Haemolytic Anaemia

Normal red cell destruction

- Red cells are removed extravascularly by the macrophages of the reticuloendothelial (RE) system, especially in the marrow but also in the liver and spleen. As the cells have no nucleus, red cell metabolism gradually deteriorates as enzymes are degraded and not replaced and the cells become non-viable.

- The breakdown of haem from red cells liberates iron for recirculation via plasma transferrin to marrow erytheroblasts, and protoporphyrin which is broken down to bilirubin.

- Bilirubin circulates to the liver where it is conjugated to glucuronides which are excreted into the gut via bile and converted to stercobilinogen and stercobilin (excreted in faeces) (Fig. 5.1).

- Stercobilinogen and stercobilin are partly reabsorbed and excreted in urine as urobilinogen and urobilin.

- Globin chains are broken down to amino acids which are reutilized for general protein synthesis in the body.

- Haptoglobins are proteins present in normal plasma capable of binding haemoglobin. The haemoglobin –haptoglobin complex is removed from plasma by the RE system. Intravascular haemolysis (breakdown of red cells within blood vessels) plays little or no part in normal red cell destruction.

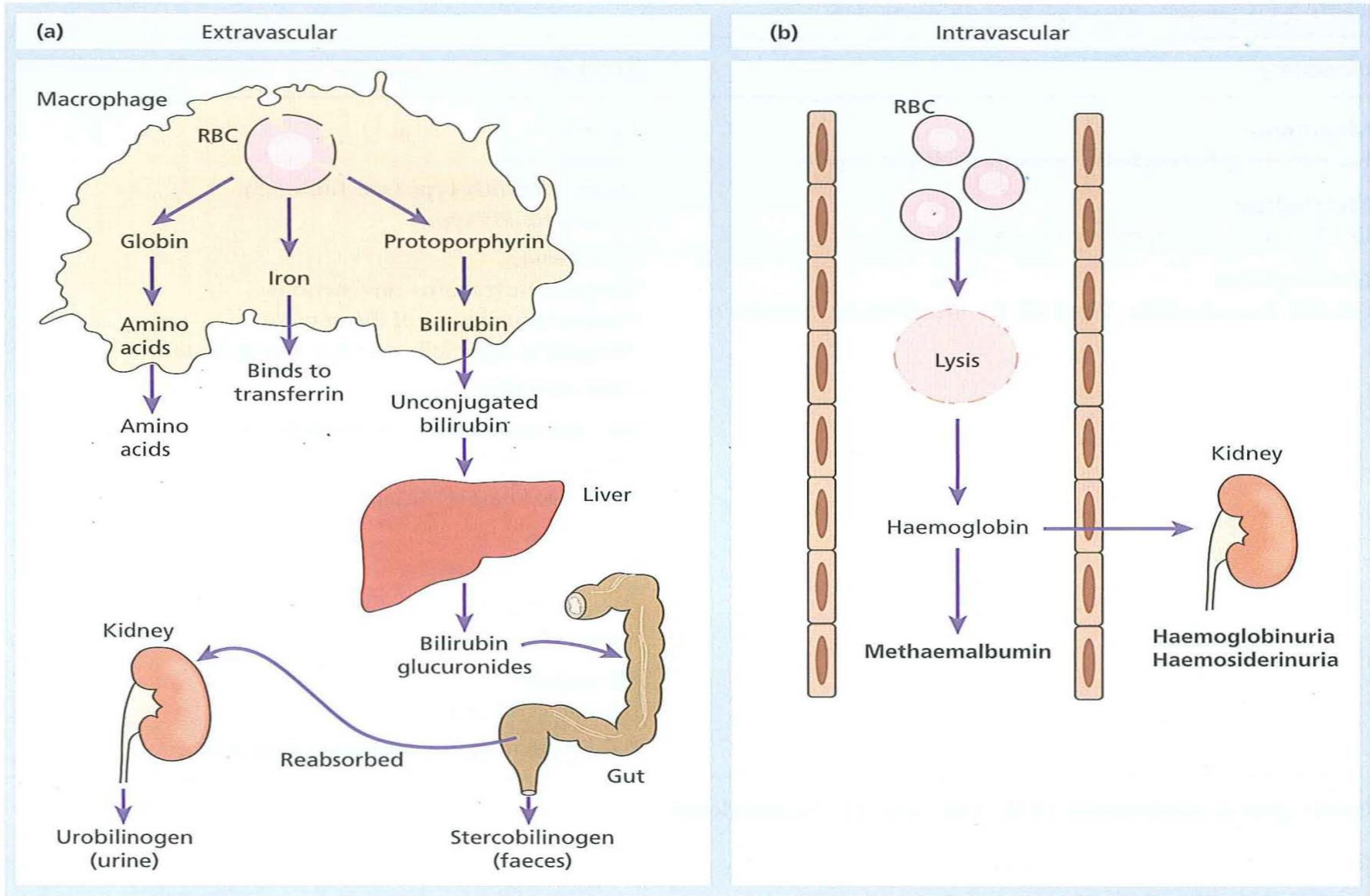

Fig. 5.1 **(a)** Normal red blood cell (RBC) breakdown. This takes place extravascularly in the macrophages of the reticuloendothelial system. **(b)** Intravascular haemolysis occurs in some pathological disorders.

Intravascular and extravascular haemolysis

- There are two main mechanisms whereby red cells are destroyed in haemolytic anaemia. There may be excessive removal of red cells by cells of the RE system (extravascular haemolysis) or they may be broken down directly in the circulation in a process known as intravascular haemolysis (Fig. 5.1; Table 5.2). In intravascular haemolysis, free haemoglobin is released which rapidly saturates plasma haptoglobins and the excess free haemoglobin is filtered by the glomerulus.
- If the rate of haemolysis saturates the renal tubular reabsorptive capacity, free haemoglobin enters urine as iron is released, the renal tubules become loaded with haemosiderin. Methaemalbumin and haemopexin are also formed from the process of intravascular haemolysis.

Table 5.2 Causes of intravascular haemolysis.

1. Mismatched blood transfusion (usually ABO)
2. G6PD deficiency with oxidant stress
3. Red cell fragmentation syndromes
4. Some autoimrmme haemolytic anaemias
5. Some drug- and infection-induced haemolytic anaemias
6. Paroxysmal nocturnal haemoglobinuria
7. March haemoglobinuria
8. Unstable haemoglobin
9. G6PD, glucose-6-phosphate dehydrogenase

Membrane defects

Hereditary spherocytosis

- Hereditary spherocytosis (HS) is the most common hereditary haemolytic anaemia in northern Europeans.
- *Pathogenesis:* HS is usually caused by defects in the proteins involved in the vertical interactions between the membrane skeleton and the lipid bilayer of the red cell.
- The loss of membrane may be caused by the release of parts of the lipid bilayer that are not supported by the skeleton. The marrow produces red cells of normal biconcave shape but these lose membrane and become increasingly spherical (loss of surface area relative to volume) as they circulate through the spleen and the rest of the RE system.
- Ultimately, the spherocytes are unable to pass through the splenic microcirculation where they die prematurely.

Hereditary elliptocytosis

- This has similar clinical and laboratory features to HS except tor the appearance of the blood film but it is usually a clinically miilder disorder.
- It is usually discovered by chance on a blood film and there may be no evidence of haemolysis.
- Occasional patients require splenectomy. \
- The basic defect is a failure of spectrin heterodimers to selfassociate into heterotetramers.
- A number of genetic mutations affecting horizontal interactions have been detected (Table 5.3).
- Patients with homozygous or doubly heterozygous elliptocytosis present with a severe haemolytic anaemia with microspherocytes, poikilocytes and splenomegaly (hereditary pyropoikilocytosis).

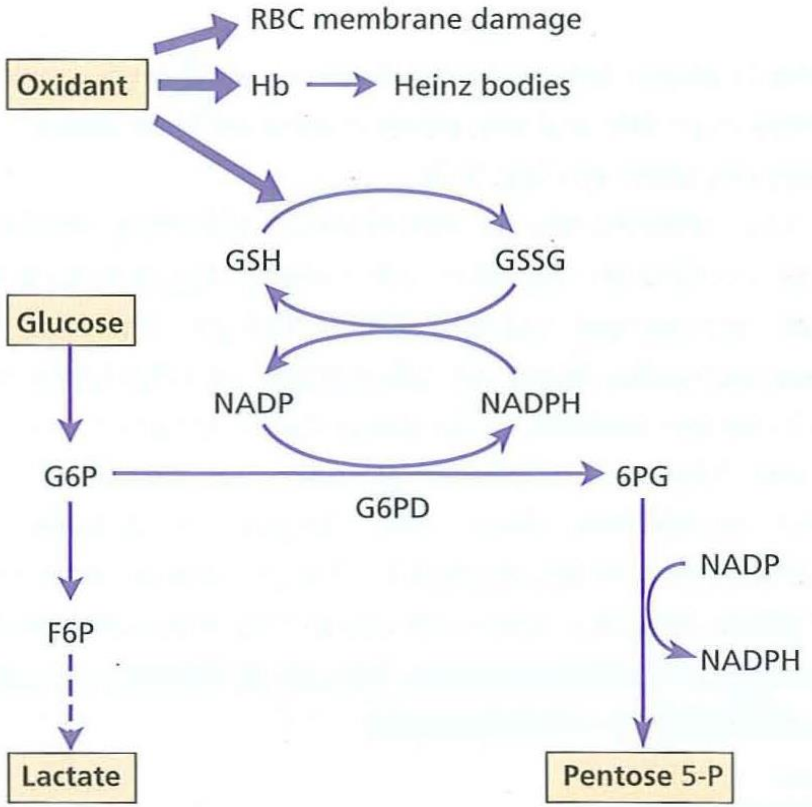

Fig. 5.6 Haemoglobin and red blood cell (RBC) membranes are usually protected from oxidant stress by reduced glutathione (GSH). In G6PD deficiency, NADPH and GSH synthesis is impaired. F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GSSG, glutathione (oxidized form); NADP, NADPH, nicotinamide adenine dinucleotide phosphate.

- **Defective red cell metabolism**
- **<u>GIucose-6-phosphate dehydrogenase deficiency</u>**
- Glucose-6-phosphate dehydrogenase (G6PD) functions to reduce nicotinamide adenine dinucleotide phosphate (NADP) while oxidizing glucose-6-phosphate.
- It is the only source of NADP in red cells

- NADP is needed for the production of reduced glutathione a deficiency renders the red cell susceptible to oxidant stress (Fig. 5.6).

Epidemiology

- There is a wide variety of normal genetic variants of the enzyme G6PD, the most common being type B

(Western) and type A in Africans.

- In addition, more than 400 variants caused by point mutations or deletions of the enzyme G6PD have been characterized which show less activity than normal and worldwide over 400 million people are G6PD deficient in enzyme activity.
- The 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.
- The main races affected are in West Africa, the Mediterranean, the Middle East and South-East Asia.
- The degree of deficiency varies, often being mild (10-15% of normal activity) in black Africans, more severe in Orientals and most severe in Mediterraneans.
- Severe deficiency occurs occasionally in white people.

Glutathione deficiency and other syndromes

- Other defects in the pentose phosphate pathway leading to similar syndromes to G6PD deficiency have been described-particularly glutathione deficiency.

Glycolytic (Embden-Meyerhof) pathway defects

- These are all uncommon and lead to a congenital non-spherocytic haemolytic anaemia.
- In some there are defects of other systems (e.g. a myopathy). The most frequently encountered is pyruvate kinase deficiency.

Pyruvate kinase deficiency

- This is inherited as an autosomal recessive, the affected patients being homozygous or doubly heterozygous.
- Over 100 different mutations have been described.
- The red cells become rigid as a result of reduced adenosine triphosphate (ATP) formation.
- The severity of the anaemia varies Widely (haemoglobin 4-10 g/dL).
- Causes relatively mild symptoms because of a shift to the right in the oxygen (02) dissociation curve caused by a rise in intracellular 2,3-diphosphoglycerate (2,3DPG).
- Clinically, jaundice is usual and gallstones frequent. Frontal bossing may be present. The blood film shows poikilocytosis and distorted 'prickle’ cells, particulal'ly post-splenectomy.
- Splenectomy may alleviate the anaemia.

Acquired haemolytic anaemias

- **Immune haemolytic anaemias**: Autoimmune haemolytic anaemias
- Autoimmtme haemolytic anaemias (AIHAs) are caused by antibody production 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 autoimmllne haemolytic anaemias

- The red cells are coated with immunoglobulin (Ig), usually immunoglobulin G (IgG) alone or with complement, and are therefore taken up by RE macrophages which have receptors for the Ig Fc fragment.
- Part of the coated membrane is lost so the cell becomes progressively more spherical to maintain the same volume and is ultimately prematurely destroyed, predominantly in the spleen.
- When the cells are coated with IgG and complement (C3d, the degraded fragment of C3) or complement alone, red cell destruction occurs more generally in the RE system.

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 a microangiopathic haemolytic anaemia.
- This is caused by red cells passing through abnormal small vessels. The latter may be caused by deposition of fibrin strands often associated with disseminated intravascular coagulation (DIC) or platelet adherence as in thrombotic thrombocytopenic purpura (TIP) or vasculitis e.g.polyarteritis nodosa.
- The peripheral blood contains many deeply staining red cell fragments
- Clotting abnormalities typical of DIC with a low platelet count are also present when DIC underlies the haemolysis.

March haemoglobinuria

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

Paroxysmal nocturnal haemoglobinuria

- PNH is a rare, acquired, clonal disorder of marrow stem cells in which there is deficient synthesis of the glycosylphosphatidylinositol (CPI) anchor, a structure that attaches several surface proteins to the cell membrane.
- It results from mutations in the X chromosome gene coding for phosphatidylinositol glycan protein A (PIG-A) which is essential for the formation of the GPI anchor.
- The net result is that CPI-linked proteins (such as CD55 and CD59) are absent from the cell surface of all the cells derived from the abnormal stem cell.
- It render red cells sensitive to lysis by complement and the result is chronic intravascular haemolysis. Haemosiderinuria is a constant feature. The other main clinical problem seen in PNH is thrombosis and patients may develop recurrent thromboses of large veins including portal and hepatic veins.
- PNH is almost invariably associated with some form of bone marrow hypoplasia.
- PNH may be diagnosed by flow cytometry which shows loss of expression of the CPI-linked proteins, CD55 (DAF) and CD59 (MIRL). The disease occasionally remits but the median survival is approximately 10 years.