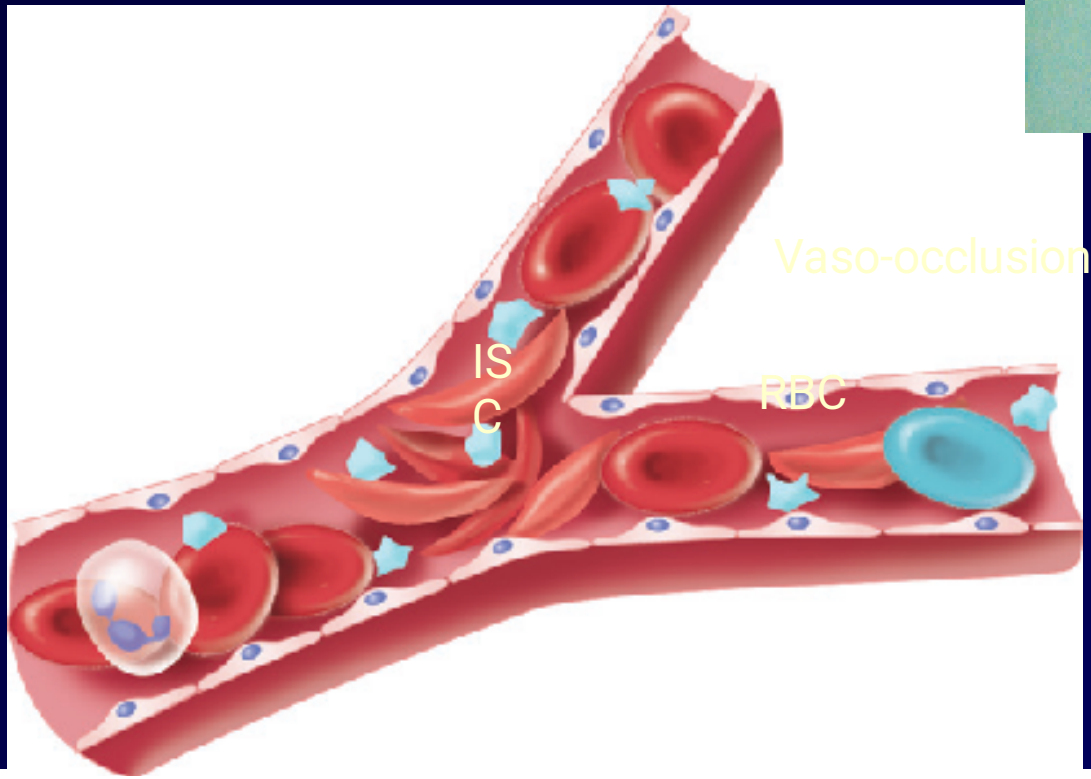
- In oxygenated states HB S is normally soluble.
- Deoxygenated Hb S molecules are insoluble and polymerize. The flexibility of the cells is decreased and they become rigid and take up their characteristic sickle appearance. This process is initially reversible but, with repeated sickling, the cells eventually lose their membrane flexibility and become irreversibly sickled.
- Sickling can produce:

1. shortened red cell survival (destroyed in RES)
2. impaired passage of cells through the microcirculation, leading to obstruction of small vessels and infarction.

- Sickling is precipitated by infection, dehydration, cold, acidosis or hypoxia.






Vaso-occlusion
IS
C
RBC

Clinical features

I. Anaemia ➢

Chronic haemolysis produces a stable haemoglobin level, usually in the 6-8 g/dL range but an acute fall in the haemoglobin level can occur owing to:

1) splenic sequestration
2) bone marrow aplasia
3) further haemolysis.

II. Crises ➢

A- Vaso-occlusive crises

- The earliest presentation in the first few years of life is acute pain in the hands and feet (dactylitis) owing to **vasooccclusion of the small vessels.**

- Severe pain in other bones as femur, humerus, vertebrae, ribs, pelvis, occurs in older children/adults.

- **Mesenteric vascular occlusion**.
- **Autosplenectomy** (hyoposplenism) due to multiple painful splenic infarctions.
- **Renal, pulmonary and cerebral infarctions**.

B- Splenic sequestration crises

Vaso-occlusion produces an acute painful enlargement of the spleen. There is splenic pooling and sequestration of red cells and hypovolaemia, leading in some to circulatory collapse and death.

The condition occurs in childhood before multiple infarctions have occured.

C- Aplastic crises

This most commonly occurs following viral infection. There is a rapid fall in haemoglobin with no reticulocytes in the peripheral blood, because of the failure of erythropoiesis in the marrow.

D- Hemolytic crises

due to drugs, acute infection or associated G6PD deficiency also occurs. Anaemia can also result from folate deficiency.

Sickle cell anaemia
Long-term problems

In adults, nearly every organ is involved;

1) ***Delayed growth and development.***
2) ***Bones*** are a common site for vaso-occlusive episodes, leading to chronic infarcts. Avascular necrosis of hips, shoulders. Osteomyelitis is commoner in sickle cell disease. *Leg ulcers* occur spontaneously (vaso-occlusive episodes) or following trauma and are usually over the medial or lateral malleoli.
3) ***Respiratory:***

pulmonary hypertension

Acute sickle chest syndrome; It is caused by infection or pulmonary infarction. It comprises dyspnea, chest pain, hypoxia, and chest X-ray shows consolidation. Gradual or very rapid onset, leading to death in a few hours. Initial management is with oxygen, antibiotics and exchange transfusion to reduce the amount of Hb S to < 20%.

4) ***Cardiac problems;*** cardiomegaly, arrhythmias, iron overload cardiomyopathy and myocardial infarctions.

5) ***Neurological complications;*** occur in 25% of patients, with TIA, fits, cerebral infarction, cerebral haemorrhage and coma.

6) ***Cholelithiasis***. Pigment stones occur.

7) ***Liver;*** hepatomegaly and liver dysfunction are caused by trapping of sickle cells.

8) ***Renal;*** Chronic interstitial nephritis

9) ***Eye;*** retinopathy, vitreous haemorrhages and retinal detachments all occur.

10) ***Pregnancy;*** Impaired placental blood flow causes spontaneous abortion, intrauterine growth retardation, pre-eclampsia and fetal death.

Investigations

1. *Blood count.* The level of Hb is in the range 6-8 g/dL with a high reticulocyte count (10-20%).
2. *Sickling* of red cells on a blood film can be induced in the presence of sodium metabisulphite.
3. *Sickle solubility test.* A mixture of Hb S in a reducing solution such as sodium dithionite gives a turbid appearance because of precipitation of Hb S, whereas normal Hb gives a clear solution.
4. *Hb electrophoresis* is always needed to confirm the diagnosis. There is no Hb A, 80-95% Hb SS, and 2-20% Hb F.

Management

- These individuals have no symptoms unless extreme circumstances cause anoxia, such as flying in non-pressurized aircraft or problems with anaesthesia.

1) Precipitating factors should be avoided or treated quickly.
2) Acute painful attacks require supportive therapy with intravenous fluids, oxygen, antibiotics and adequate analgesia .
3) Hyposplenism:- Prophylaxis against infection by long acting penicillin and vaccination with polyvalent pneumococcal and *Haemophilus influenzae* type B vaccine.
4) Folic acid is given to all patients with haemolysis.

5) Transfusions should only be given for clear indications. Patient with steady state anaemia should not be transfused.

Indications of blood transfusions

1. Heart failure,
2. TIAs, strokes,
3. acute chest syndrome,
4. acute splenic sequestration and
5. aplastic crises.
6. repeated transfusions may be used to reduce the proportion of circulating Hb S to less than 20% to prevent sickling.
7. Exchange transfusions may be necessary in patients with severe or recurrent crises, or before emergency surgery.

6) Bone marrow transplantation:- to treat sickle cell anaemia in children and young adolescents who have severe complications.

7) Crises Treatment: Aplastic crises(packed RBCs transfusion),sequestration crises(blood transfusion & urgent splenectomy),vasoocclusive crises(hydration, repeated exchange transfusions).

8) *Gene therapy* the ultimate corrective therapy for severe Hb abnormalities. Normal Hb genes could be inserted into the patient's haemopoietic cells in vitro and these cells could be transplanted back into the patient after ablative bone marrow treatment.

9)

Prenatal screening and diagnosis of severe haemoglobin abnormalities

- 25% of the offspring of parents who both have either β-thalassaemia or sickle cell trait, will have β-thalassaemia major or sickle cell anaemia, respectively
- Antenatal diagnosis should be offered if both parents are affected.
- Fetal DNA analysis can be carried out using amniotic fluid, chorionic villus or fetal blood samples.

METABOLIC DISORDERS OF THE RED CELL

Red cell metabolism

The mature red cell have only limited enzyme systems to maintain their viability and function. Energy is required in the form of ATP for:

1. the maintenance of the flexibility of the membrane to allow passage through small vessel.
2. regulation of the sodium and potassium pumps to ensure osmotic equilibrium.
3. Maintain Hb in the reduced state.

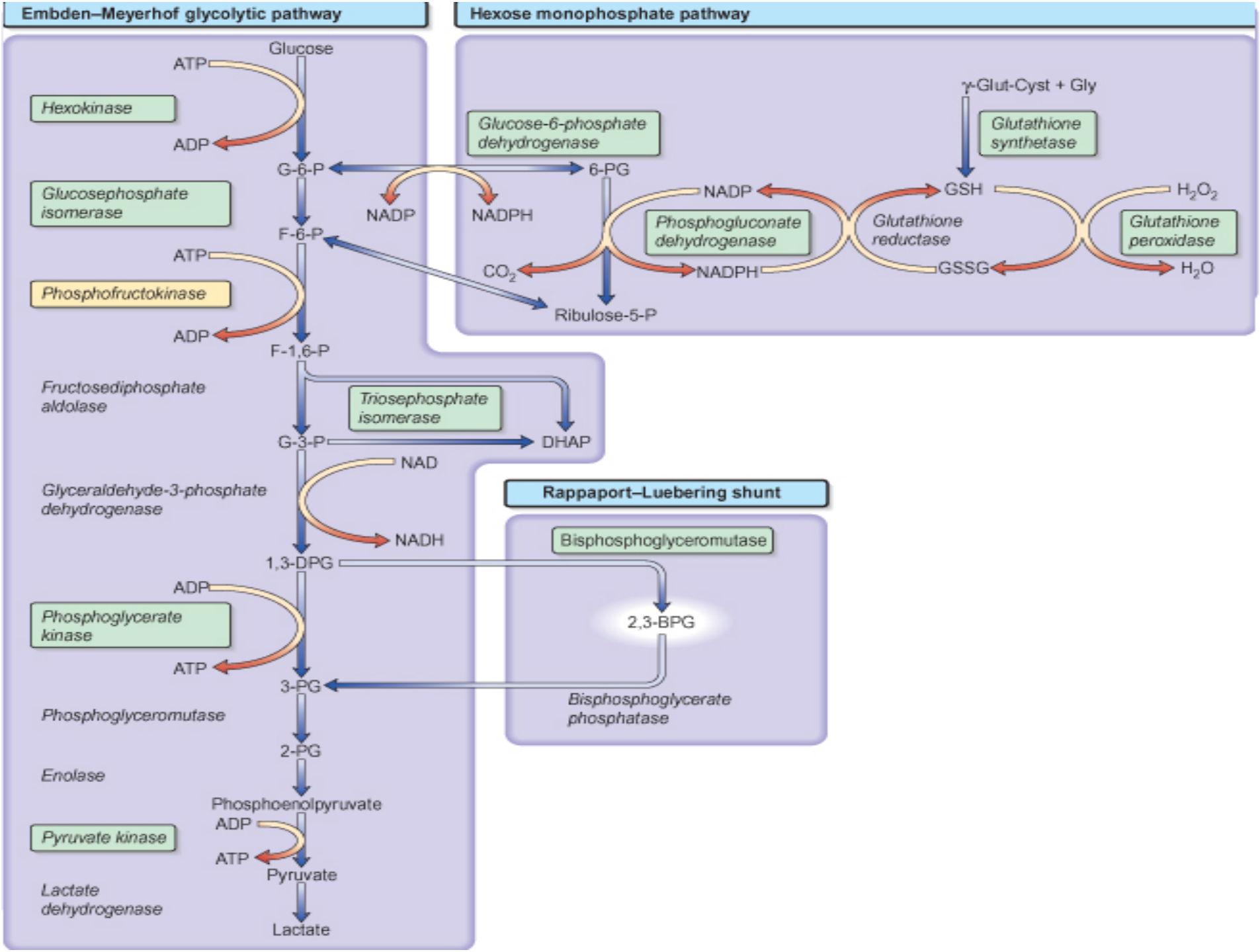
METABOLIC DISORDERS OF THE RED CELL

- The red cell enzyme systems responsible for producing energy includes;

1. the glycolytic pathway which produces ATP
2. the hexose monophosphate pathway, which provides reducing power for the red cell in the form of NADPH.

- NADPH maintains glutathione (GSH) in a reduced state. Glutathione is necessary to combat oxidative stress to the red cell, and failure of this mechanism may result in:

1. rigidity & decreased RBCs membrane flexibilibity
2. oxidation of the Hb molecule, producing methaemoglobin and precipitation of globin chains as Heinz bodies


Embden–Meyerhof glycolytic pathway
Hexose monophosphate pathway
Glucose
ATP
Hexokinase
ADP
G-6-P
Glucosephosphate isomerase
F-6-P
ATP
Phosphofructokinase
ADP
F-1,6-P
Fructosediphosphate aldolase
Triosephosphate isomerase
G-3-P
DHAP
NAD
Glyceraldehyde-3-phosphate dehydrogenase
NADH
1,3-DPG
ADP
Phosphoglycerate kinase
ATP
3-PG
Phosphoglyceromutase
2-PG
Enolase
Phosphoenolpyruvate
ADP
Pyruvate kinase
ATP
Pyruvate
Lactate dehydrogenase
Lactate
NADP
NADPH
Glucose-6-phosphate dehydrogenase
6-PG
NADP
Phosphogluconate dehydrogenase
CO_2
NADPH
Ribulose-5-P
γ-Glut-Cyst + Gly
Glutathione synthetase
GSH
Glutathione reductase
GSSG
H_2O_2
Glutathione peroxidase
H_2O
Rappaport–Luebering shunt
Bisphosphoglyceromutase
2,3-BPG
Bisphosphoglycerate phosphatase

(G6PD) deficiency

- The enzyme G6PD oxidizes glucose-6-phosphate to 6-phosphoglycerate with the reduction of NADP to NADPH. The reaction is the only source of NADPH, which is used via glutathione to protect the red cell from oxidative damage.
- G6PD deficiency is a common condition that presents with a haemolytic anaemia and affects millions of people throughout the world.
- The deficiency is more common in males than in females.
- There are over 400 structural types of G6PD, and mutations are mostly single amino acid substitutions.

G6PD deficiency *clinical syndromes*

1. Acute drug-induced haemolysis usually dose related. Mothballs containing naphthalene can also cause haemolysis.
2. Favism (ingestion of fava beans)
3. Chronic haemolytic anaemia
4. Neonatal jaundice
5. Infections and acute illnesses will also precipitate haemolysis in patients with G6PD deficiency.

- *The clinical features are due to rapid intravascular haemolysis with symptoms of anaemia, jaundice and haemoglobinuria.*

G6PD deficiency

Drugs causing haemolysis

1. Analgesics, such as: Aspirin ,Phenacetin
2. Antimalarials, such as: Primaquine, Pyrimethamine, Quinine, Chloroquine, Pamaquin.
3. Antibacterials, such as: Most sulphonamides, Dapsone, Nitrofurantoin, Chloramphenicol, Quinolones.
4. Miscellaneous drugs, such as: Vitamin K, Probenecid, Nalidixic acid, Quinidine, Dimercaprol, Phenylhydrazine.

G6PD deficiency,*Investigations*

1. **Blood count** is normal between attacks.
2. **During an attack the blood film** may show cells with abnormal shapes, Heinz bodies and reticulocytosis.
3. **Haemolysis** is evident.
4. **G6PD deficiency can be detected**

The level of the enzyme may also be directly assayed..

Treatment

1. Any offending drugs should be stopped.
2. Underlying infection should be treated.
3. Blood transfusion may be life-saving.
4. Splenectomy is not usually helpful.