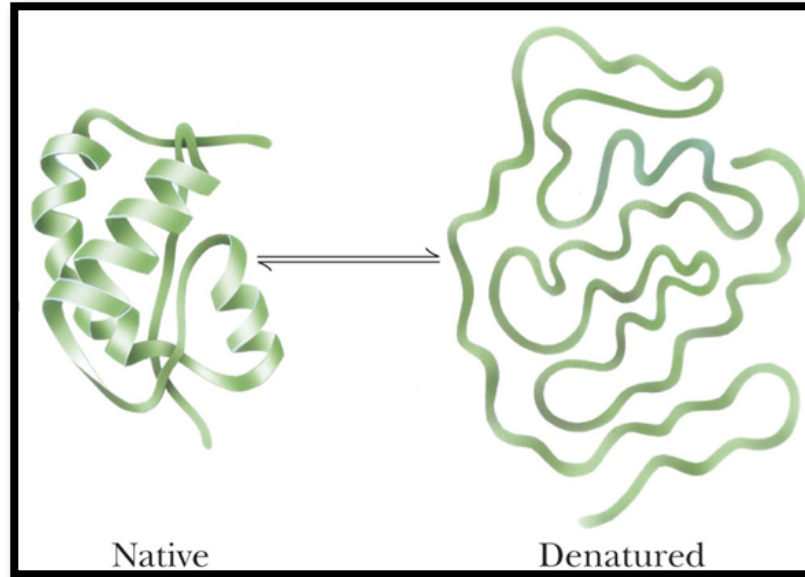


Hemolytic Anemia: G6PD Deficiency
for the USMLE Step One Exam

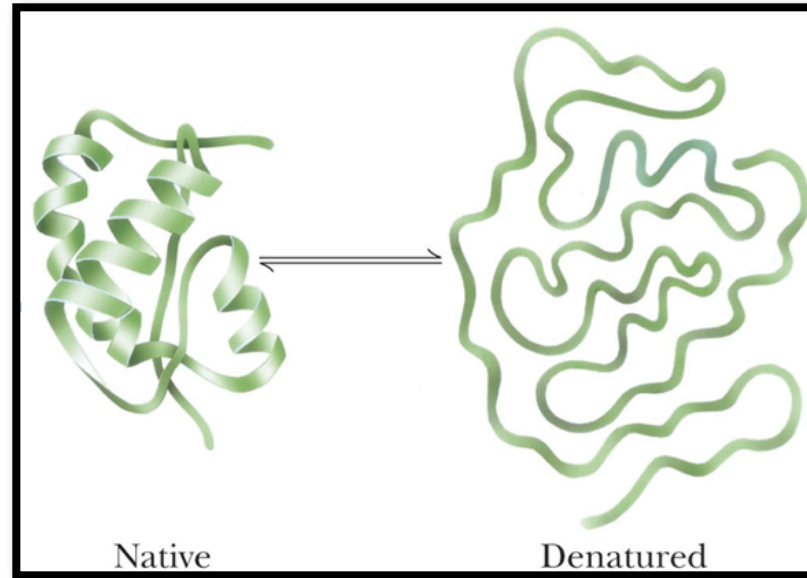


Howard Sachs, MD

www.12DaysinMarch.com

E-mail: Howard@12daysinmarch.com

Hemolytic Anemia: G6PD Deficiency
for the USMLE Step One Exam

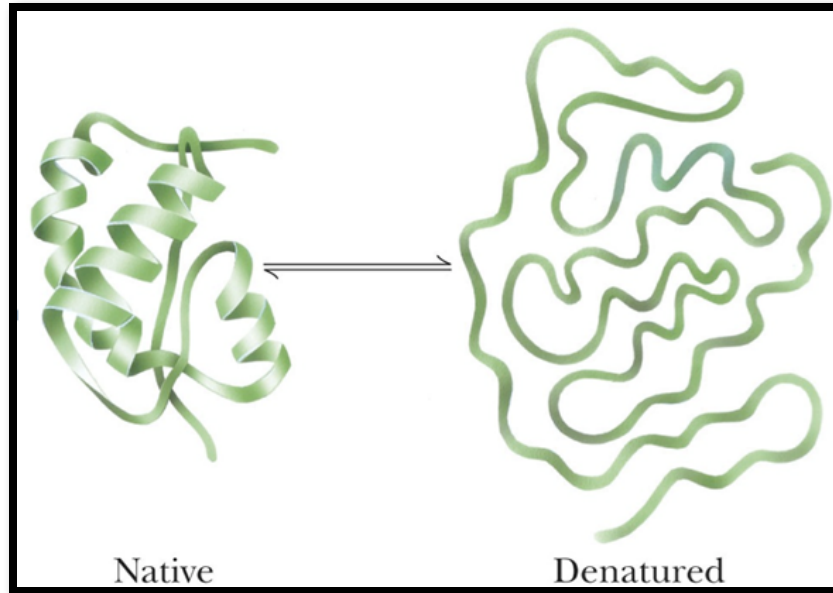


12DaysinMarch

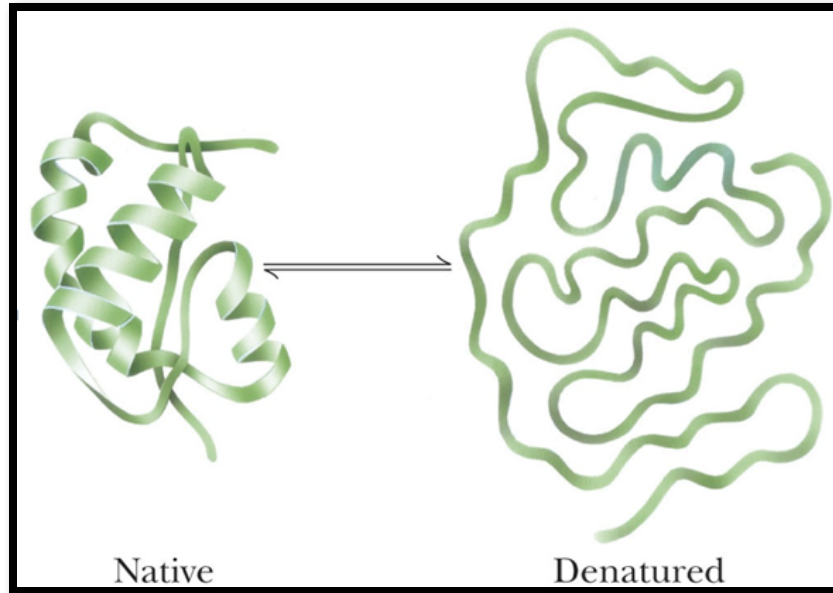
Tutorial Services
(see website for details)

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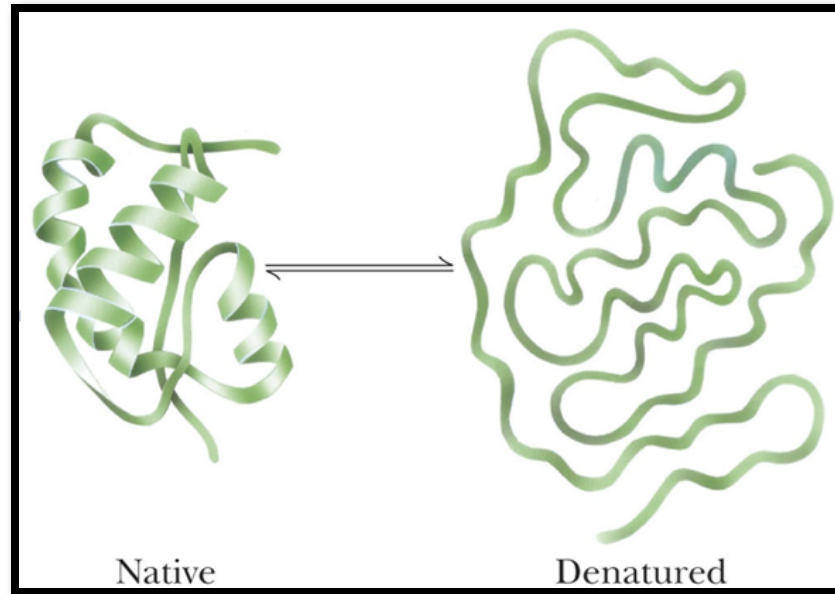
G6PD Deficiency Insufficiency



G6PD Insufficiency → Denatured Hemoglobin

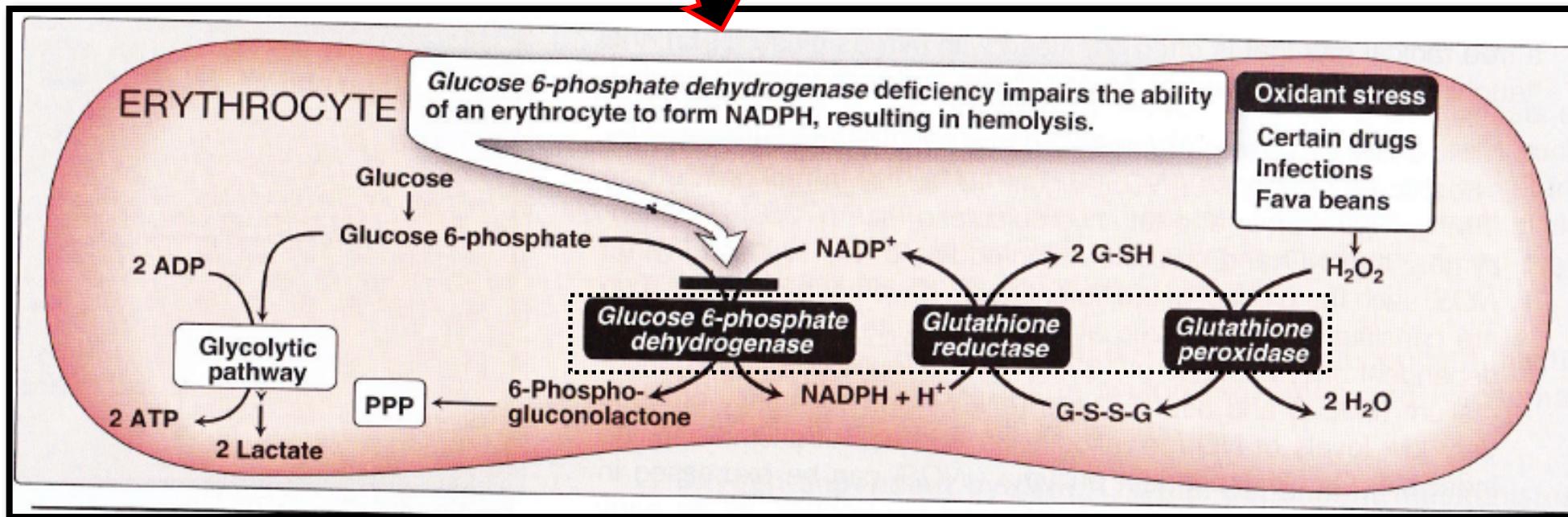


G6PD Insufficiency → Denatured Hemoglobin



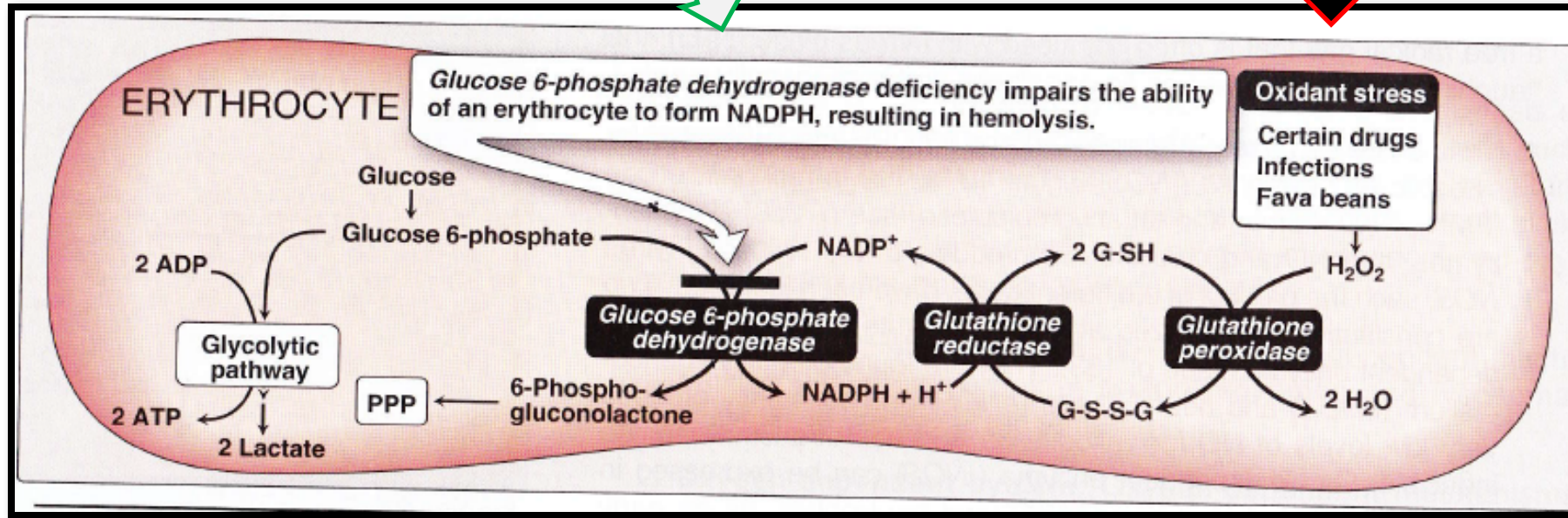
1. Why it becomes denatured?
2. The consequence of denatured Hgb?

Here it is in a nutshell.
Failure to generate NADPH
due to deficiency of G6PD



Here it is in a nutshell.
Failure to generate NADPH
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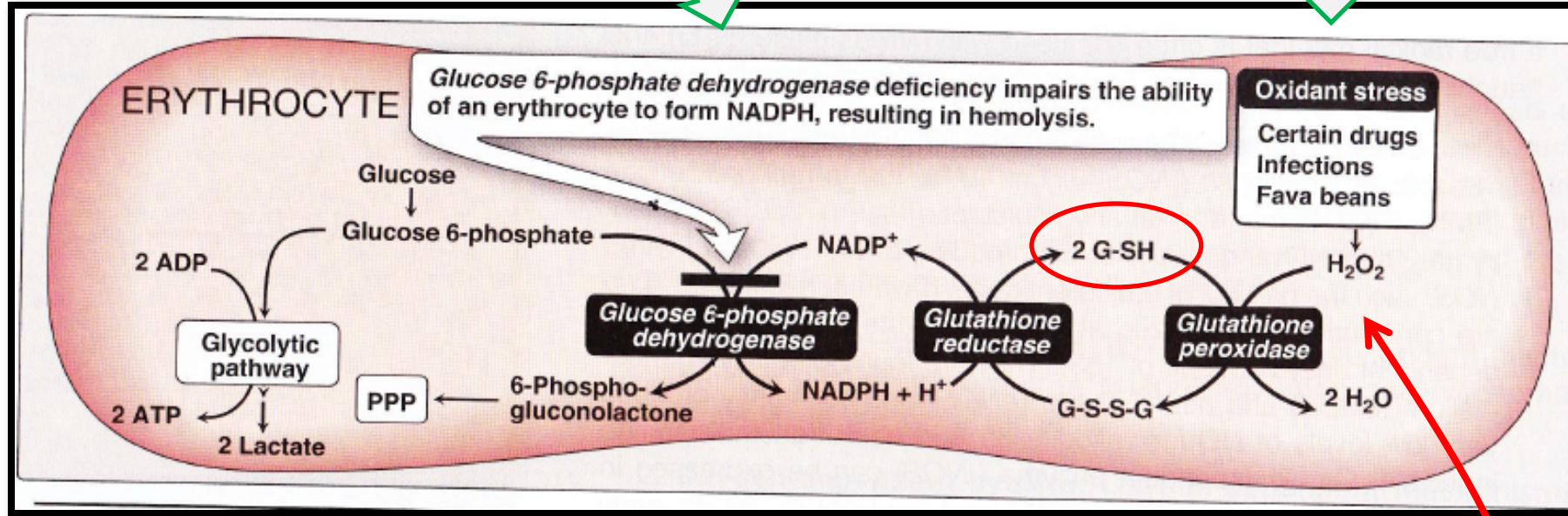
These are the triggers for
the 'oxidative stress' →
hemolysis



- Sulfa/Dapsone, Primaquine (anti-malarial)
- Fava beans

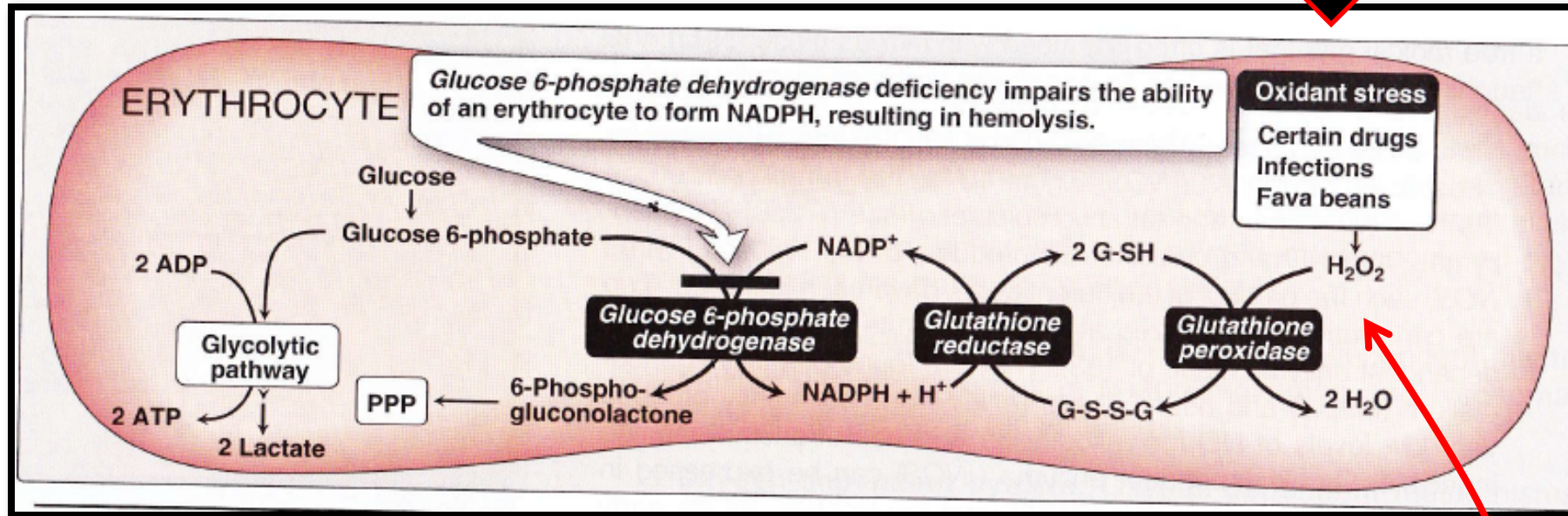
Here it is in a nutshell.
Failure to generate NADPH
due to deficiency of G6PD

These are the triggers for
the 'oxidative stress' →
hemolysis



H₂O₂ is the villain
in this story

These are the triggers for the 'oxidative stress' → hemolysis



No NADPH due to deficiency of G6PD



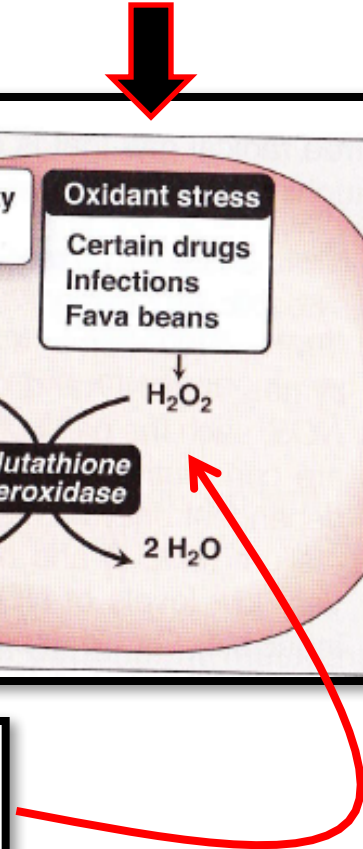
No Glutathione



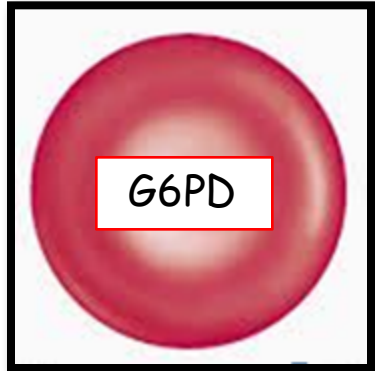
No Reduction of H₂O₂



H₂O₂ is the villain in this story

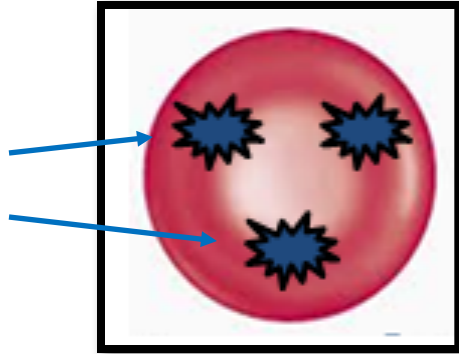


Who cares about this stupid enzyme?



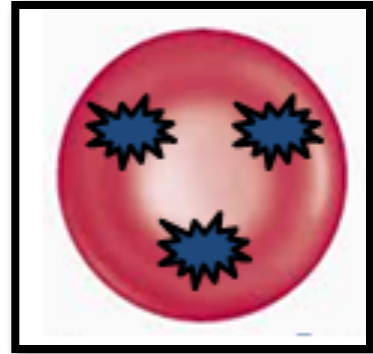
This guy

Why does he care?

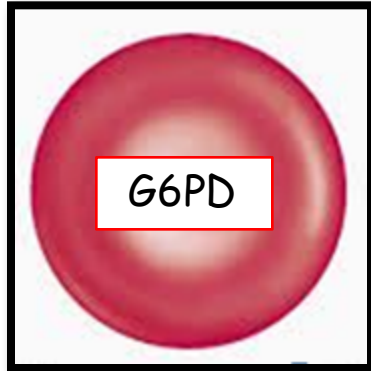


Because of these guys

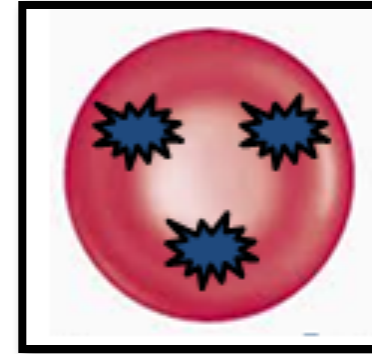
It looks like acne.



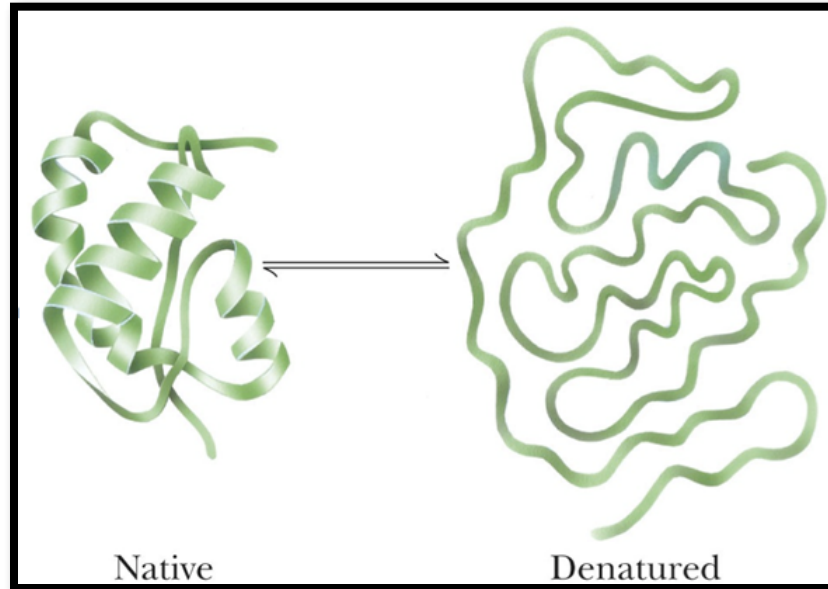
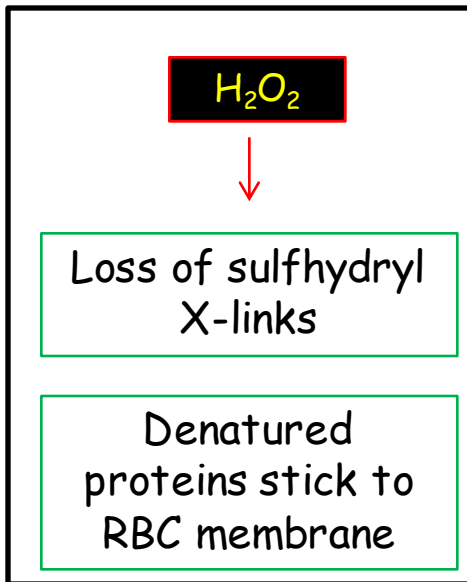
It's not.
It's 'denatured Hgb'

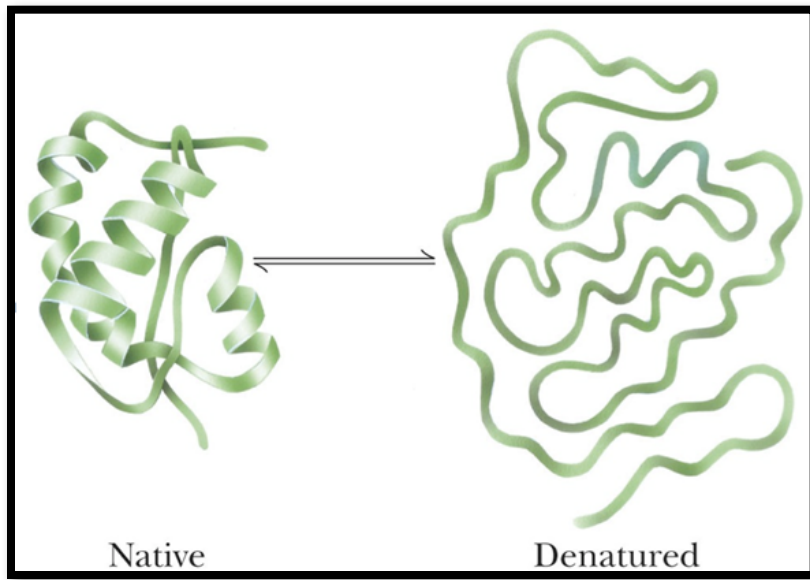


Deficiency
Oxidative Stress



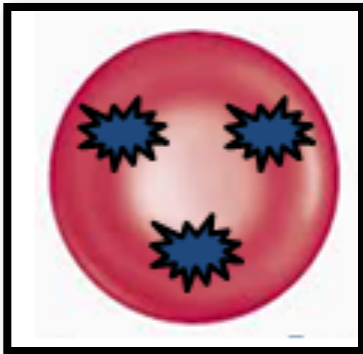
'Denatured Hgb'





And why does this happen?

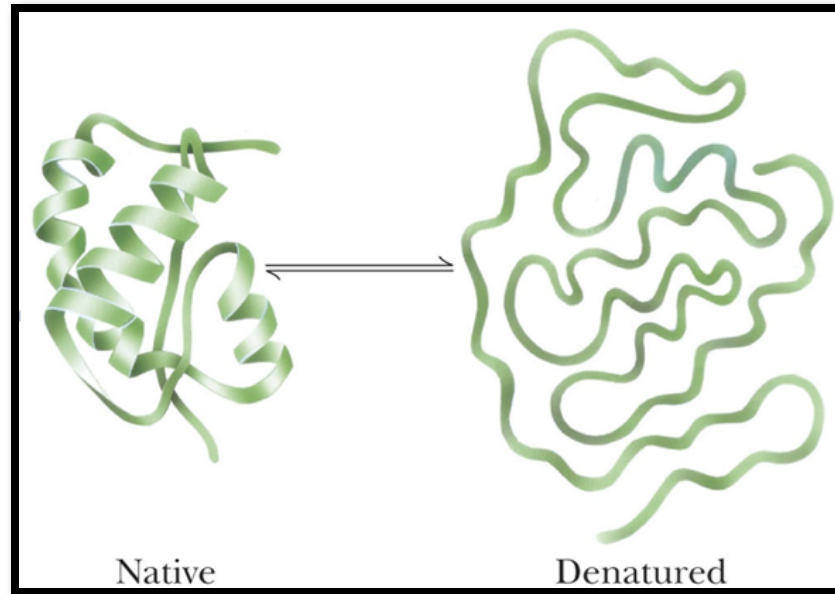
Hydrogen Peroxide (and other reactive oxygen species) are toxic to cell, enzymes, **proteins**, pumps, etc.



What happens to these RBCs?

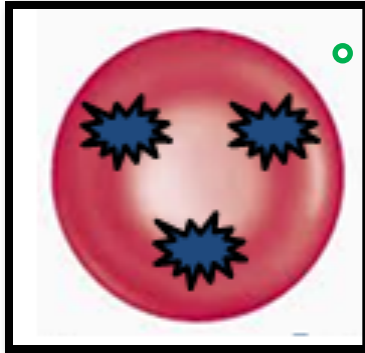


G6PD Insufficiency → Denatured Hemoglobin



1. Why it becomes denatured?
2. The consequence of denatured Hgb?

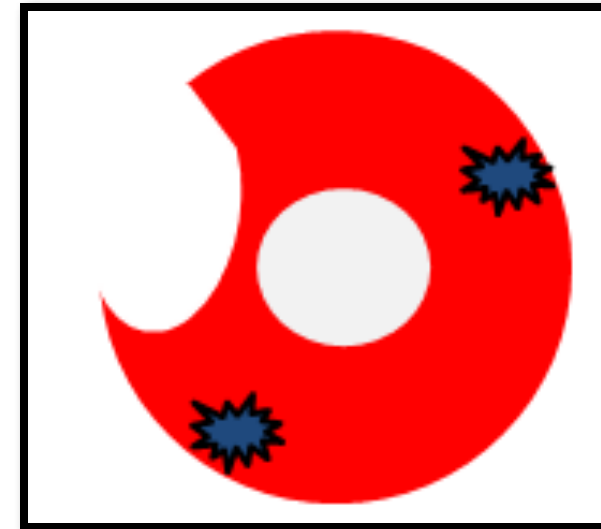
Help! I'm ugly
and denatured.



Not a problem Ma'am.
This is our job.



That's a tite squeeze...
Membrane is less deformable

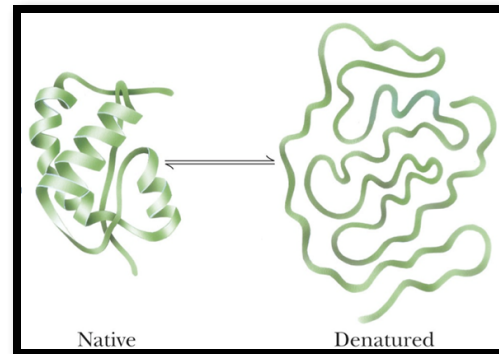


Ouch! Someone
bit me!

Enzyme Defect: G6PD Deficiency

(↓ NADPH → ↓ Glutathione levels → ↑ free radical/peroxidase)

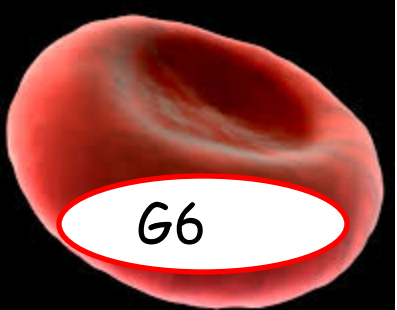
- Background
 - Enzyme defect (**X-linked, AR**); 400 different mutations
 - Variable degrees of expression (i.e. $T \frac{1}{2}$ varies → Δ severity)
 - Mature RBCs do not synthesize proteins so enzyme levels fall quickly as cell age
 - Enzyme **insufficiency** offers inadequate protection against oxidant stress
 - Hemolytic episodes occur following exposures that generate (unprotected) oxidative stress.
 - Oxidative stress leads to denaturing of proteins (Hgb, membrane proteins)



G6PD Sufficiency



Newborn RBC



$T_{1/2} = 62 \text{ d}$

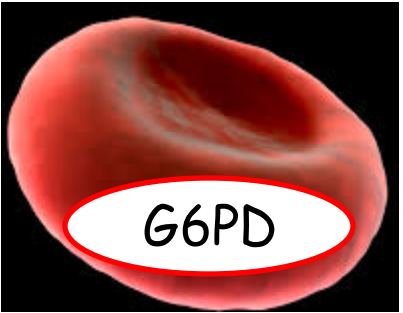


Geriatric

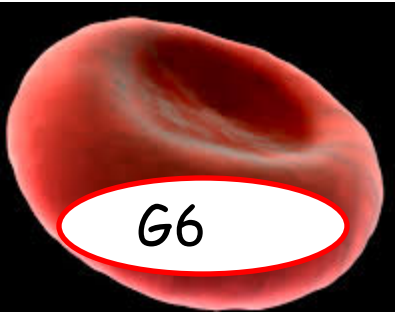
Protect the old fella

'Vulnerable'

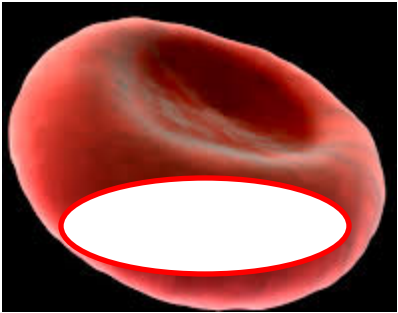
G6PD InSufficiency



Newborn RBC



$T_{\frac{1}{2}} = 62 \text{ d}$



Geriatric



Newborn RBC

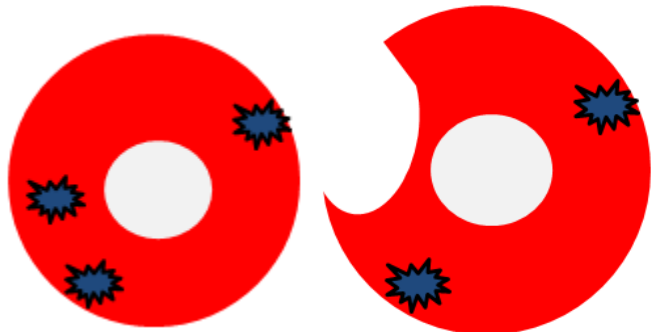


Hrs - Days

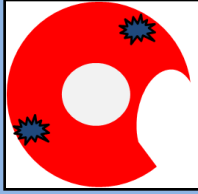
G6PD InSufficiency

Trigger

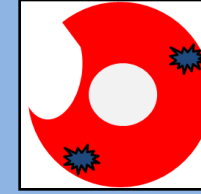
No GSH



Until Newborn RBCs Arrive

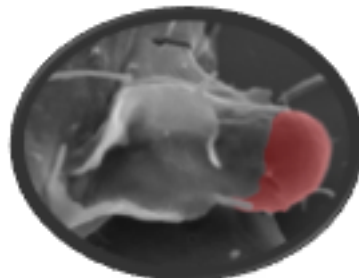


Denatured, oxidized Hgb



- Mechanism

- Oxidants → **X-linking** of reactive sulfhydryl groups on globin chains.
- The globin chains become denatured and form **membrane-bound precipitates** (Heinz bodies - oxidized, denatured Hgb).
 - Heinz bodies can damage the RBC membrane sufficiently to cause **intravascular hemolysis**.
 - Less severe injury can lead to ↓ in **RBC deformability**
 - Splenic MΦ take a 'bite' out of cell trying to remove Heinz Hgb bodies.



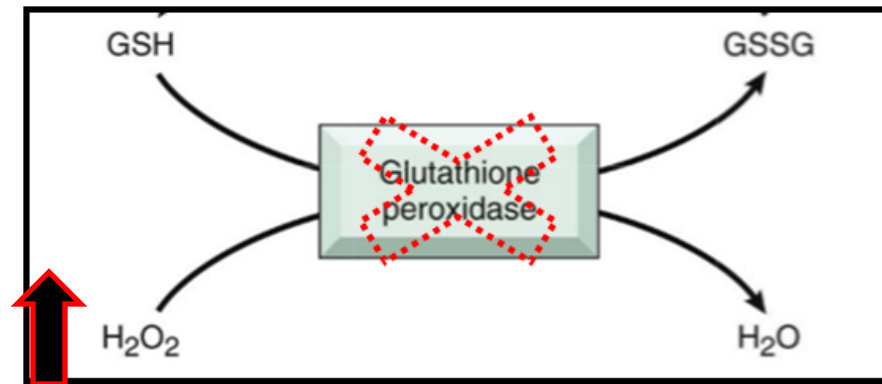
'dark, intracellular inclusions'

Enzyme Defect: G6PD Deficiency

(↓ NADPH → ↓ Glutathione levels → ↑ free radical/peroxidase)

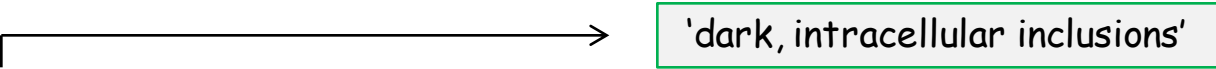
- Pathogenesis

- Triggers: infection, **drugs** (sulfa/dapsone, anti-malarials), **foods** (fava **beans**), DKA.
- The triggers lead to oxidative stress/injury in the absence of reduced glutathione



Please recognize the **code names** of the triggers...
(e.g. treated for UTI, ate a bean he never had before, etc)

- Clinical: (asx most frequent)
 - Self-limited episodes of hemolysis 2-3 d after exposure
 - Older cells affected: when they are depleted → episode subsides
 - No splenomegaly/stones due to self-limited episodes (new cells have adequate enzyme)
 - Hemolysis: hemoglobinuria (intravascular), jaundice, symptoms of anemia

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 - Hemolysis: hemoglobinuria (intravascular), jaundice, symptoms of anemia
- Diagnostics 
 - Smear: Heinz bodies, bite cells, hemolysis, spherocytes.
 - Labs: Anemia, Hemolysis indices (LDH/retic/indirect bili/haptoglobin)
 - Coomb's negative hemolytic anemia
 - Urine: hemoglobinuria (intravascular)

- **Clinical: (asx most frequent)**
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- **Diagnostics** → 'dark, intracellular inclusions'
 - Smear: Heinz bodies, bite cells, hemolysis, spherocytes.
 - Labs: Anemia, Hemolysis indices (LDH/retic/indirect bili/haptoglobin)
 - Coomb's negative hemolytic anemia
 - Urine: hemoglobinuria (intravascular)
 - **Spot test**: fluorescence of NADPH after NADP and G6P are added to hemolysate of test RBC

Spot test rxn:
NADPH fluoresces
under UV light

No fluorescence if deficient

Glucose-6-Phosphate + NADP
(Not fluorescent)

G6PDH

↓ Patient's
blood

6-Phosphogluconate + NADPH
(Fluorescent)



• Clinical: (asx most fr...)

– Self-limited episode

- Older cells affected
- No splenomegaly/stones due to self-limited episodes (new cells have adequate enzyme)

– Hemolysis: hemoglobin

Spot test rxn:

exposure

episode subsides

ce, symptoms of anemia

• Diagnostics

– Smear: Heinz bodies

– Labs: Anemia, Hemo

– Coomb's negative he

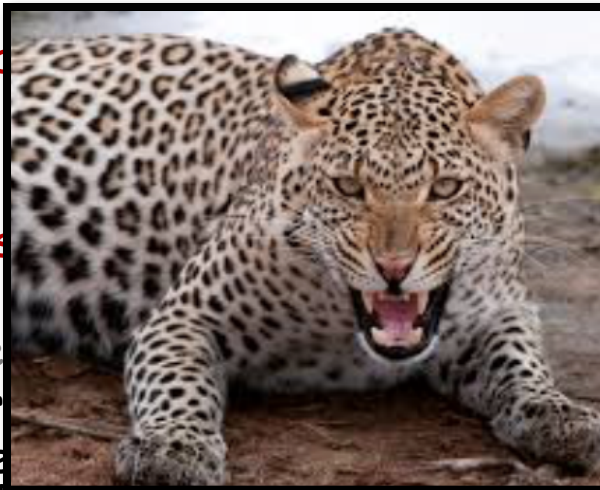
– Urine: hemoglobinur

– Spot test: fluoresce
hemolysate of test RBC

ocytes.

irect bili/haptoglobin)

and G6P are added to



Glucose-6-Phosphate + NADP
(Not fluorescent)

G6PDH

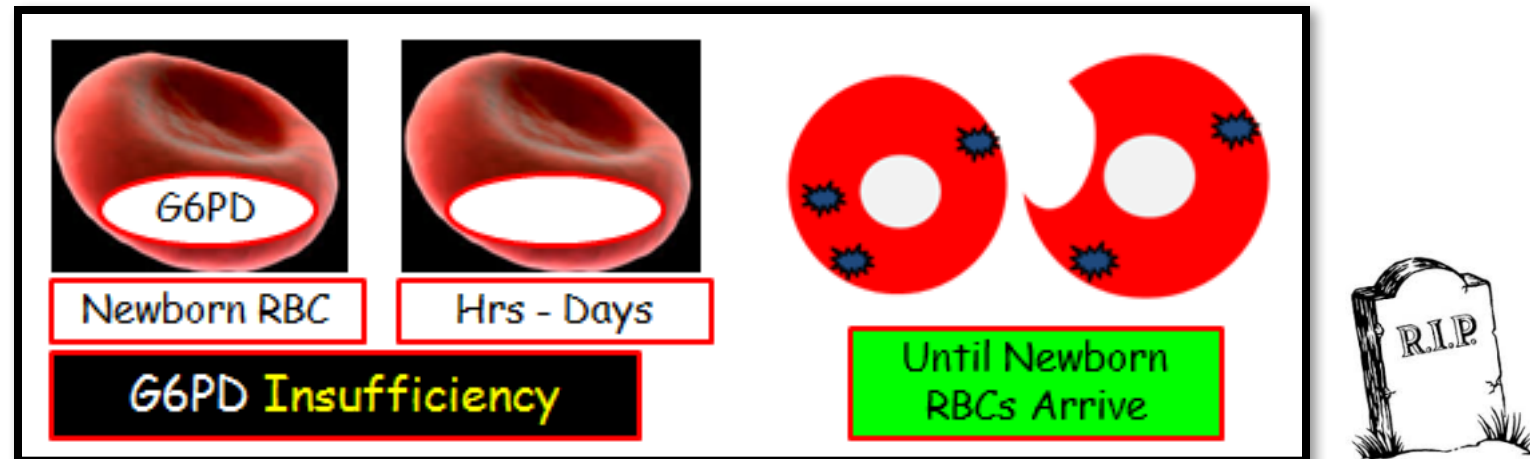
↓ Patient's
blood

6-Phosphogluconate + NADPH
(Fluorescent)

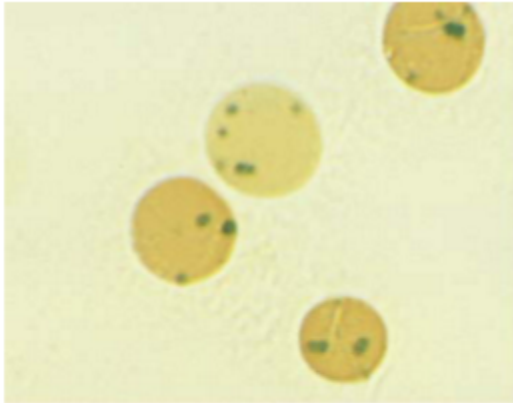
No fluorescence if deficient



- Rx: Stop offending drug/exposure
- **Special notes**
 - Between episodes, blood count is normal.
 - During crisis, G6PD level may be normal as young (surviving) cells do have normal levels (or, if you prefer, most deficient cells have already hemolyzed).
 - **Test 2-3 months after episode**

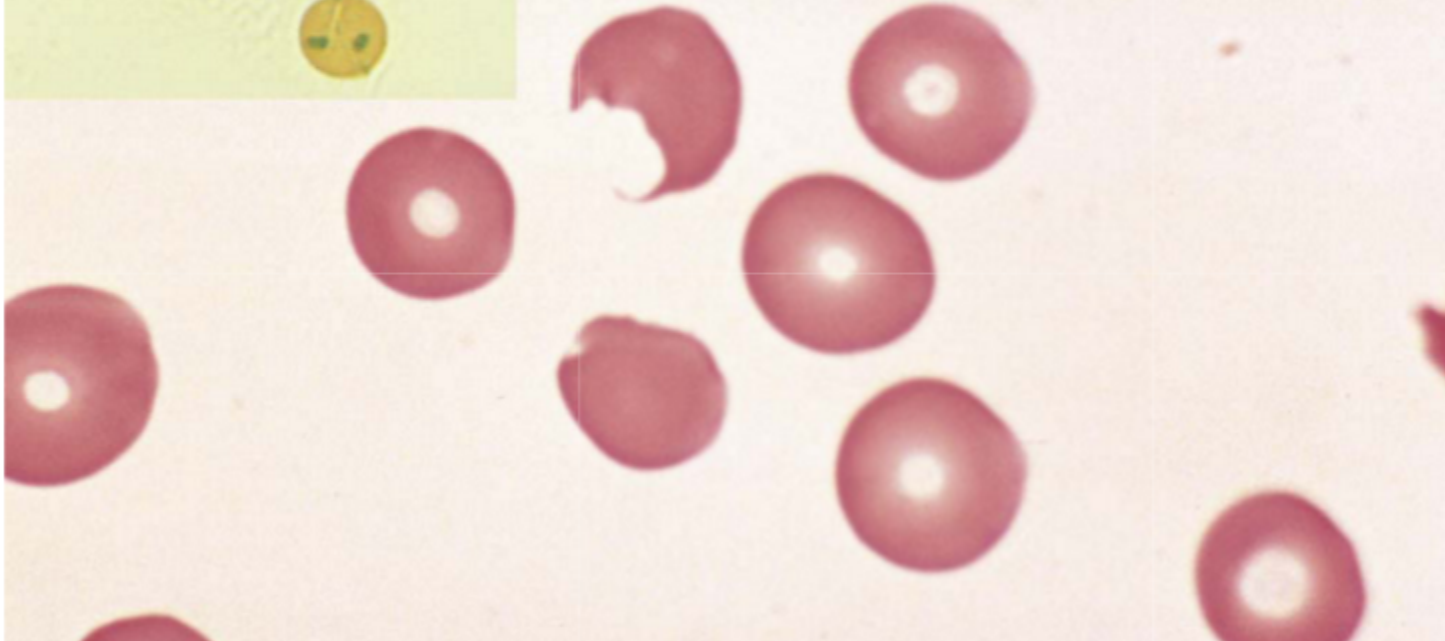


These guys are dead...
need to wait for new cells to test.

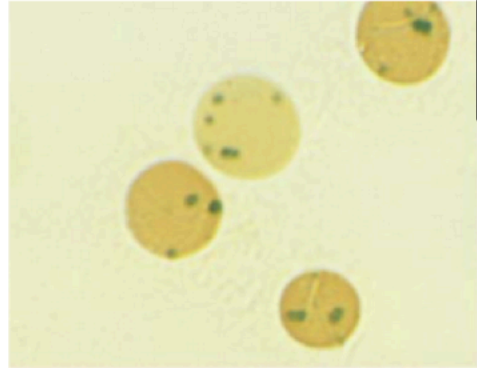


Supravital staining:
'dark, intranuclear inclusions'

Crystal violet:
'irregular, dark, purple granules'

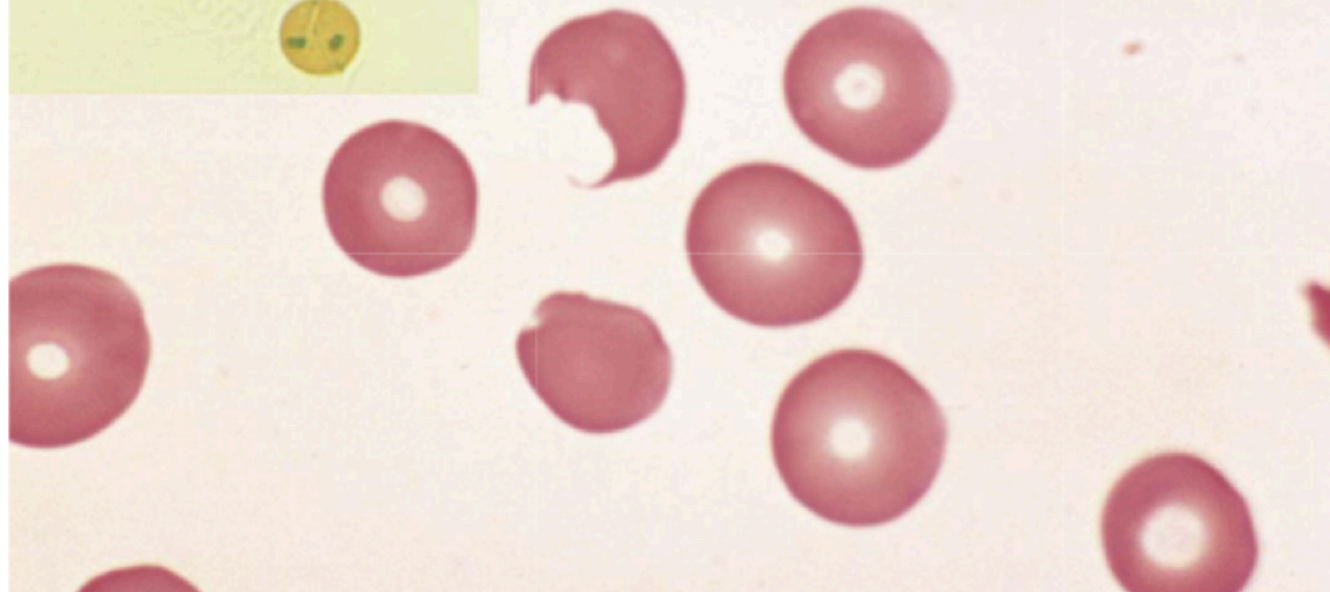


Splenic MΦ pluck out Heinz body → 'bite cells'

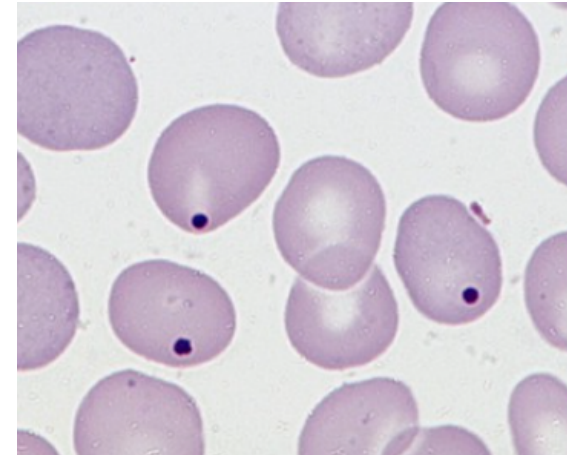


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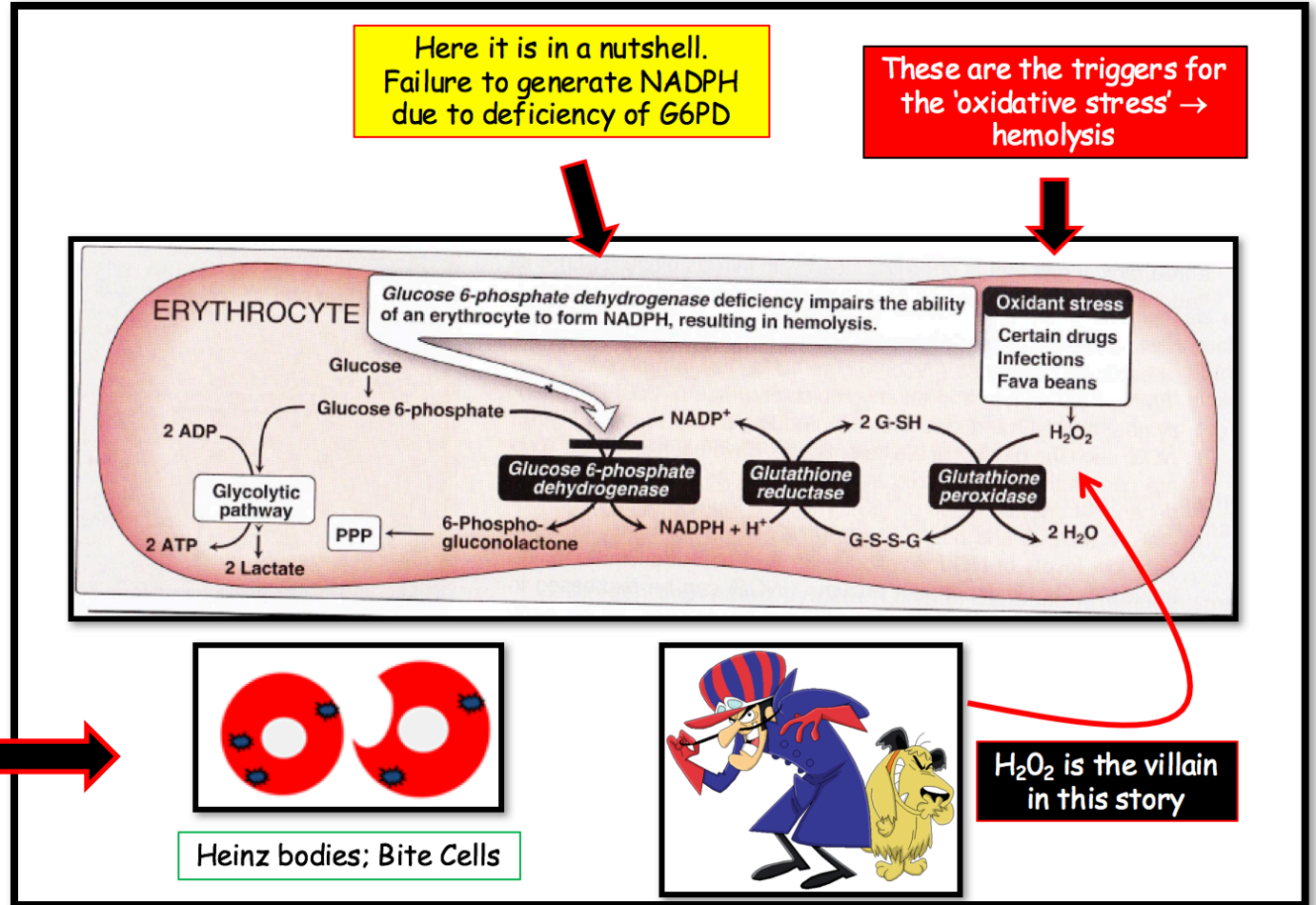
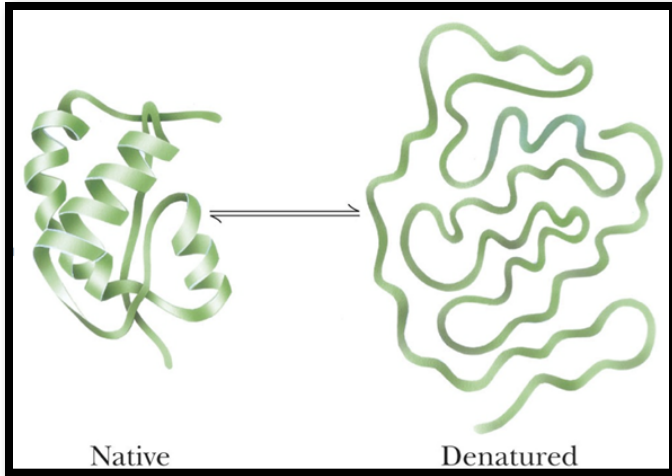


Splenic M Φ pluck out Heinz body \rightarrow 'bite cells'



Howell Jolly Bodies
(DNA remnants in asplenia)

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