Glucose-6-Phosphate Dehydrogenase (G6PD) and Malaria

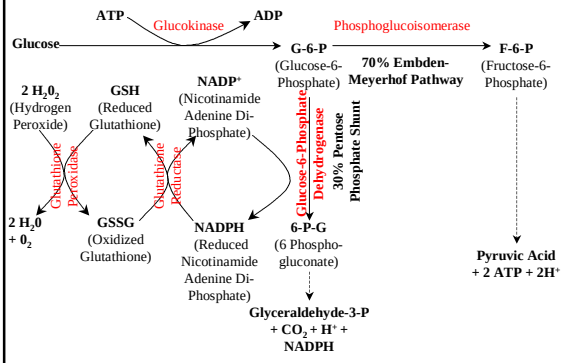
Structure of G6PD

- The enzyme, Glucose-6-Phosphate Dehydrogenase, is comprised of a dimer or tetramer of identical polypeptide chains
  - Each unit consists of 515 amino acids
- The single G6PD locus in humans is located on the telomeric region of the long arm of the X-chromosome
  - Females have two X chromosomes, hence two copies of G6PD, while males have only one X chromosome and one copy of G6PD

Function of G6PD

- G6PD is present in the cytoplasm of all cells of the body
  - In Red Blood Cells (RBC), which lack nuclei, mitochondria, and other organelles, G6PD is particularly significant
    - G6PD is involved in the first step of the Pentose Phosphate Shunt
      - Catalyzes the oxidation of Glucose-6-Phosphate to 6-Phosphogluconolactone (Phosphogluconate)
      - Only source of NADPH and GSH, necessary for the reduction of hydrogen peroxide
        - Hydrogen Peroxide is a strong oxidant that will degrade the RBC and cause hemolysis if it is not reduced

Red Blood Cell Metabolism



Familial Genetics of G6PD

- Five genotypes can form from combinations of one normal (Gd^B) and one deficient form (e.g., Gd^{A-} or Gd^{Med}) of G6PD
  - Females
    - Gd^B Gd^B, Homozygous Normal; "Normal"
    - Gd^B Gd^{A-}, Heterozygous; "Heterozygote"
    - Gd^{A-} Gd^{A-}, Homozygous Deficient; "G6PD Deficient"
  - Males
    - Gd^B, Hemizygous Normal; "Normal"
    - Gd^{A-}, Hemizygous Deficient; "G6PD Deficient"

Mendelian Transmission

	Males	
Females	Gd^B	Y
Gd^B	Gd^B Gd^B	Gd^B
Gd^{Med}	Gd^B Gd^{Med}	Gd^{Med}
	Daughters	Sons

G6PD Heterozygotes

- Because of the random inactivation of one X chromosome in each female body cell, heterozygotes have two kinds of Red Blood Cells
  - G6PD Normal
  - G6PD Deficient
    - Depending on which X chromosome was inactivated in the stem cell giving rise to the particular RBC

G6PD Variants

Four most common variants out of 300+ known		
Gd^{B}	Normal Activity	All World Populations
Gd^{A}	Normal Activity; Aspartic acid substituted for asparagine at position 126, Guanine for adenine at DNA position 376	Africa (most common variant)
Gd^{A-}	8 - 20% Normal Activity; Methionine for Valine at position 67 and Aspartic Acid for Asparagine at position 126, Adenine for Guanine at position 202 and Guanine for Adenine at position 376	Africa
Gd^{Med}	< 5% Normal Activity; Phenylalanine for Serine at position 188; Thymine for Cytosine at position 563	Iran, Iraq, India, Pakistan, Greece, Sardinia

G6PD Activity

- Declines with age of RBC
  - Gd^{B} has 62 day half-life for decay of activity
    - Sustains GSH levels for 100 to 120 day RBC life span
  - Gd^{A-} has normal activity when new, but the activity half-life is only 13 days
    - Deficiency is due to instability of the enzyme
  - Gd^{Med} has greater instability with 8 day half-life
    - New cells already have reduced activity, and mature RBC have enzyme levels < 1% normal activity

Symptoms of G6PD deficiency

- G6PD deficiency is manifested as anemia, with RBCs being prematurely destroyed
  - RBCs are also extremely susceptible to oxidative stress
  - Neonatal jaundice is a yellowish discoloration of the whites of the eyes, skin, and mucous membranes caused by deposition of bile salts in these tissues
    - A severe form of this is a direct result of insufficient activity of the G6PD enzyme in the liver
      - In some cases, the neonatal jaundice is severe enough to cause death or permanent neurologic damage (Beutler, 1994).

Symptoms of G6PD deficiency, 2

- Outside areas where dietary components cause hemolytic crises, infection is the most common cause of hemolysis and anemia in subjects with G6PD deficiency
  - Oxidative metabolites produced by bacterial, viral, and rickettsial infections cause an anemic response
    - Viral hepatitis, pneumonia, and typhoid fever are particularly likely to precipitate a hemolytic episode in G6PD deficient individuals

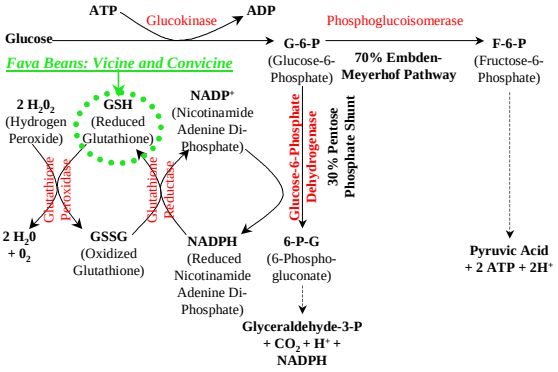
G6PD Hemolysis

- Red blood cells will hemolyze or burst when the oxidant stress level becomes too high
  - Hemolysis occurs in G6PD deficient individuals due to the consumption of certain foods or drugs
    - Substances that increase the oxidation of glutathione, thereby diminishing the available GSH for oxidation of peroxide, creating a potential for hemolysis
      - Fava Beans contains vicine and convicine whose metabolites can cause a hemolytic crisis in Gd^{Med} individuals
      - Many anti-malarial drugs, sulfonamides, sulfones and other drugs produce the same reaction in severely deficient individuals
  - Can also cause the oxidation of hemoglobin, making it lose the ability to be a reversible oxygen carrier

Favism

- The Fava Bean (*Vicia faba*) is a favored cultigen in areas where the Gd^{Med} allele is common
  - Vicine and convicine make up about 0.5% of the wet weight of the Fava bean
    - These compounds metabolize to divicine and isouramil in the intestine
      - These metabolites decrease RBC reduced glutathione (GSH)
        - Increase the production of hydrogen peroxide and free radicals
      - Creates a severe oxidant stress in G6PD deficient cells

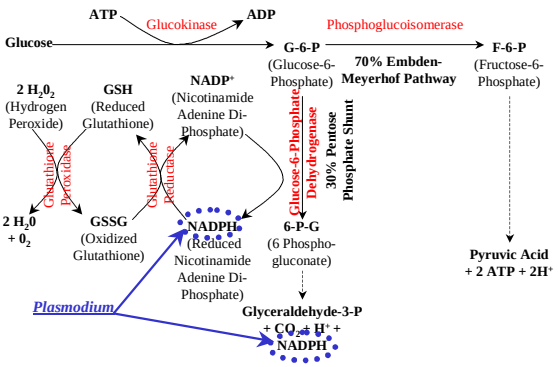
G6PD and Fava Beans



Plasmodium in the RBC

- *Plasmodium* protozoans preferentially attack immature RBC but *P. falciparum* can invade RBC of all ages
  - *Plasmodium* oxidizes RBC NADPH from the Pentose Phosphate pathway for its metabolism
    - This results in a deficiency of RBC GSH, most severe in G6PD deficient individuals, leading to peroxide-induced hemolysis which curtails the development of *Plasmodium*
      - After several cell cycles the *Plasmodium* can adapt to produce its own G6PD, reducing the adaptive benefit of G6PD deficiency

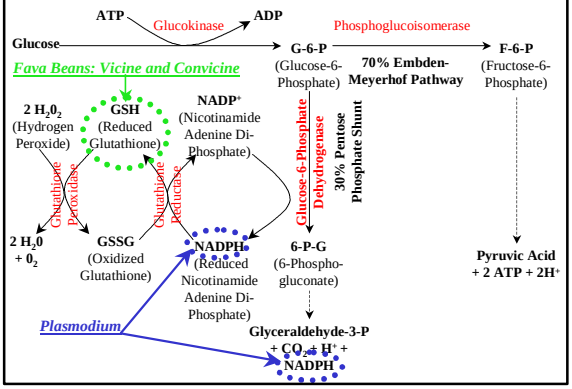
G6PD and Malaria



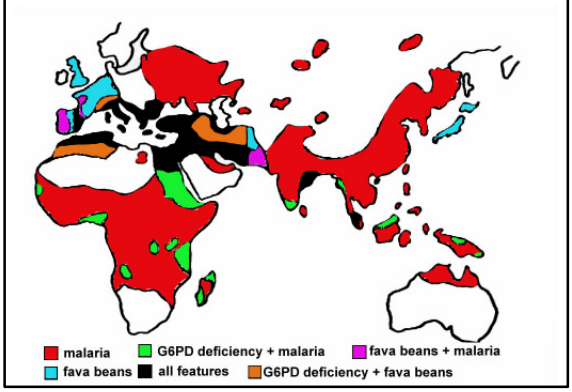
Fava Beans and Malaria

- Recall that fava beans contain compounds that metabolize to powerful oxidants
  - In a cell that is oxidant-stressed by *Plasmodium* infection, the addition of another strong oxidant can lead to a rapid build-up of peroxide
  - *In vitro* and *in vivo* (mouse) studies indicate a mild suppressant effect of divicine and isouramil on *Plasmodium* **in G6PD normals**
    - This effect is even greater in G6PD deficient individuals

G6PD, Fava Beans, and Malaria



Distribution of G6PD, Fava Beans, and Malaria



Case Study: Gd^{Med} and Favism

- Fava bean cultivation is widespread, especially throughout the circum-Mediterranean region
- There is substantial overlap between the cultivation of fava beans and the Gd^{Med} allele
  - Serious cases of hemolytic favism are described more than 2,000 years ago by Greeks
    - About 1 in 12 cases of favism results in mortality
    - Mostly affects children (up to 95% of cases)
- Why continue to cultivate fava beans?

Nutrition and Fava Beans

- Fava beans are only one of several legumes cultivated in the Mediterranean including chick peas, kidney beans, and lentils
  - Fava beans are a highly productive crop and produce a high yield of protein by dry weight
    - However, kidney beans and chick peas are more efficient in terms of the ratio of weight of protein consumed to weight gained in growing individuals
    - Lentils are as efficient as fava beans
  - Continued use where Favism rates are high must be due to other factors

Responses to Favism

- Mediterranean populations have developed several responses including food taboos, preparation techniques, and folk remedies
  - Highly susceptible groups including children and pregnant women are frequently forbidden to consume fava beans
  - Drying, soaking, and removing the skins appear to reduce toxicity
  - Increasing sugar consumption reduces the severity of an impending hemolytic crisis

Continued Cultivation

- There are three lines of evidence that suggest continued cultivation of Fava Beans in the face of Favism is related to malaria
  - The association of divicine and isouramil with the suppression of *Plasmodium* growth
  - The clinal association of fava beans cultivation and malaria
  - The overlap of the peak fava bean harvest and consumption times with the peak *Anopheles* mosquito breeding season

Selection in Males

- Males are G6PD deficient or Normal
  - Malaria alone:
    - Select for an increase in Gd^{Med} because of resistance to *Plasmodium* in G6PD RBCs
  - Combination of Malaria and Fava beans:
    - Select against Gd^{Med} through favism and hemolytic anemia
      - Cooking and preparation techniques may buffer the favism selection
      - Other genes may also buffer favism
        - Acid Phosphatase B and β-Thallasemia reduce severity
    - Decreased selection against Gd^{B} through increased resistance to *Plasmodium* from fava beans

Selection in Females

- Malaria alone favors the heterozygotes
  - Selects against Gd^B/Gd^B, most susceptible genotype to *Plasmodium*
  - Anemia selects against Gd^{Med}/Gd^{Med}, but they are resistant to severe malaria symptoms
  - Heterozygotes (Gd^B/Gd^{Med}) are favored
    - Increased resistance to malaria compared to Gd^B/Gd^B
    - Less susceptible to hemolytic crises from diet or infection than Gd^{Med}/Gd^{Med}
    - The balance is complicated by the random deactivation of an X chromosome in the cells producing the RBCs
      - Heterozygotes will range widely from about 20% normal to about 80% normal RBCs, and the response to malaria and other hemolytic crises will vary accordingly

Selection in Females, 2

- Combination of Malaria and Fava beans complicates selection
  - Fava beans intensify selection against Gd^{Med}/Gd^{Med}
    - Favism and increased incidence of hemolytic anemia
      - Cooking, preparation techniques, and other genes may buffer Gd^{Med}/Gd^{Med} from severe hemolytic crises
  - Decreased selection against Gd^B/Gd^B
    - Increased resistance to *Plasmodium* from fava beans
  - Heterozygotes (Gd^B/Gd^{Med}) are still most fit
    - The differential between Gd^B/Gd^{Med} and Gd^B/Gd^B resistance to malaria is reduced (selection is weaker)
    - Selection differential may be stronger against Gd^{Med}/Gd^{Med} because of the increased incidence of hemolytic crises due to favism