# Hemolytic anemias

DEFINITION

Hemolytic anemia (HA) is defined as anemia caused by a shortened lifespan of mature red blood cells (RBCs) in the peripheral circulation. Hemolysis and accelerated destruction of RBCs can take place within the vasculature (i.e., intravascular hemolysis) or mainly in the liver and the spleen (i.e., extravascular hemolysis). HA can be the consequence of an intrinsic and often genetically determined defect of the RBC membrane or an RBC constituent (hemoglobin [Hb] structure or enzyme machinery, or HA can result from an extrinsic and usually acquired disorder of the RBC membrane (immune, infectious, toxic)

# HEMOLYTIC ANEMIAS: RED BLOOD CELL MEMBRANE AND METABOLIC DEFECTS

- The mature erythrocyte differs from all other cells in the body. Lacking a nucleus, DNA, RNA, and ribosomes, it cannot synthesize RNA, DNA, or protein.
- It does not divide, it has no mitochondria, it cannot perform the Krebs cycle, and it lacks an electron transport system for oxidative phosphorylation.
- After enucleation, the reticulocyte, the precursor of the mature erythrocyte, leaves the marrow and enters the circulation equipped with a full complement of enzymes, transporters, signaling molecules, and all other proteins necessary to perform the essential functions of the red blood cell (RBC) during its lifespan.
- The erythrocyte membrane accounts for only about 1% of the total weight of an RBC, yet it plays a critical role in the maintenance of normal RBC homeostasis through a number of mechanisms. These include retention of vital compounds and removal of metabolic waste, regulation of erythrocyte metabolism and pH, and import of iron required for hemoglobin (Hb
- The membrane skeleton, a network of proteins on the inner surface of the RBC, provides the strength and flexibility needed to maintain the normal shape and deformability of the erythrocyte.

- The principal functions of erythrocyte metabolism in the mature erythrocyte include

1. maintenance of adequate supplies of adenosine triphosphate (ATP),

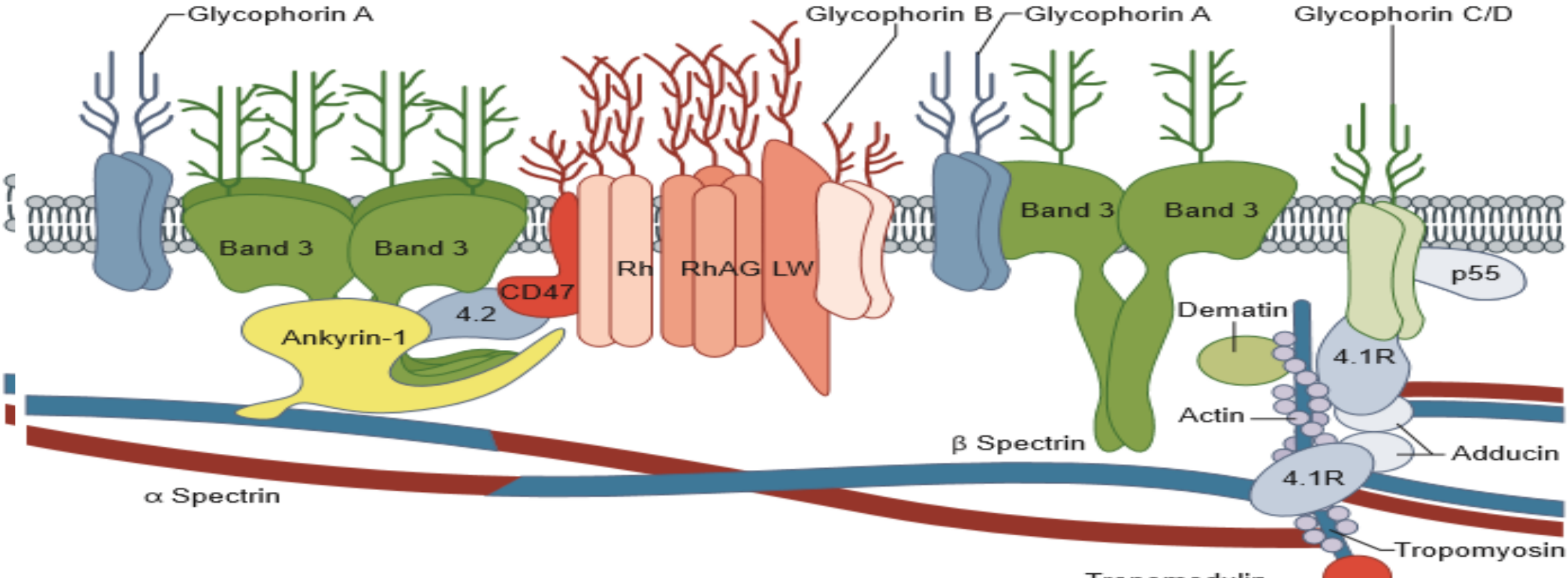
2. production of reducing substances to act as antioxidants,

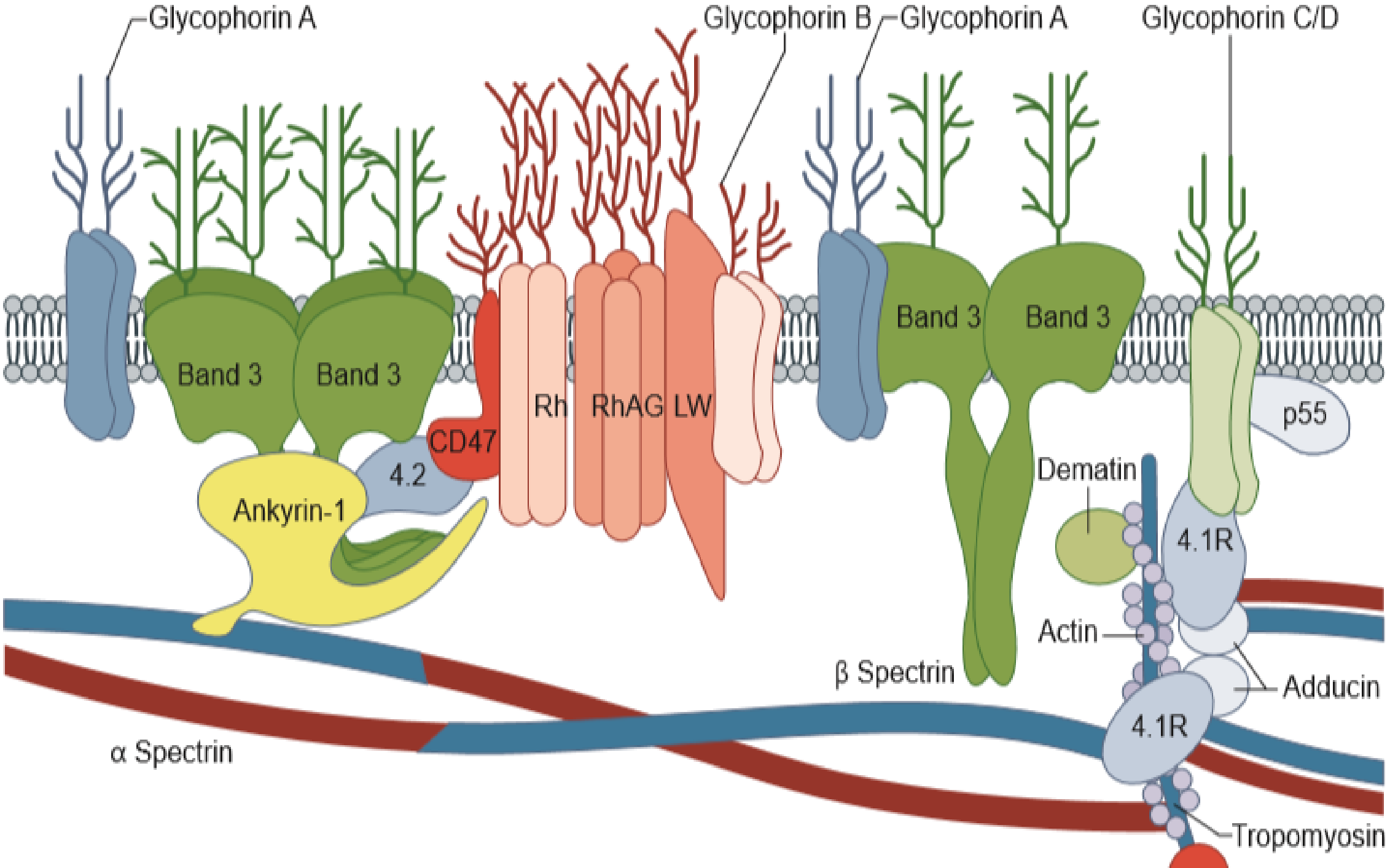
3. and control of oxygen affinity of Hb by production of adequate amounts of 2,3-diphosphoglycerate (2,3DPG).

Because the mature erythrocyte has lost its ability to perform oxidative phosphorylation, its energy is supplied by anaerobic glycolysis though the Embden Meyerhof pathway, by oxidative glycolysis through the hexose monophosphate (HMP) shunt, and through nucleotide salvage pathways

- THE ERYTHROCYTE MEMBRANE

Composed of a lipid bilayer and an underlying cortical membrane skeleton, the membrane provides the erythrocyte the deformability and stability required to withstand its travels through the circulation. In one circulatory cycle throughout the body, an erythrocyte is subjected to high sheer stress in the arterial system, dramatic size and shape changes in the microcirculation with capillary diameters as small as 7.5 µm and marked fluctuations in tonicity, pH, and Po2.




Glycophorin A
Glycophorin B
Glycophorin A
Glycophorin C/D
Band 3
Band 3
Band 3
Band 3
Rh
RhAG
LW
CD47
4.2
Ankyrin-1
p55
Dematin
4.1R
Actin
β Spectrin
Adducin
4.1R
α Spectrin
Tropomyosin

# Hereditary Spherocytosis

## DEFINITION

Hereditary spherocytosis is a group of disorders characterized by spherical erythrocytes on the peripheral blood smear. Clinical, laboratory, and genetic heterogeneity characterize this group of disorders

# DISORDERS WITH SPHEROCYTES ON PERIPHERAL BLOOD FILM

- ✓Hereditary spherocytosis
- ✓Autoimmune hemolytic anemia
- ✓Thermal injuries Microangiopathic and macroangiopathic hemolytic anemias
- ✓Hepatic disease
- ✓Clostridial septicemia
- ✓Transfusion reactions with hemolysis
- ✓Poisoning with certain snake, spider, and Hymenoptera venoms
- ✓ Severe hypophosphatemia Heinz body anemias ABO incompatibility (neonates)

# Hereditary spherocytosis

## Epidemeology:

- Hereditary spherocytosis affects approximately one in 2000 to 3000 individuals of northern European ancestry. Found worldwide, it is much more common in whites than individuals of African ancestry

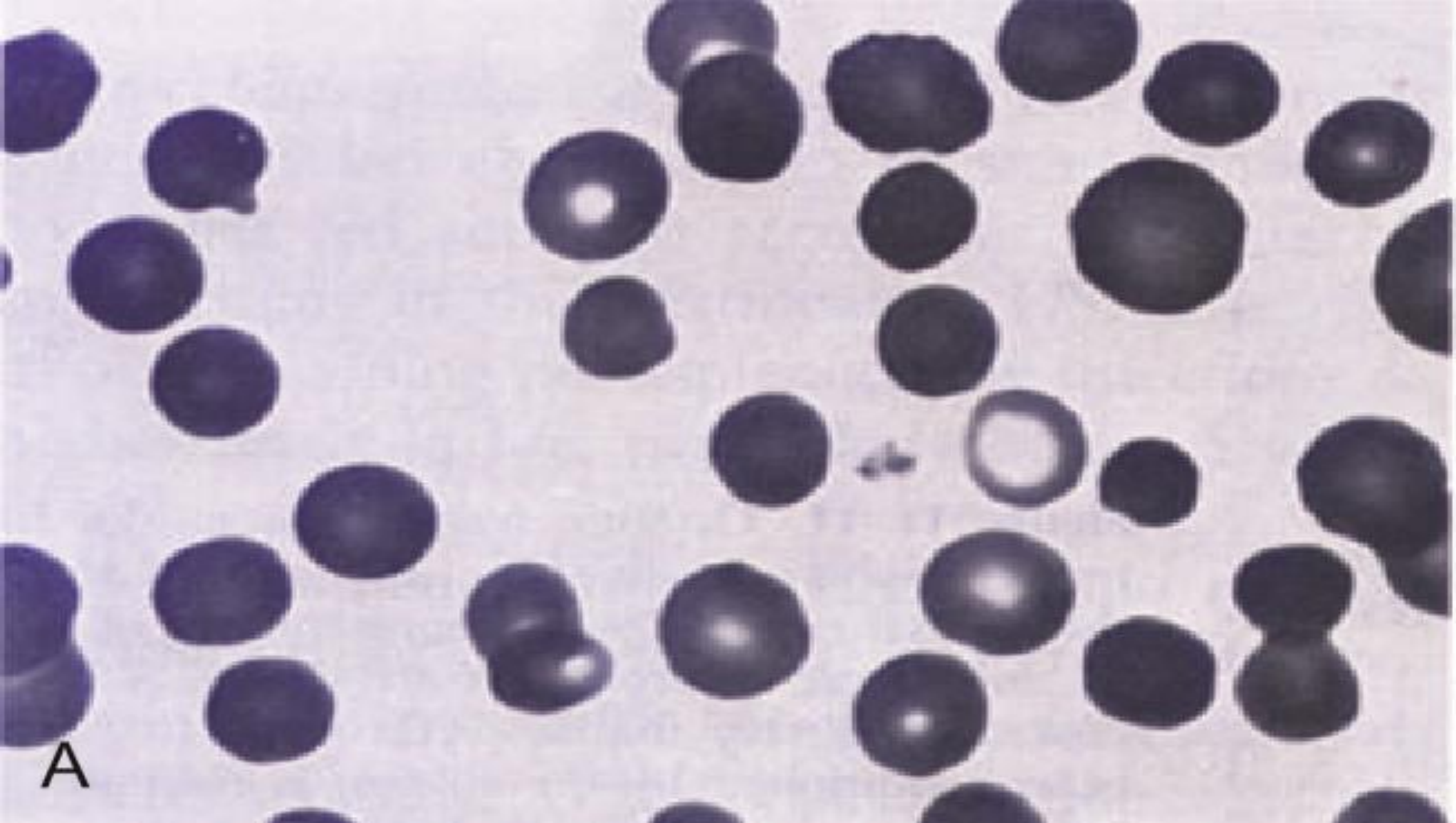
# PATHOBIOLOGY

- The primary defect in HS is the loss of erythrocyte membrane surface area caused by defects in erythrocyte membrane proteins, including α spectrin, β spectrin, ankyrin, band 3, and protein 4.2. Qualitative or quantitative defects of one or more of these membrane proteins lead to membrane instability, which, in turn, leads to membrane loss.
- In approximately two thirds of HS patients, inheritance is autosomal dominant. In the remaining patients, inheritance is non dominant owing to a de novo mutation or autosomal recessive inheritance.
- Splenic destruction of poorly deformable spherocytes is the primary cause of hemolysis experienced by HS patients. Abnormal erythrocytes are trapped in the splenic microcirculation and ingested by phagocytes. Moreover, the splenic environment is hostile to erythrocytes, with low pH, low glucose, and low ATP concentrations and high local concentrations of toxic free radicals produced by adjacent phagocytes, all contributing to membrane damage.

## CLINICAL MANIFESTATIONS

- The clinical manifestations of the spherocytosis syndromes vary widely. The classic triad of HS is anemia, jaundice, and splenomegaly. Rarely, patients may have severe hemolytic anemia presenting in utero or shortly after birth and continuing through the first year of life. These patients may require multiple blood transfusions, and in some cases, splenectomy in the first year of life.
- Many patients with HS escape detection throughout childhood. In these patients, the diagnosis of HS may not be made until they are being evaluated for unrelated disorders later in life or when complications related to anemia or chronic hemolysis occur. Although the lifespan of an erythrocyte in these patients may be shortened to only 20 to 30 days, they adequately compensate for their hemolysis with increased bone marrow erythropoiesis.
- Chronic hemolysis leads to the formation of bilirubinate gallstones, the most frequently reported complication in patients with HS. Although gallstones have been observed in early childhood, most appear in adolescents and young adults. Routine interval ultrasonography to detect gallstones should be performed even if patients are asymptomatic.
- Other complications of HS include aplastic, hemolytic, and megaloblastic crises.
- Aplastic crises occur after virally induced bone marrow suppression and present with anemia, jaundice, fever, and vomiting. The most common etiologic agent in these cases is parvovirus B19. Hemolytic crises, usually associated with viral illnesses and occurring before 6 years of age, are generally mild and present with jaundice, increased spleen size, and a decrease in hematocrit. Megaloblastic crises occur in HS patients with increased folate demands, such as the pregnant patient, growing children, or patients recovering from an aplastic crisis

- Uncommon manifestations of HS include skin ulceration, gout, chronic leg dermatitis, cardiomyopathy, spinal cord dysfunction, movement disorders, and extramedullary erythropoiesis. In patients with untreated severe HS, poor growth and findings attributable to extramedullary hematopoiesis, such as hand and skull deformities, may be found.


A

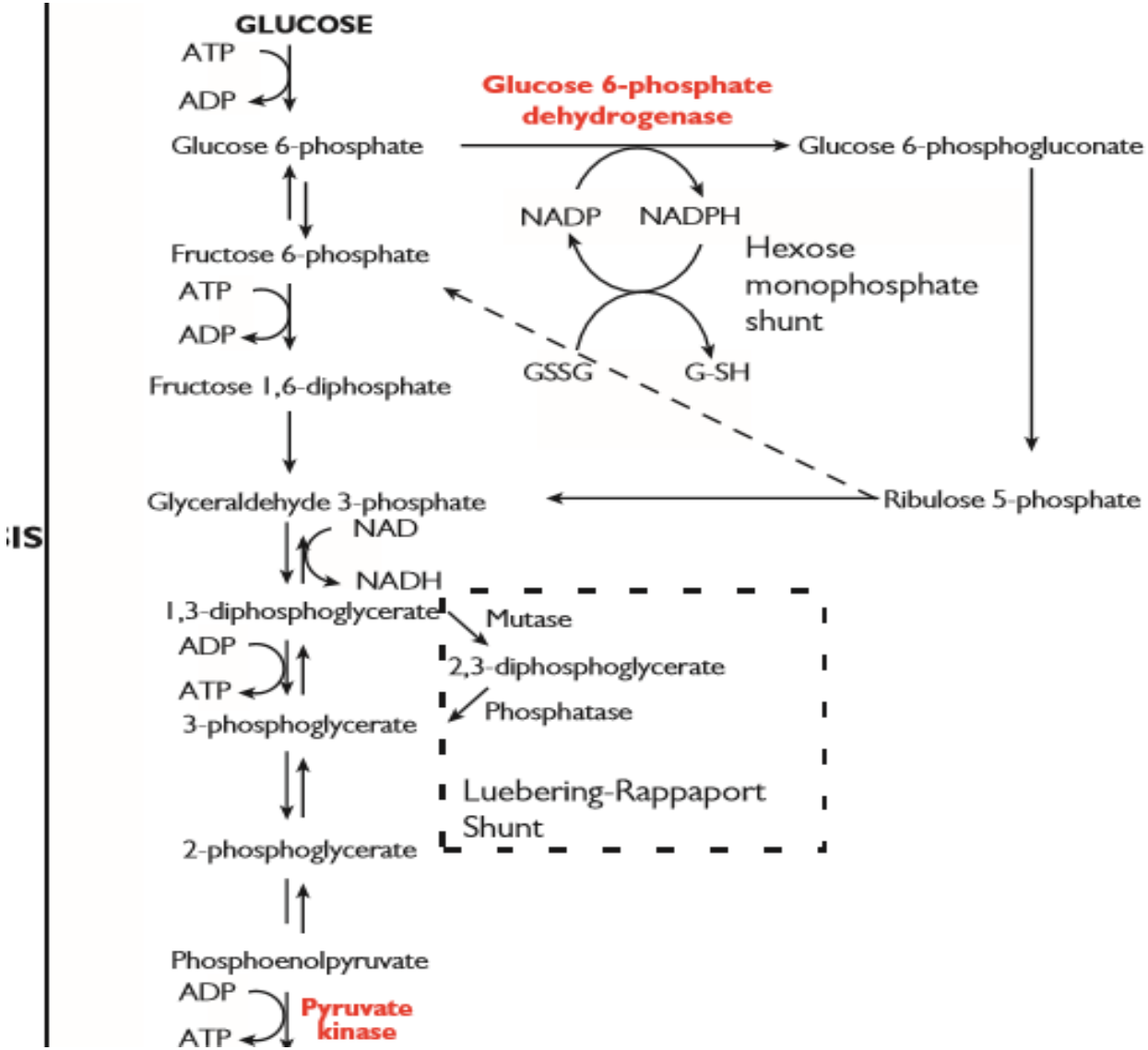
DIAGNOSIS:

- Patients with HS may present at any age, usually with anemia, hyperbilirubinemia, or an abnormal blood smear. In evaluating a patient with suspected HS, particular attention should be paid to the family history, including questions about anemia, jaundice, gallstones, and splenectomy.
- The initial laboratory investigation should include a complete blood count with a peripheral smear, reticulocyte count, direct antiglobulin test (Coombs test), and serum bilirubin. When the peripheral smear or family history is suggestive of HS, an incubated osmotic fragility test or flow cytometric analysis of eosin-5maleimide–labeled erythrocytes (EMA binding) should be obtained. Rarely, additional, specialized testing is required to confirm the diagnosis.

# GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

- G6PD deficiency is the most common inherited disorder of erythrocyte metabolism, affecting more than 400 million people worldwide. The high prevalence of G6PD deficiency is thought to be attributable to genetic selection because G6PDdeficient erythrocytes have a selective advantage against invasion by the malaria parasite Plasmodium falciparum.

# Glycolysis


GLUCOSE
ATP
ADP
Glucose 6-phosphate
Glucose 6-phosphate dehydrogenase
Glucose 6-phosphogluconate
NADP
NADPH
Hexose monophosphate shunt
Fructose 6-phosphate
ATP
ADP
Fructose 1,6-diphosphate
GSSG
G-SH
Glyceraldehyde 3-phosphate
Ribulose 5-phosphate
NAD
NADH
1,3-diphosphoglycerate
Mutase
2,3-diphosphoglycerate
ADP
ATP
Phosphatase
3-phosphoglycerate
Luebering-Rappaport Shunt
2-phosphoglycerate
Phosphoenolpyruvate
ADP
ATP
Pyruvate kinase

- EPIDEMIOLOGY AND PATHOBIOLOGY

G6PD is the initial and rate limiting step in the HMP shunt, which converts NADP into NADPH. NADPH is required for the generation of glutathione, a critical constituent in the prevention of oxidative damage to the cell. G6PDdeficient patients may develop acute hemolytic anemia after exposure to oxidative stress. Although G6PD is a ubiquitous enzyme, erythroid cells are particularly susceptible to oxidative stress because the HMP shunt is their only source of NADPH. Hundreds of G6PD variants have been described, but only a few are common. Variants are classified on the basis of biochemical characteristics

- electrophoretic mobility; ability to use substrate analogue, Km for NADP and G6PD; pH activity profile; and thermal stability. The normal enzyme, GdB, is present in 99% of white Americans and 70% of African Americans.
- A normal variant, GdA+, found in 20% of African Americans, has a faster electrophoretic mobility than GdB. GdA–, the most common variant associated with hemolysis, is found in about 10% of African Americans and in many Africans.
- GdA– has decreased catalytic ability compared with GdA+. GdMed, the second most common variant associated with hemolysis, is common in the Mediterranean area, in India, and in Southeast Asia, with a prevalence of up to 5% to 50%. GdMed exhibits markedly decreased catalytic activity. Gd Canton, a variant common in Asian populations, produces a clinical syndrome similar to GdA–

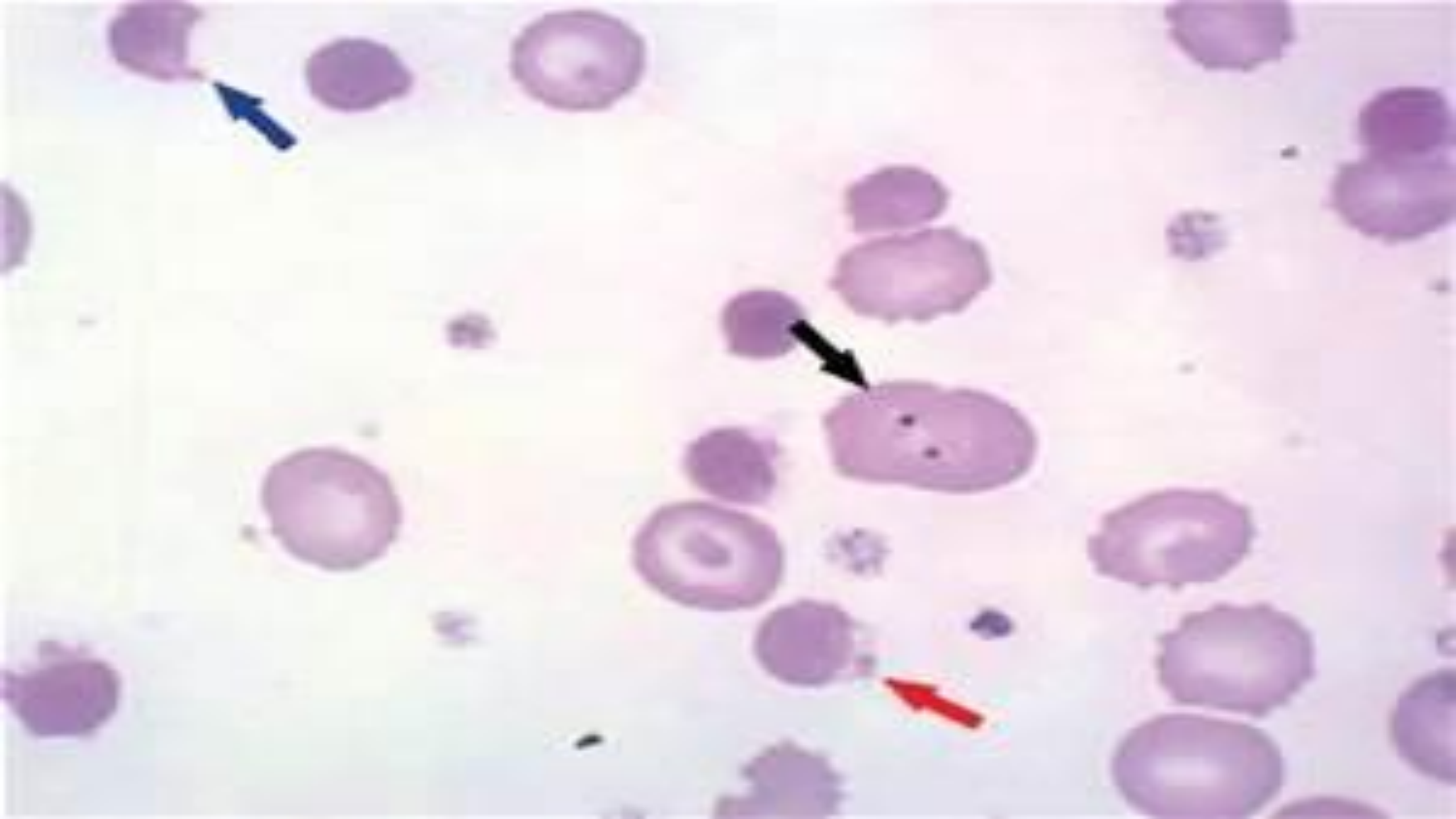
- GdB activity decreases as normal cells age, with a half life of approximately 60 days. Despite very low levels of or no active G6PD, older erythrocytes maintain the ability to produce NADPH and maintain a GSH response to oxidative stress.
- The GdA– variant has a half life of only 13 days, so young cells have a normal amount of enzyme activity, but older RBCs are grossly deficient. Because of this heterogeneity in G6PD levels, individuals with the GdA– variant experience only limited hemolysis after oxidant exposure.,
- G6PD deficiency primarily affects males. Males have only one G6PD allele and express only one G6PD type. Females can express one or two G6PD types. The Lyon hypothesis specifies that only one X chromosome is active in any given cell; thus, any given cell in a heterozygous female is either normal or deficient.
- In females who are heterozygous for G6PD deficiency, average G6PD activity may be normal or mildly, moderately, or severely reduced, depending on the degree of lyonization. G6PDdeficient erythrocytes in heterozygous females are susceptible to the same oxidant stress as G6PDdeficient cells in males, but, typically, the overall degree of hemolysis is less because there is a smaller population of vulnerable cells.

Features

- Haemolysis after exposure to oxidants or infection.
- Chronic non-spherocytic haemolytic anaemia.
- Acute episodes of haemolysis with fava beans (termed favism).
- Methaemoglobinaemia.
- Neonatal jaundice

3 main forms of the disease, those associated with:

1. Acute intermittent haemolytic anaemia.
2. Chronic haemolytic anaemia.
3. No risk of haemolytic anaemia.



Mechanism Oxidants - denatured Hb - methaemoglobin - Heinz bodies - RBC less deformable - destroyed by spleen.

2 main forms of the enzyme

- Normal enzyme is G6PD-B, most prevalent form worldwide.
- 20% of Africans are type A.
- A and B differ by 1 amino acid.
- Mutant enzyme with normal activity = G6PD A(+), find only in black individuals.
- G6PD A(−) is main defect in African origin; d stability of enzyme in vivo; 5–15% normal activity. • 400+ variants but only 2 are relevant clinically:
- Type A(−) = Africans (10% enzyme activity).
- Mediterranean (with 1–3% activity).

Drug-induced haemolysis in G6PD deficiency

- Begins 1–3d after ingestion of drug.
- Anaemia most severe 7–10d after ingestion.
- Associated with low back and abdominal pain.
- Urine becomes dark (black sometimes).
- Red cells develop Heinz body inclusions (cleared later by spleen).
- Haemolysis is typically self-limiting.
- But heterogeneous; variable sensitivity to drugs.
- Risk and severity are dose related.

## Haemolysis due to infection and fever

- 1–2d after onset of fever.
- Mild anaemia develops.
- Commonly seen in pneumonic illnesses.

## Favism

- Hours/days after ingestion of fava beans (broad beans).
- Beans contain oxidants vicine and convicine - free radicals - oxidize glutathione.
- Urine becomes red or very dark.
- Shock may develop—may be fatal.

## Risk of haemolysis in G6PD-deficient individuals Definite risk Possible risk

- Antmalarial drugs
- Primaquine
- Pamaquine
- Chloroquine
- Probenecid
- Quinine and quinidine (acceptable in acute malaria

## Analgesic drugs

- Aspirin
- Phenacetin Others
- Dapsone
- Methylthioninium chloride (methylene blue)
- Nitrofurantoin
- 4-quinolones (e.g.ciprofloxacin, nalidixic acid)
- Sulfonamides (e.g. co-trimoxazole

## Neonatal jaundice

- May develop kernicterus (possible permanent brain damage).
- Rare in A(−) variants.
- More common in Mediterranean and Chinese variants.

Laboratory investigation

- In steady state (i.e. no haemolysis) the RBCs appear normal.
- Heinz bodies in drug-induced haemolysis (methyl violet stain).
- Spherocytes and RBC fragments on blood film if severe haemolysis.
- increased reticulocytes.
- increased unconjugated bilirubin, LDH, and urinary urobilinogen.
- decreased  haptoglobins.
- DAT −ve(direct antiglobulin test).

- Diagnosis

Demonstrate enzyme deficiency. In suspected RBC enzymopathy, assay G6PD and PK first, then look for unstable Hb. Diagnosis is difficult during haemolytic episode since reticulocytes have iilevels of enzyme and may get erroneously normal result; wait until steady state (7-6 weeks after episode of haemolysis). Family studies are helpful.

Management

- Avoid oxidant drugs.
- Transfuse in severe haemolysis or symptomatic anaemia.
- IV fluids to maintain good urine output.
- ± exchange transfusion in infants.
- Splenectomy may be of value in severe recurrent haemolysis.
- Folic acid supplements (?proven value).
- Avoid Fe unless definite Fe deficiency.