Deficiencies of Glycolytic Pathway
Mature RBCs have the capacity for a limited number of enzymatic reactions

The mature RBC is completely dependent on glucose as a source of energy. Glucose usually (90%) is catabolized to pyruvate and lactate in the major anaerobic glycolytic pathway (Embden-Myerhof Pathway). In the process, ATP is generated to play its major role in maintaining a cation gradient in the RBC, thus protecting it from premature death.

No mitochondria in RBC, so it depends entirely on anaerobic glycolysis to produce energy.

Hereditary deficiency of some of the glycolytic enzymes have been documented, and several cause a hereditary nonspherocytic hemolytic anemia (HNSHA), whereas others cause multisystem disease

Most are rare

Pyruvate kinase deficiency is the most common and comprises 90% of affected patients
Deficiencies of Glycolytic pathway

- Hexokinase (AR) Mild-severe
- Glucose-6-isomerase (AR) Mod-severe
- Phosphofructokinase (AR) Mild myopathy
- Aldolase (AR) Mild-mod
- Phosphoglucone kinase (X-linked) retardation
- Pyruvate kinase (AR) Mod-severe
Pyruvate Kinase Deficiency

-AR, patient must be homozygous for the trait to be expressed fully

-Detected in infancy or childhood due to anemia, jaundice, splenomegaly, gallstones. The severity of the condition is widely variable, even among patients with the same level of deficiency.

Lab studies

-Norchromic normocytic, or macrocytic anemia with reticulocytosis in the absence of blood loss, is suggestive of hemolysis.

-Enzyme assay and, more recently, DNA analysis by PCR or single-strand polymorphism are available to confirm the diagnosis and to identify the carrier state if the need arises.
Fluorescent Screening test for Pyruvate kinase deficiency:

**Principle:** Reduced pyridine nucleotide (NADH) fluoresces when illuminated with long-wave UV light.

\[
\text{Phosphoenolpyruvate (PEP) + ADP} \rightarrow \text{Pyruvate + ATP} \\
\text{Pyruvate} \rightarrow \text{Lactate + NAD}^+ \\
\text{LDH is present in excess of PK, NAD production is limited by PK levels. Thus, there should be a time dependent loss of fluorescence as NADH is oxidized to NAD when PK is normal.}
\]
**Specimen:** Heparin or EDTA blood, suitable for several days at 4 C, and 1 day at RT

**Procedure:** Centrifuged, plasma and buffy coat aspirated. RBC suspension lysed by buffered hypotonic screening mixture (that provides PEP, ADP, NADH, MgCl₂).

Spotted on a filter paper immediately after mixing and every 15 minutes after. Dried, examined under long-wave UV light (Control: healthy person)

**Interpretation:** First spot should fluoresce brightly. Normal fluorescence disappears after 15 minutes of incubation. In PK deficiency, fluorescence fails to disappear even after 45-60 minutes of incubation

**Precautions:** False negative with recent RBC transfusion. Some patients with reticulocytosis may have normal screening test
RBC enzyme activity assays

-Spectrophotometric techniques that depend on the absorption of light of the reduced pyridine nucleotide, NADPH, or NADH at 340nm. Reduction results in formation of NADPH or NADH, with increase in absorbance at 340 nm, and oxidation results in formation of NADP or NAD with a decrease in absorbance. The change in absorbance may be used to calculate enzyme activity.

Molecular Identification of Enzyme defects:

Southern blot and mutation analysis
Deficiencies of Hexose Monophosphate Shunt Pathway
Hexose monophosphate Shunt (HMP)

G6PD deficiency Common
Gamma-Glutamylcysteine synthase Rare
GSH synthetase Rare
Glutathione reductase Rare
G6PD Deficiency:

- G6PD enzyme located on X chromosome

-> 30 different mutations give rise to a variety of clinical diseases

-Normally 2 isotypes of G6PD: A and B. Can be differentiated based on electrophoretic mobility

-B isoform most common type of enzyme found in all population groups

-A isoform, found in 20% black men in US, migrates more rapidly on electrophoretic gels than B. It has similar enzyme activity as B, and does not cause disease

-11% of US black men have G6PD variant (G6PD A⁻). It has same electrophoretic mobility as A, but is unstable, resulting in enzyme loss and ultimate enzyme deficiency. Older RBC have only 5-15% enzyme

-G6PD A⁻ most clinically significant type of abnormal G6PD among US Blacks

-Other G6PD variants predominate in other racial groups: G6PD\textsuperscript{MED} in Sicilians, Greeks, Sepharic Jews, Arabs. G6PD\textsuperscript{CANTON} or G6PD\textsuperscript{MAHIDOL} in Asian population
- X linked, so sex-linked inheritance pattern.
- Effect fully seen in affected men
- Carrier women are variable affected depending X chromosome inactivation

**Clinical findings**

- Episode of hemolysis following infection or ingestion of an oxidant drug
- In G6PD A- def, the hemolytic anemia is self-limited as the young RBC produced in response to hemolysis have nearly normal enzyme.
- Five classes of G-6-PD deficiency exist based on enzyme activity levels, as follows:
  1. Enzyme deficiency with chronic nonspherocytic hemolytic anemia
  2. Severe enzyme deficiency (enzyme level <10%)
  3. Moderate-to-mild enzyme deficiency (enzyme level 10-60%)
  4. Very mild-to-no enzyme deficiency (enzyme level > 60%)
  5. Increased enzyme activity
Lab:

- Acute hemolysis is associated with formation of Heinz bodies (denatured Hgb).
- Bite cells (eccentrocytes) may be seen

**Fluorescent Screening test for G6PD Deficiency:**

In the presence of NADP⁺, G6PD is oxidized to form 6-phosphogluconate and NADPH. Then

\[
\text{6-Phosphogluconate + NADP}^+ \overset{6\text{-phosphogluconate dehydrogenase}}{\rightarrow} \text{Ribulose-5-P + NADPH + H}^+ 
\]

NADPH can be detected by fluorescence

When G6PD is low, less NADPH is formed.

Normal sample shows a bright fluorescence after 5-10 minutes of incubation, whereas deficient sample shows no fluorescence.
Quantitative G6PD assay:

-Formation of NADPH from NADP$^+$ from G6P is measurable by a change in absorbance created by NADPH at 340 nm in a spectrophotometer.

Heinz body test:

Crystal violet or neutral red is added to blood.

Reduced Glutathione determination:

RBC GSH levels are often decreased in patients with HMP shunt or GSH synthetic pathway deficiencies. Dithiol compound, dithio-bisnitrobenzoic acid (DTNB) is reduced by GSH to form yellow anion, the optical density is measured at 412 nm.