G6PD deficiency anaemia

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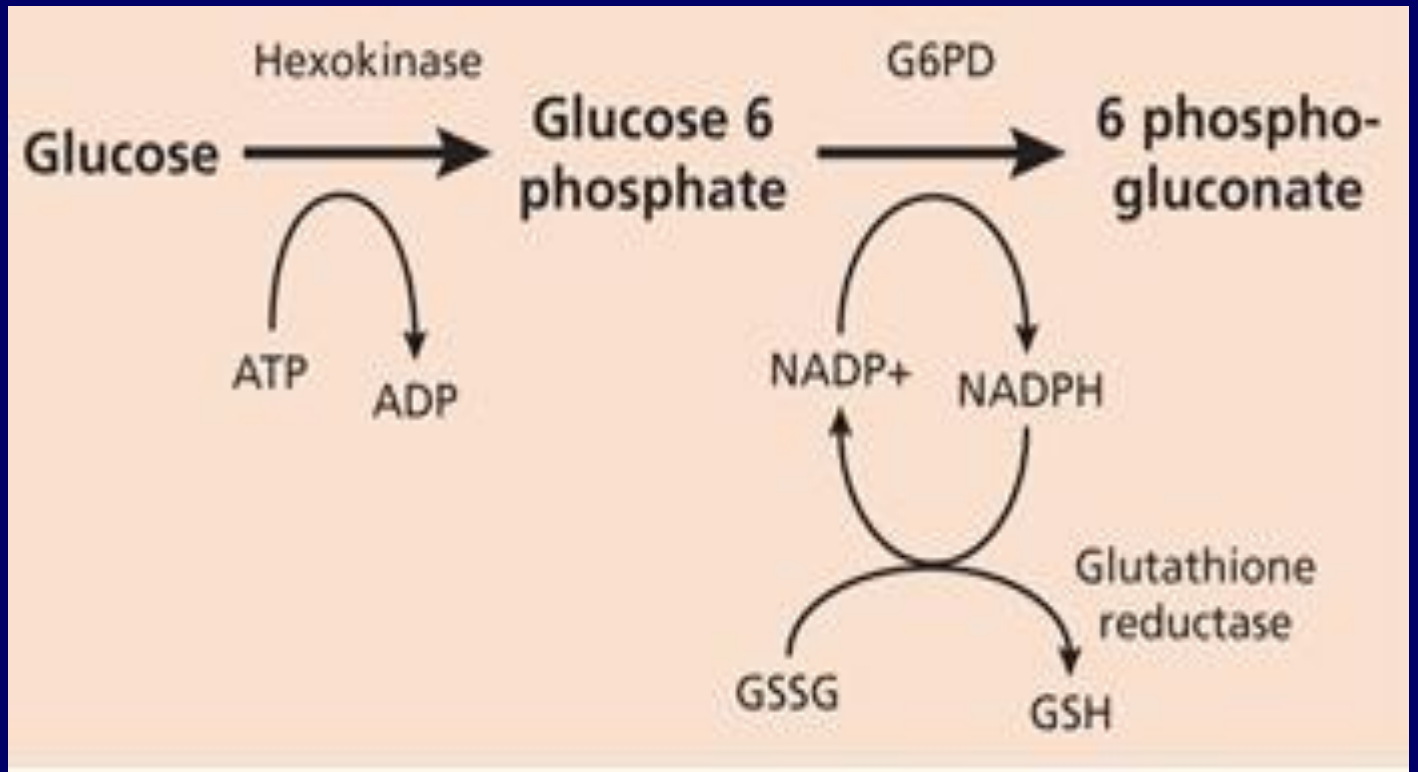
Hexose monophosphate pathway

- Also called pentose phosphate pathway ‘PPP’.
- About 10% of glycolysis occurs by this pathway.
- In which:



G6PD deficiency anaemia

- It is the most common red cell enzymopathy associated with haemolysis.

- The disorders results from the inheritance of any of a large number of abnormal genes that code for the G6PD enzyme.
- Protection against Malaria parasite.

Inheritance

- G6PD is an x-linked inherited disease, so it is fully expressed in males with the genetic abnormalities.
- In females it is fully expressed only when two mutant genes are inherited (homozygous).
- The heterozygous women has two populations of cells, one population with normal enzyme activity and the other with deficient enzyme activity.
- However, most heterozygous women are clinically normal and have normal G6PD activity.

Demographics

G6PD deficiency is most common in:

- West Africa
- Middle East
- Southeast Asia
- Mediterranean

Pathophysiology

- G6PD catalyzes the first step in PPP pathway:

- NADPH (nicotin amide-adenine dinucleotide phosphate) is important intracellular reducing agent convert glutathione (GSSG) to its reduced form (GSH)
- GSH protects enzymes and Hb against oxidative denaturation.

How does it work?

- G6PD is required by all cells to protect from damage by oxidation. It catalyses the first step in the HMP pathway (glucose-6-P oxidized to 6-phosphogluconate) which is linked to the reduction of NADP to NADPH, which is used to generate reduced glutathione.

For the red cell, this is the sole source of protection against oxidant damage in the form of free radicals generated by the conversion of oxy- to deoxyhemoglobin and by peroxides generated by phagocytosing granulocytes.

Normal red cells generate NADPH in response to oxidant stress; this capacity is impaired in patients with G6PD deficiency.

Failure to withstand oxidant stress damage to sulphydryl groups in hemoglobin and the red cell membrane causes hemolysis.

Cells in other tissues and organs have alternate pathways for the generation of NADPH and can withstand such oxidant stress. But not so in the simple RBC.

The activity of all red cell enzymes, including G6PD, is highest in young red cells (reticulocytes), and progressively declines as the cell ages.

- Under normal conditions, the individual with G6PD deficiency compensates for the shortened lifespan of the red cells because the activity of G6PD is highest in young erythrocytes and decreases with cell aging.

Oxidative stress can lead to mild-to-severe haemolytic episodes, results when G6PD deficient erythrocytes fail to produce sufficient NADPH and subsequently fail to maintain adequate levels of GSH to detoxify hydrogen peroxide.

- Hb is then oxidized to methemoglobin.
- Heme is librated from globin and globin denatures forming Heinz bodies which attach to sulfhydryl group , inducing cell rigidity.

G6PD Crisis Precipitation - Beans

Broad beans (favism)

Fava beans,

G6PD Crisis Precipitation - Oxidizing Drugs

NSAIDs (aspirin, ibuprofen)

Antibiotics (sulfonamides)

Nitrofurantoin

Antimalarials (primaquine, quinine, quinidine)

Other oxidants

Henna – primarily infants and pregnant women

Naphthlene (moth balls) – regular exposure

Favism

Results from ingestion of fava beans.

Peak incidence April/May coincident with harvest time.
Usually male children, ages 1-5.
5-24 hours after ingestion - HA, nausea, back pain, chills, fever, jaundice and hemoglobinuria. Acute fall in hemoglobin requiring transfusion.

Most commonly seen with G6PD Mediterranean variant.

The World Health Organization classifies G6PD genetic variants into

- Class I: Severe deficiency (<10% activity) with chronic (nonspherocytic) hemolytic anemia
- Class II: Severe deficiency (<10% activity), with intermittent hemolysis
- Class III: Mild deficiency (10-60% activity), hemolysis with stressors only
- Class IV: Non-deficient variant, no clinical sequelae
- Class V: Increased enzyme activity, no clinical sequelae

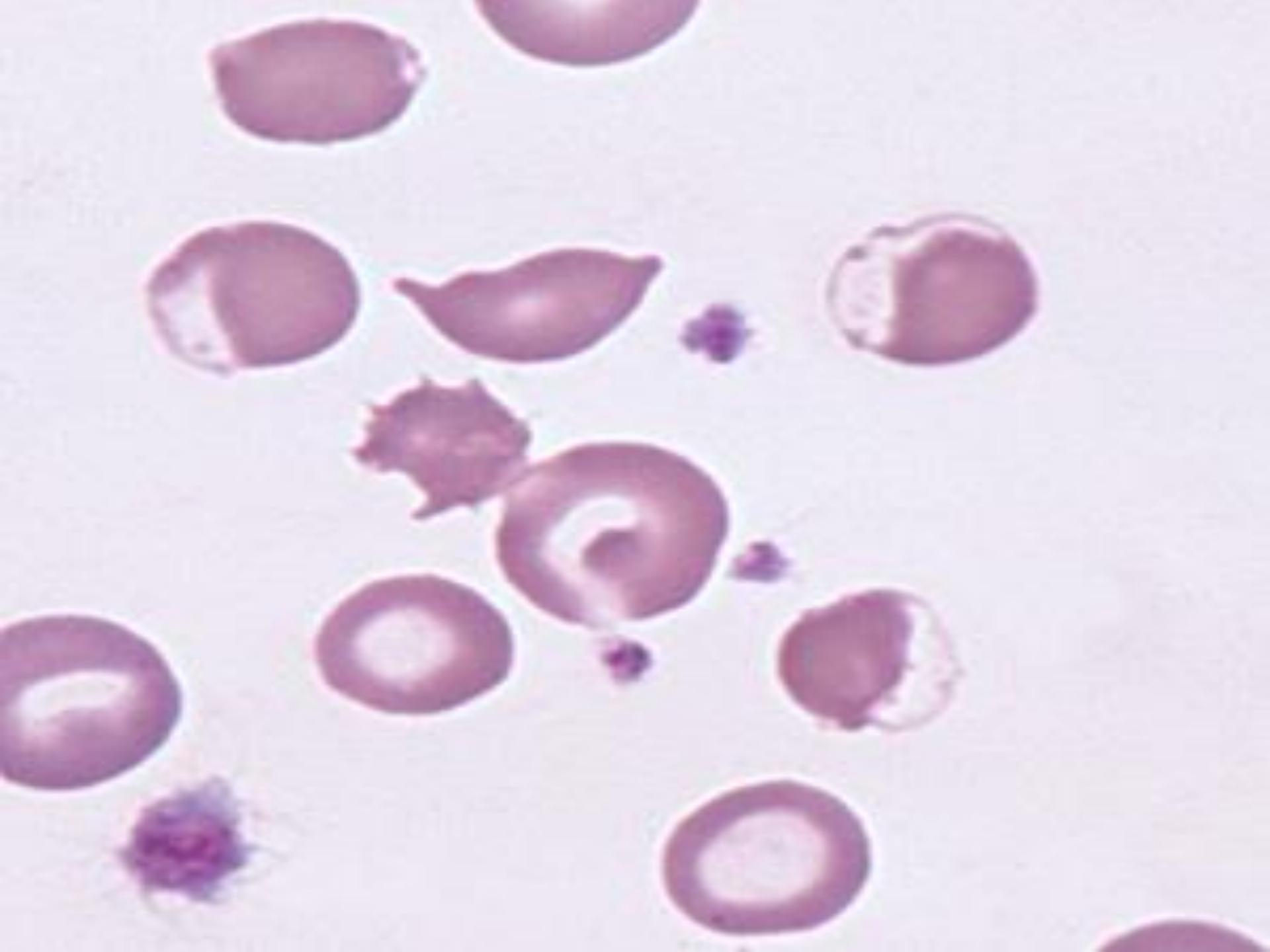
Clinical presentation

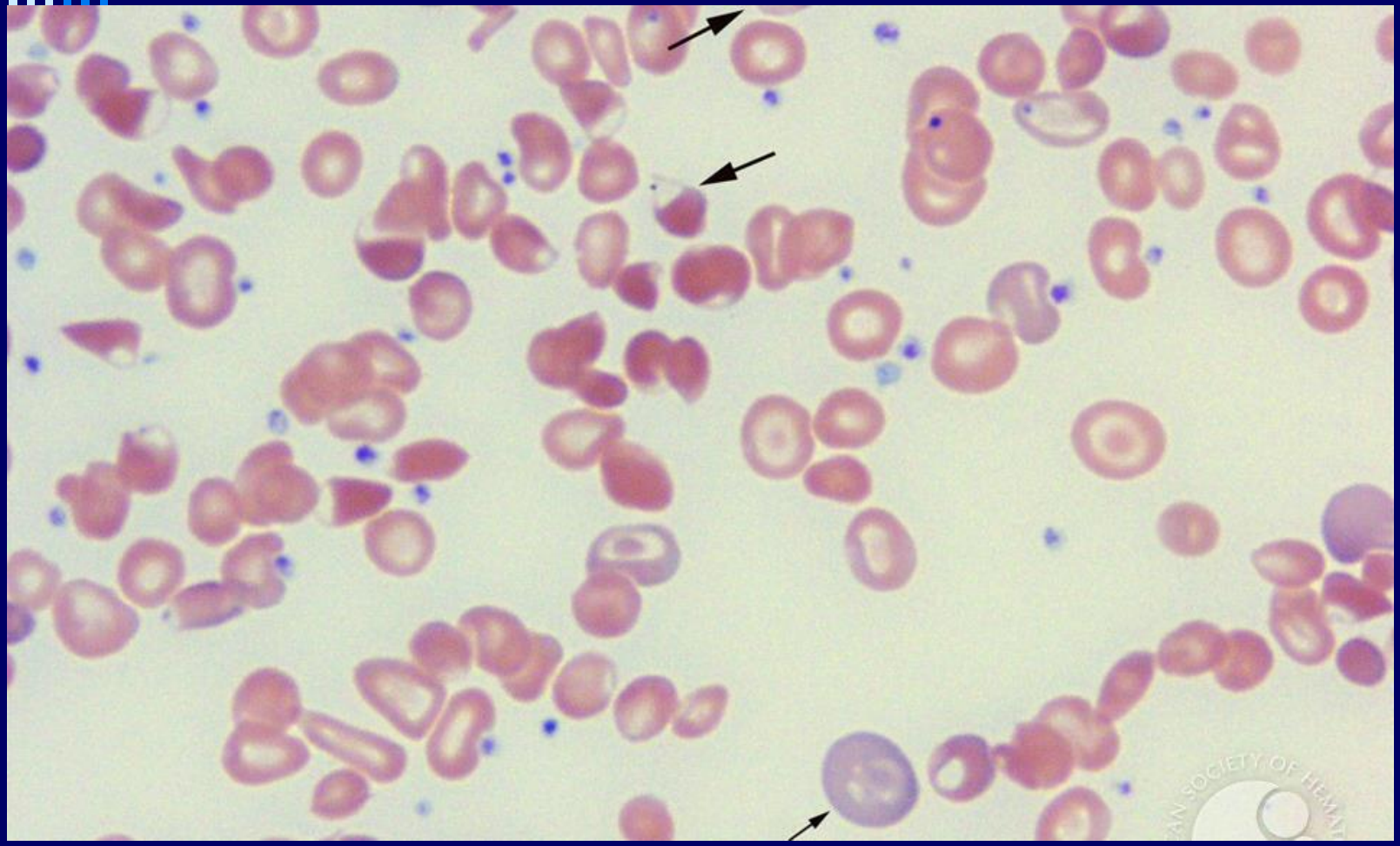
G6PD manifests itself in a number of ways:

- Prolonged neonatal icterus
- Hemolytic crises in response to :
  - oxidant drugs
  - Certain foods (eg. fava bean)
  - severe infection
- Abdominal pain
- Intravascular haemolysis resulting in haemoglobinuria
- Very severe crises can cause acute renal failure

Lab. findings

- *CBC:*
- Hb, PCV, red cell count: low
- Red cell indices: normal
- T.WBCs and PLT count: normal
- PBP:
  - Normocytic normochromic RBCs.
  - Polychromasia
  - Some spherocytes may be present
  - Blister cells and fragmented ‘bite’ cells are often seen.



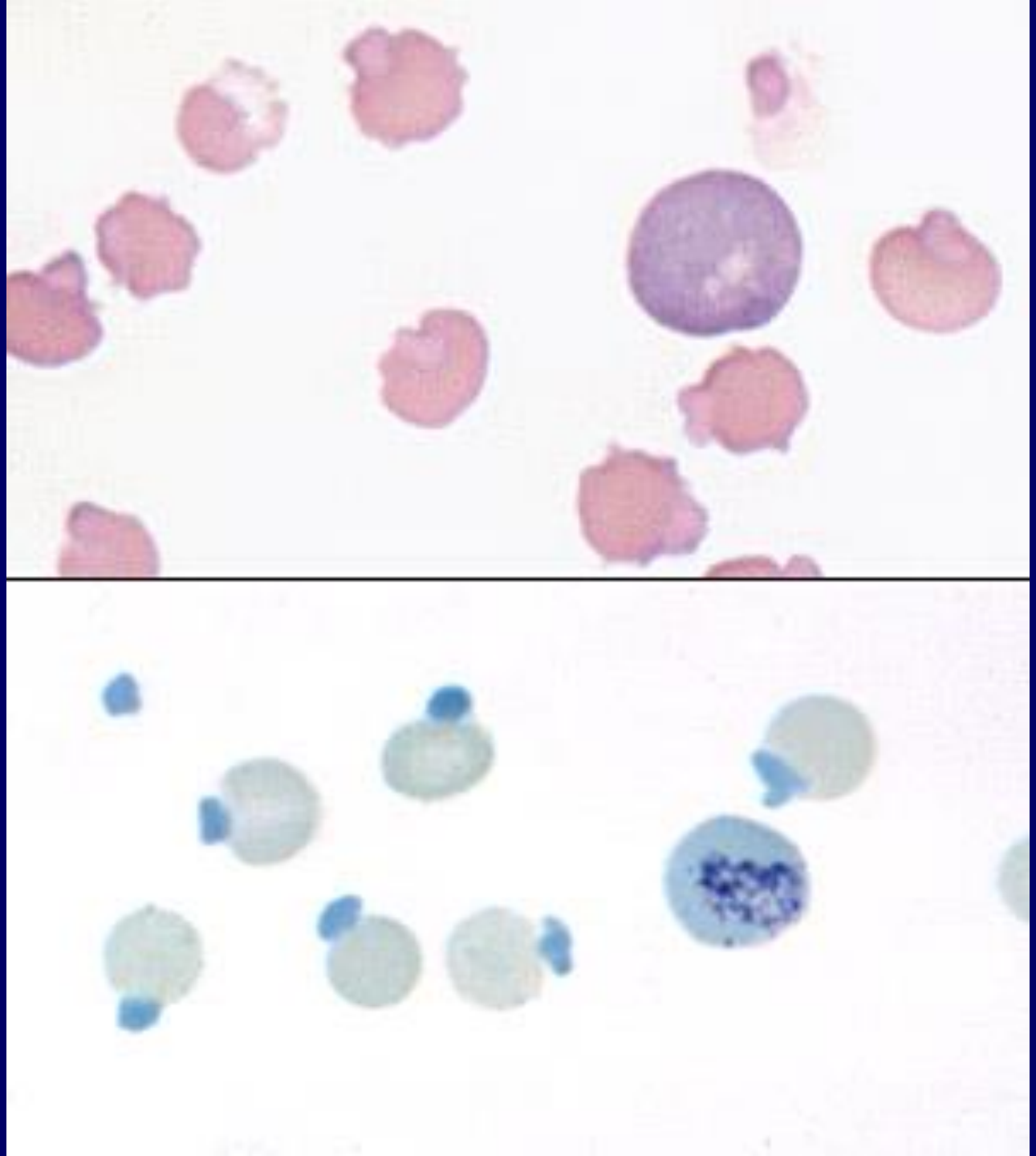


- *Reticulocyte count:* increase
- *Special haematological tests:*

- Demonstration of Heinz bodies by a supravital staining
- Methaemoglobin reduction test.
- G6PD fluorescent spot test.
- Quantitative assay of G6PD

NOTE:

- The diagnosis of G6PD deficiency during an acute haemolytic episode may be difficult, because the deficiency may be obscured by a younger RBCs population as the older G6PD deficient RBCs are destroyed.



- *Chemistry:*
  - S. bilirubin: high
  - S. haptoglobin: low
  - Haemoglobinuria
  - Haemosiderinuria
  - Urobilinogen: high

Treatment

- Avoid ingestion of oxidant drugs and fava bean.
- Blood transfusion.
- Phototherapy.
- Vaccination against certain infections.

- G6PD deficiency and malaria
- G6PD deficiency and diabetes

Methaemoglobin reduction test

Purpose: screening test for G6PD deficiency

Principle:

Sodium nitrite converts Hb to Hi. Incubation of the samples with methylene blue allows stimulation of the pentose phosphate pathway, in subjects with normal G6PD levels, the Hi is reduced during the incubation period, in G6PD-deficient subjects, the block in the pentose phosphate pathway prevents this reduction.

Reagents:

- Sodium nitrite: 180 mmol/l
- Dextrose: 280 mmol/l. Dissolve 5 g of dextrose and 1.25g of $NaNO_2$ in 100 ml of water.
- Methylene blue: 0.4 mmol/l. Dissolve 150 mg of methyl-thionine chloride (methylene blue chloride), in 1 liter of water.
- Nile blue sulphate: 22 mg in 100 ml of water. This may be used as an alternative to methylene blue.

Working reagent: mix equal volume of sodium nitrite and methylene blue or nile blue sulphate

Sample:

- Use anticoagulated blood (EDTA or ACD) and test the samples preferably within 1 h of collection if left on the bench or within 6 h if kept at 4°C.
- Blood in ACD, however, can be stored for up to 1 week, but will be unsatisfactory if there is any haemolysis.
- With blood from severely anaemic patients, adjust the PCV to 0.40 +/- 0.05.

Method:

- Add 2 ml of the test blood to the tube containing 0.2 ml of the combined reagent either freshly prepared or dried. Close the tube with a stopper and gently mix the contents by inverting it 15 times.
- Prepare control tubes by adding 2 ml of normal blood to a similar tube without reagents (normal reference tube) and to a tube containing 0.1 ml of sodium nitrite-dextrose mixture without methylene blue ('deficient' reference tube).
- Incubate the samples at 37°C for 90 min. If the blood has been heparinized, incubation should be continued for 3 h.

- After the incubation, pipette 0.1-ml volumes from the test sample, the normal reference tube and the deficient reference tube into 10 ml of water in separate, clear glass test-tubes of identical diameter. Mix the contents gently. Compare the colours in the different tubes.

Interpretation:

- Normal blood yields a colour similar to that in the normal reference tube -a clear red.
- Blood from deficient subjects gives a brown colour similar to that in the deficient reference tube.
- Heterozygotes give intermediate reactions.

Every job is a self portrait of the

person who does it”

Autograph your work with excellence…!

THANK YOU