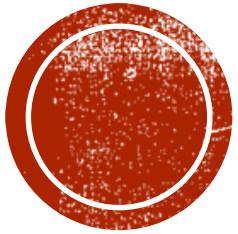


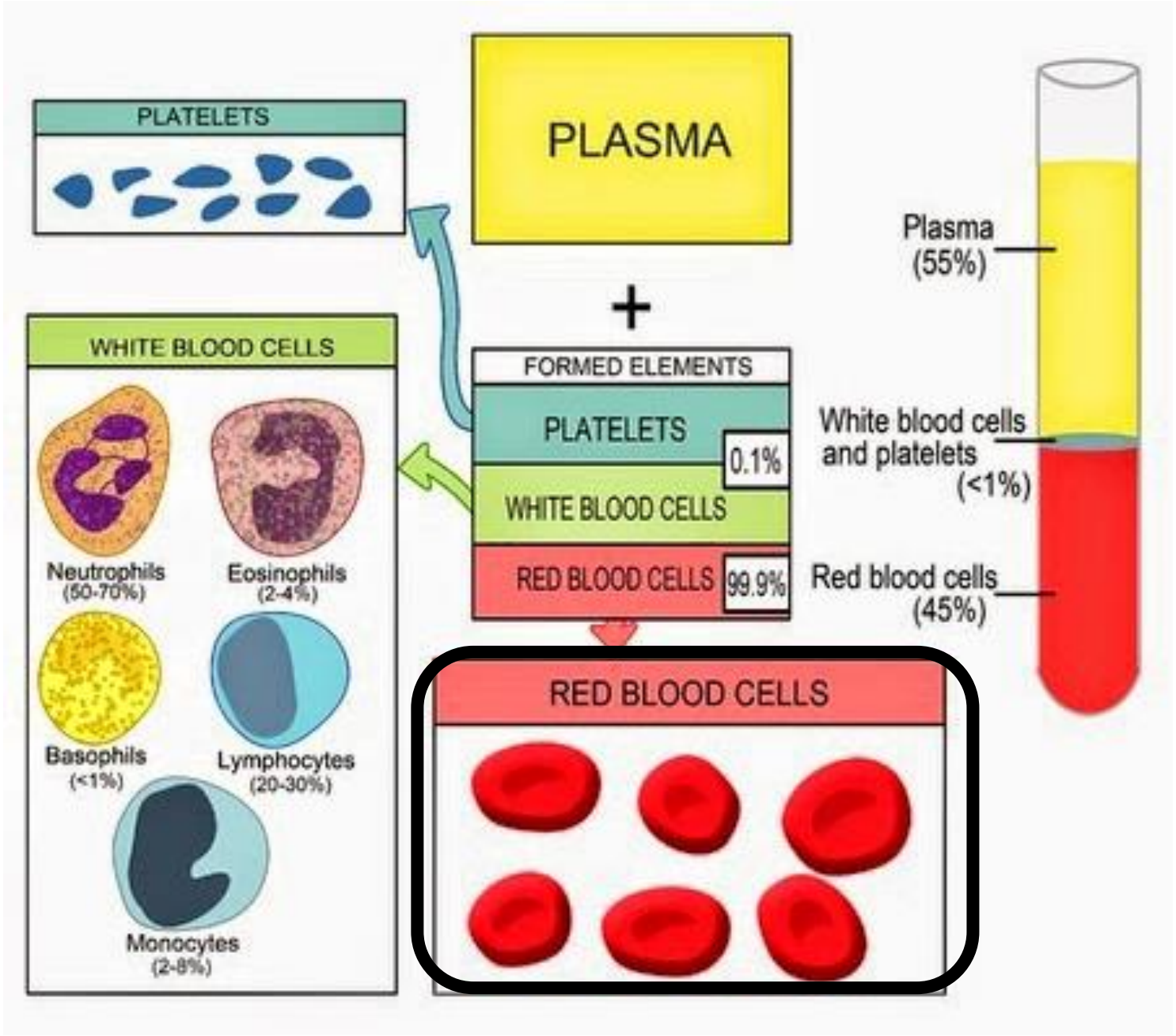
Diagnostic Approach to Anemia

Ben Heyman, MD
Associate Clinical Professor of Medicine
Medical Director Koman Outpatient Pavilion Sanford Stem Cell Clinical Center
UCSD Moores Cancer Center

LECTURE GOALS

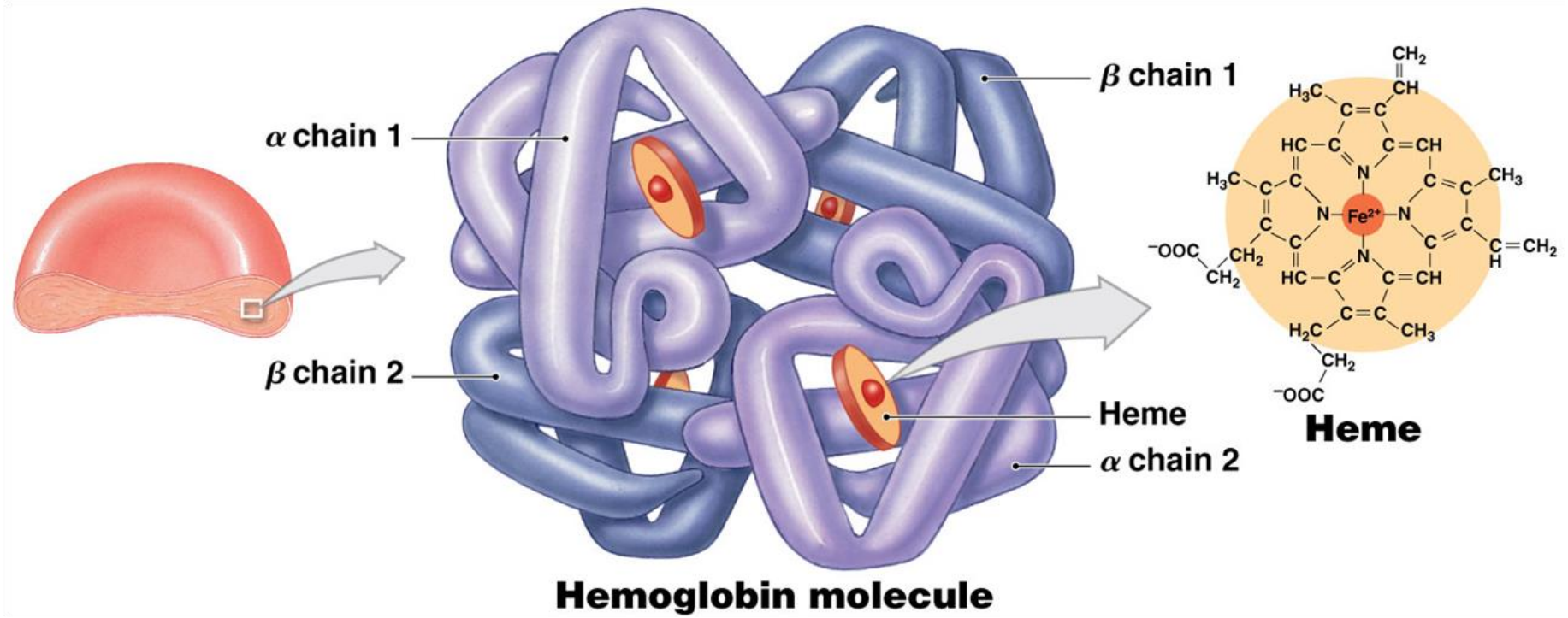
- Review basics of blood and red blood cells.
- Review Classification of Anemias
- Review Work-up of Anemia
- Review management of Anemia

THE ELEMENTS OF BLOOD



https://www.123rf.com/photo_18143356_the-elements-of-blood-useful-for-education-in-schools-and-clinics.html

THE ELEMENTS OF RED BLOOD CELLS (RBCS)

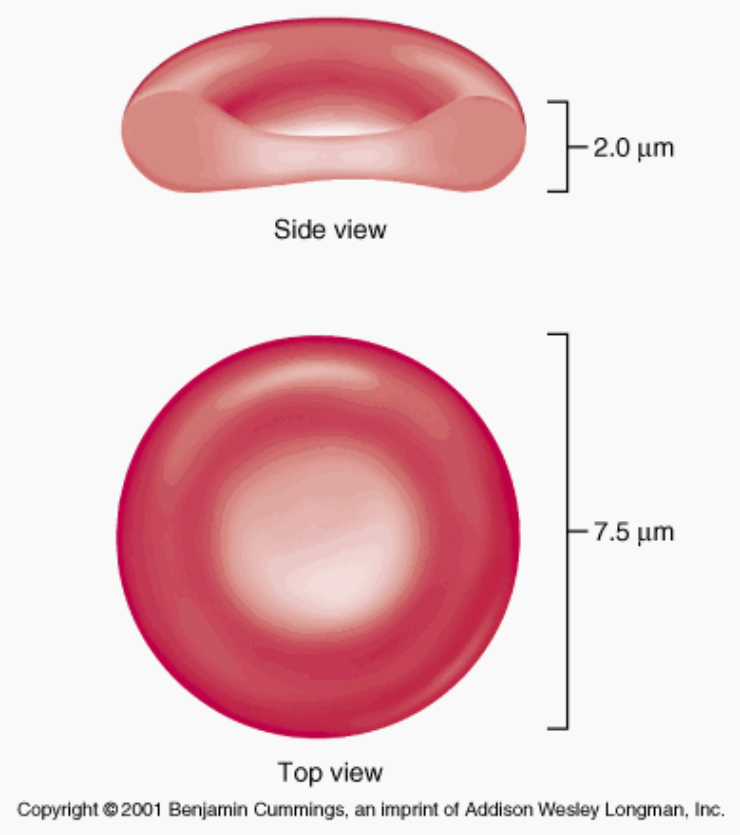


About 250 MILLION hemoglobin molecules make up each RBC!!

4 heme molecules are in 1 hemoglobin molecule

http://www.namrata.co/category/hemoglobin-and-hemoglobinopathies/structure-of-normal-hemoglobin/

BASICS OF RBCS

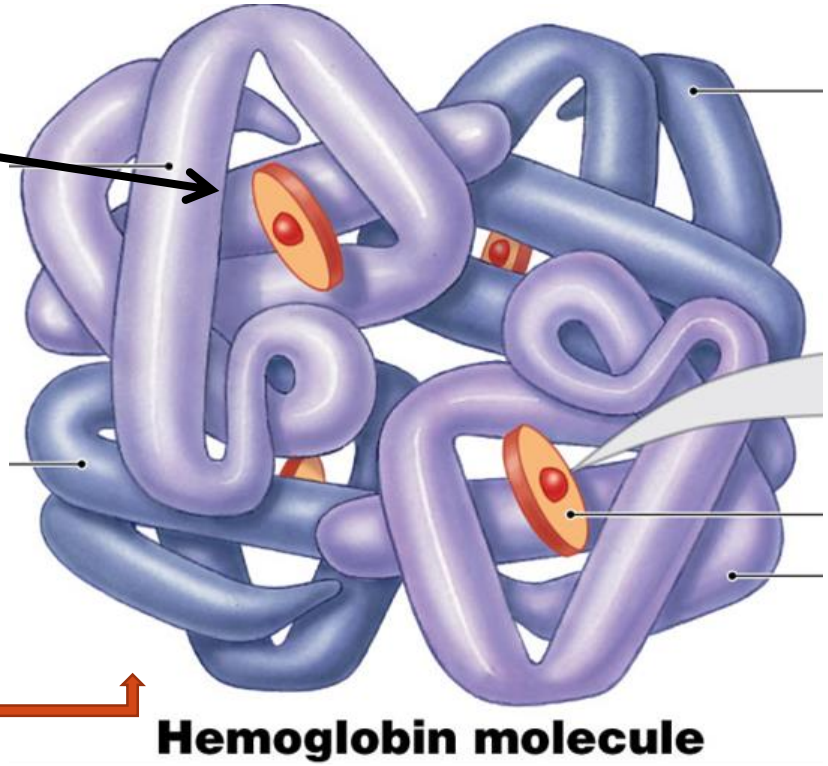


- Biconcave, disc-shaped
  - Flexible membrane
  - High surface-to-volume ratio → bends through the smallest vessels
  - Maximum surface area to transport oxygen and carbon dioxide
- Anuclear
  - Loses the nucleus before leaving the marrow
  - (never "normal" to have a RBC with a nucleus in the blood!)

GLOBIN + HEME = HEMOGLOBIN (HB)

Hemoglobin

- Heme
- Globin (4 chains)
  - Alpha (2 chains)
  - Beta * (2 chains)



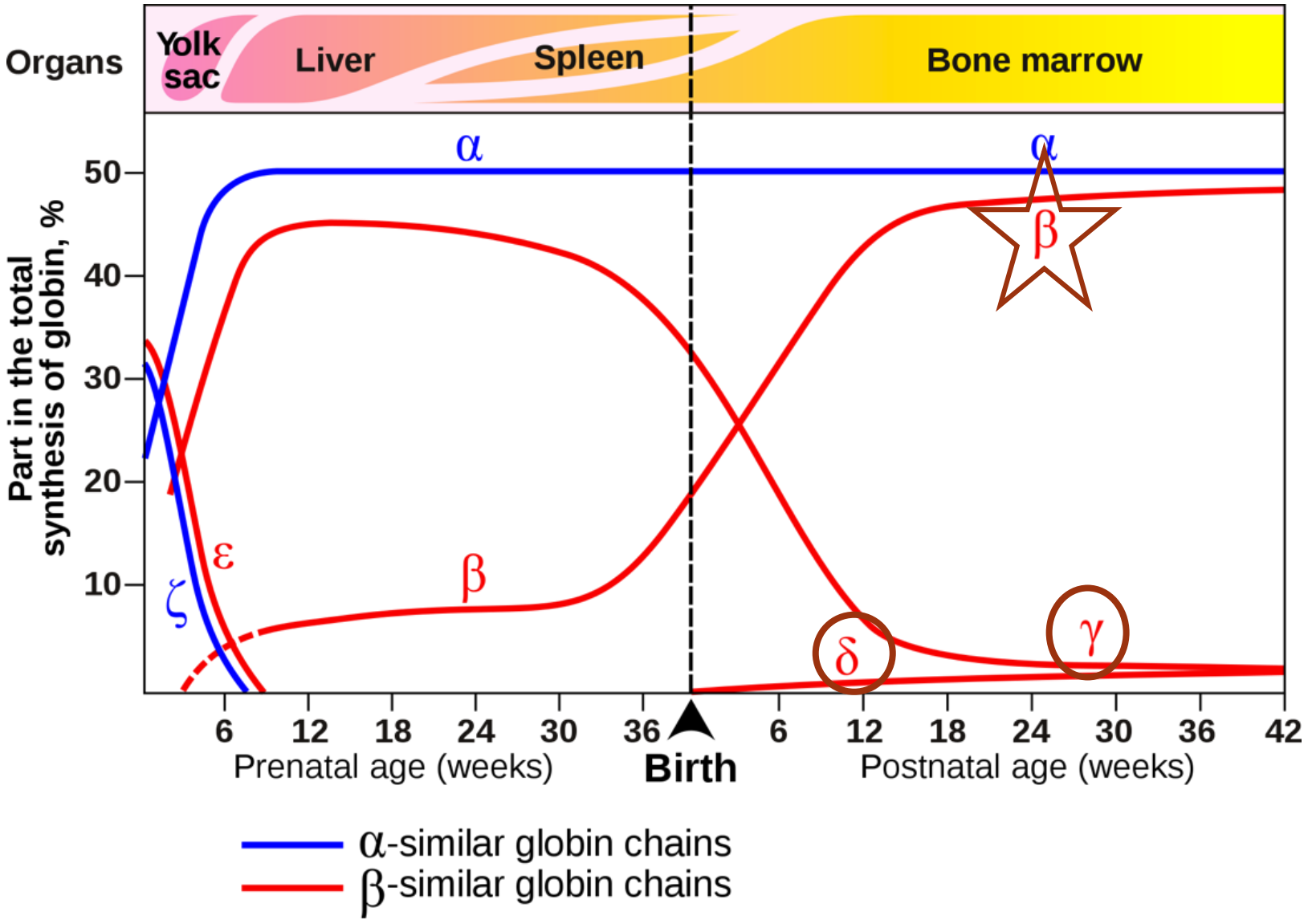
* The main partner in adult Hb

ADULT HB

Hb	Globin components	Normal Percentages
A	$\alpha\alpha\beta\beta$	~96%
A2	$\alpha\alpha\delta\delta$	<3%
F	$\alpha\alpha\gamma\gamma$	<1%
Total		100%

* Changes to these percentages (or new types of Hb) tell you there may be a hemoglobinopathy

GLOBIN CHAINS

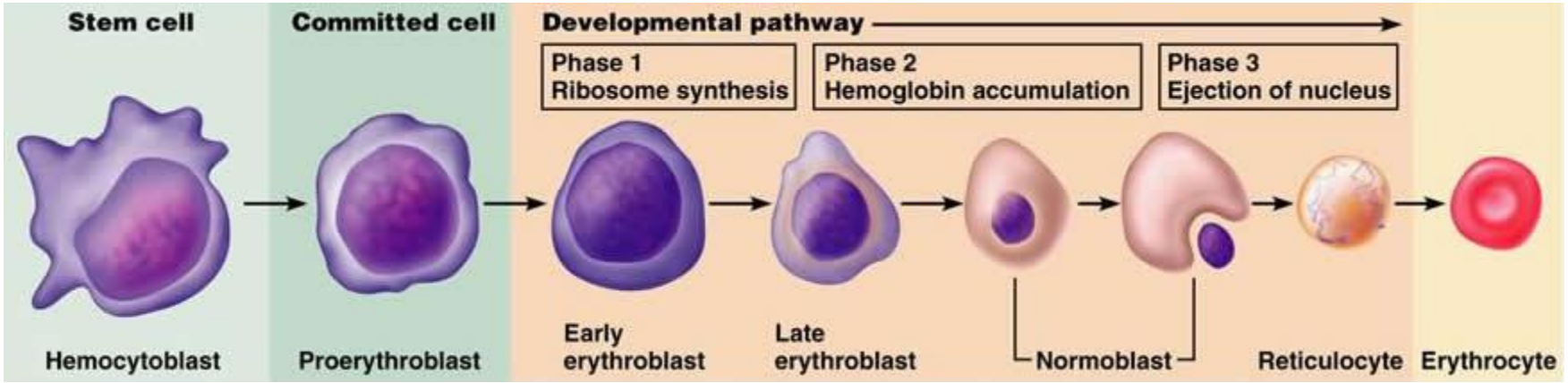


https://en.wikipedia.org/wiki/Hemoglobin

NORMAL RBC PRODUCTION

- Production of RBCs = Erythrocytosis

- Made in the bone marrow as an adult

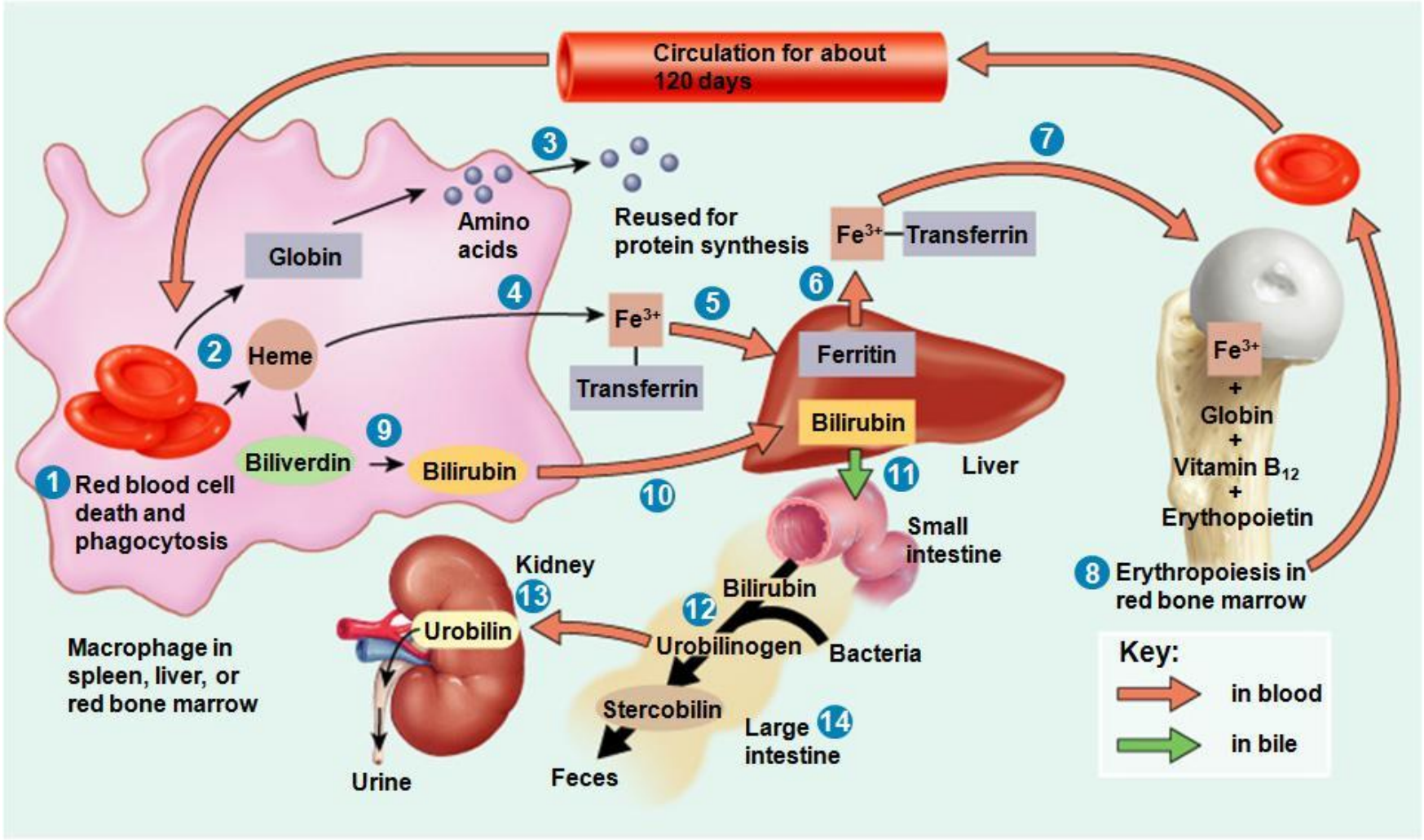


All occurs in the bone marrow

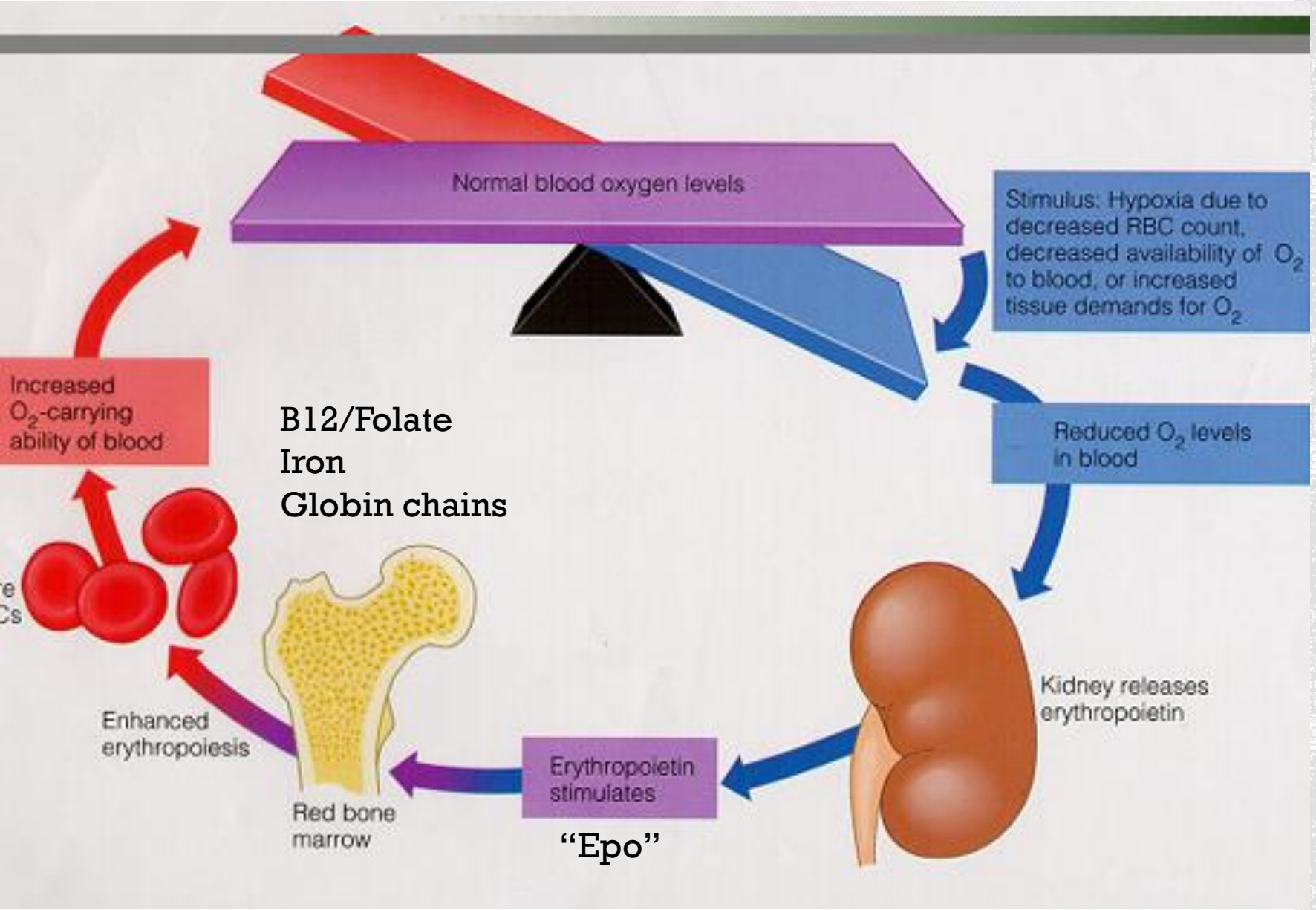
The only cells that should be in the peripheral blood

http://www.interactive-biology.com/3969/erythropoiesis-formation-of-red-blood-cells/

RBC LIFE CYCLE



http://spaces.imperial.edu/thomas.morrell/cha_13_tortora_blood.htm



B12/Folate
Iron
Globin chains

"Epo"

NORMAL CYCLE OF RBC PRODUCTION

RBC NEEDS:

Iron

Globin

B12

Folate

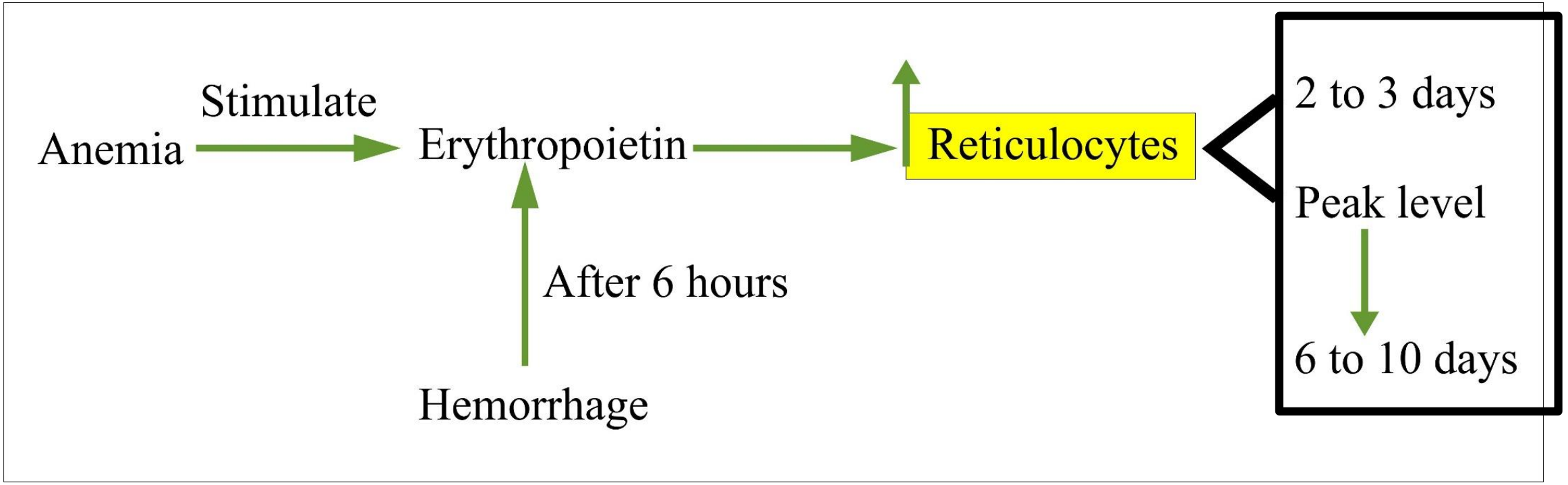
Erythropoietin (EPO)

Working marrow

Right environment

All reasons for anemia…

RETICULOCYTOSIS

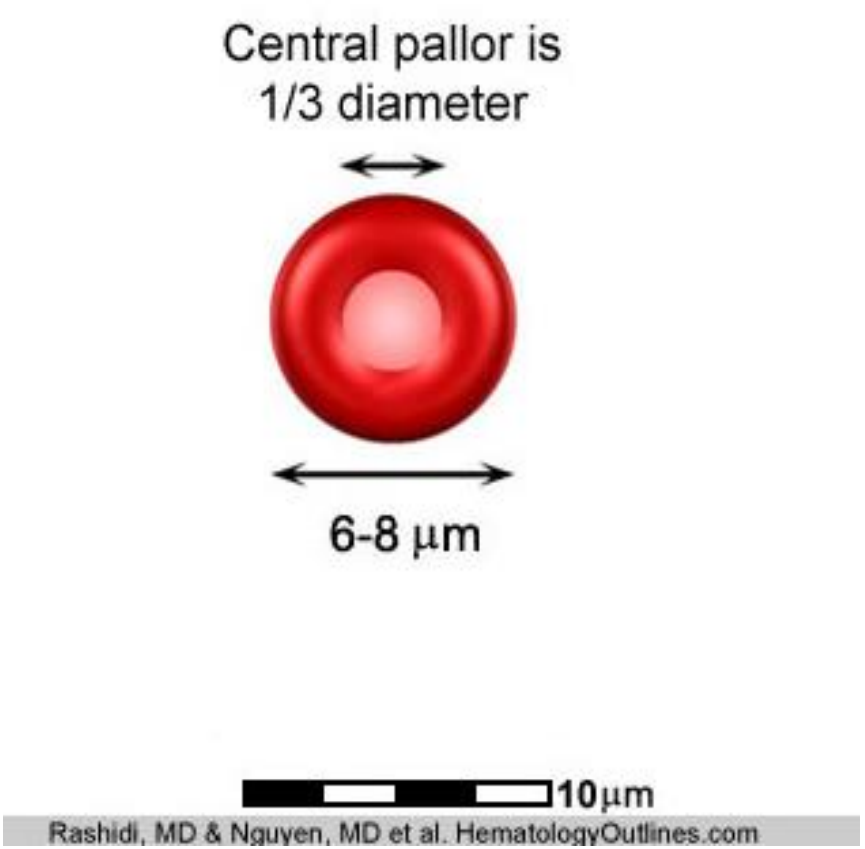


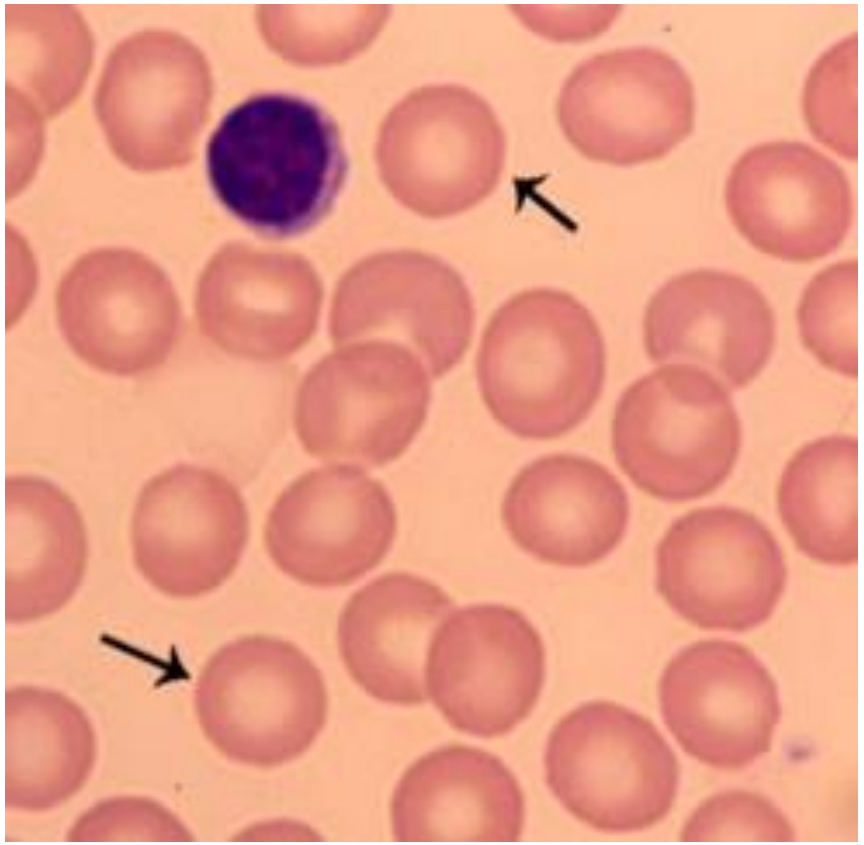
http://www.labpedia.net/test/130

RED BLOOD CELL INDICES

Term	Definition	Normal value
Hgb	Concentration of Hgb	12.3 - 15.3 g/dL 14 - 17.4 g/dL
Hct	% of RBCs, by volume, in whole blood	38-46% 42-54%
Mean corpuscular volume (MCV)	Average volume of the patient's RBCs	80-100 (femtoliters, fL)
Mean corpuscular hemoglobin (MCH)	Average Hgb content in RBC	26-31 (picograms [pg]/cell)
Red cell distribution width (RDW)	Measure of the variation in RBC size (high = lots of variation)	13-15

NORMAL RBC

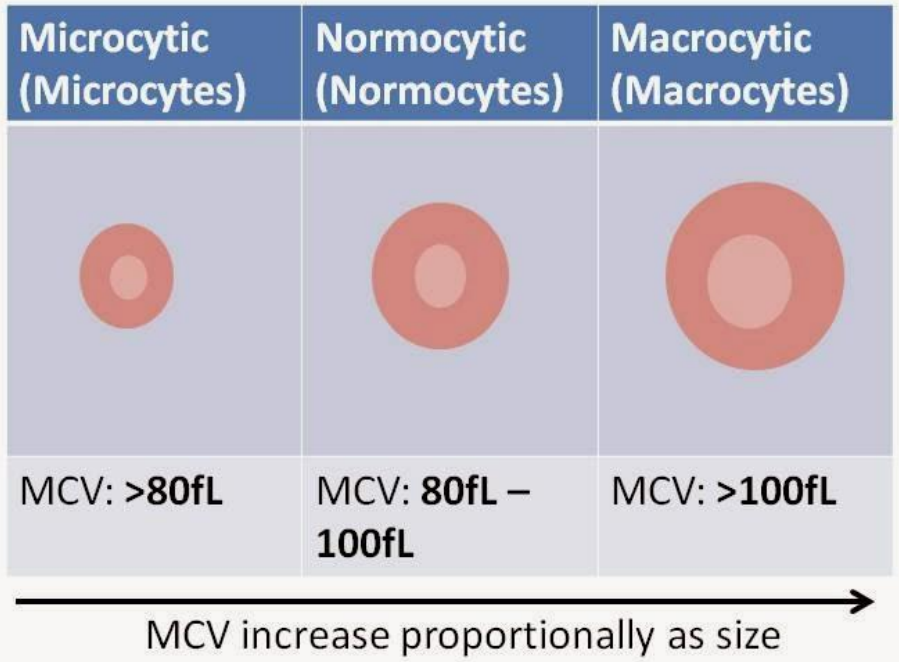




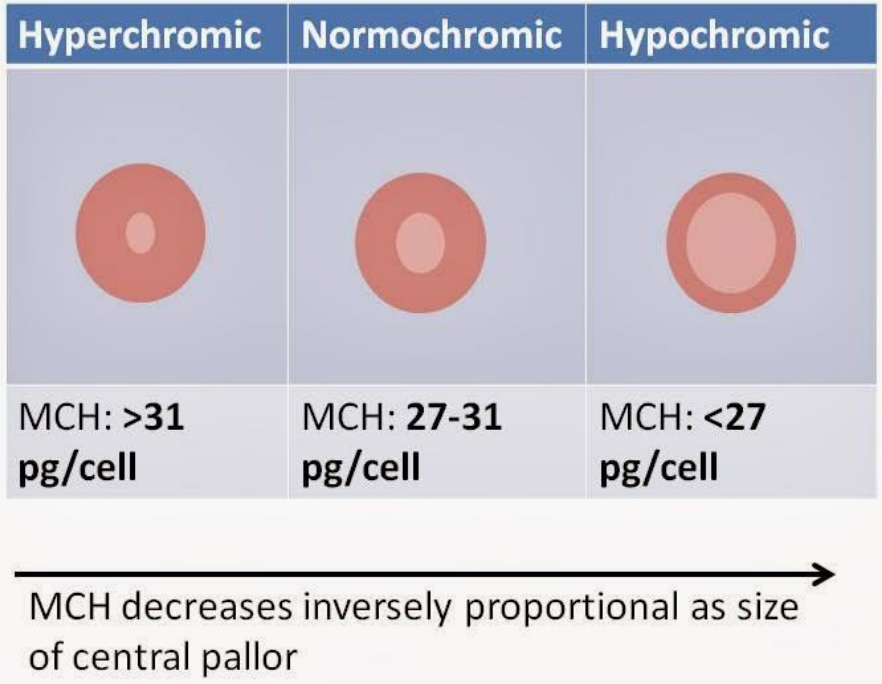
These RBCs would have a NORMAL MCV, NORMAL MCH, NORMAL RDW

RED BLOOD CELL INDICES

MCV

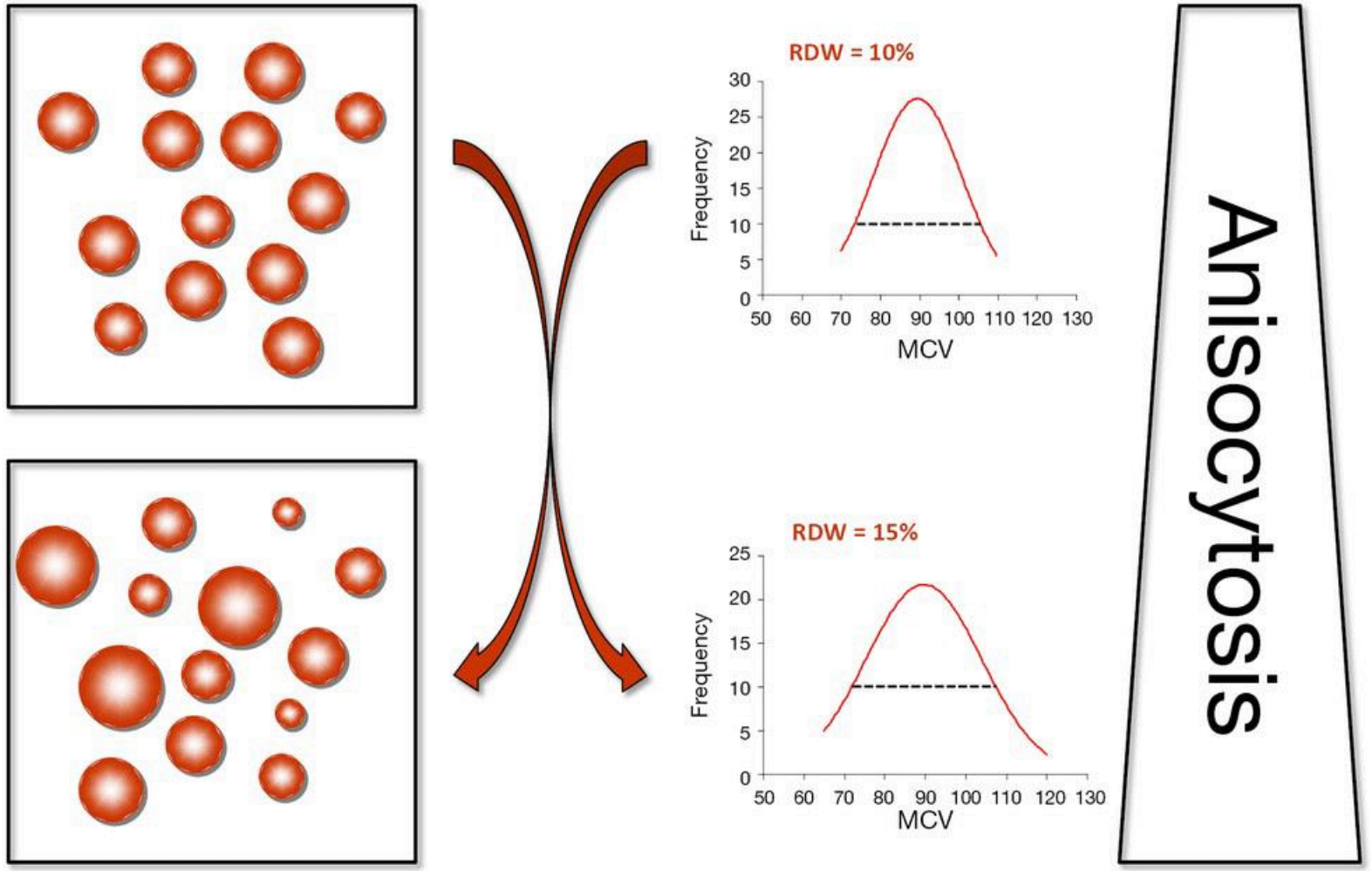


MCH



http://textbookhaematology4medical-scientist.blogspot.com/2014/03/red-cell-indices.html

RDW



http://jtd.amegroups.com/article/view/5455/html

HOW TO APPROACH ANEMIA

- TAKE A GOOD HISTORY AND PHYSICAL!!
  - Age
  - Sex
  - History
  - Symptoms
  - Medical and family history including ethnicity
  - Medications
  - Physical exam

Anemia is not a disease, but rather the expression of an underlying disorder or disease.

- Labs
  - CBC+differential (with previous values to compare)
  - Red blood cell indices, specifically MCV
  - Reticulocytes
  - LFTs, chemistry panel
  - Peripheral smear
  - Signs of destruction of RBCs

- Other labs and interventions only after coming up with a short differential based on the history, physical and labs

DEFINITION OF ANEMIA

	Anemic Hgb (g/dL)	Normal Hgb (g/dL)
Women	<12	12.3 - 15.3
Men	<13*	14 - 17.4

Hgb = grams of Hgb per 100mL of whole blood
Hct = % RBCs in whole blood (spun down)
*Based on WHO, though other textbooks will say <14

SYMPTOMS OF ANEMIA

- **Non-specific!!!**
- **Asymptomatic if mild anemia**
- Fatigue
- Shortness of breath
- Dizziness or lightheadedness
- Syncope
- Palpitations
- Chest pain
- Headaches

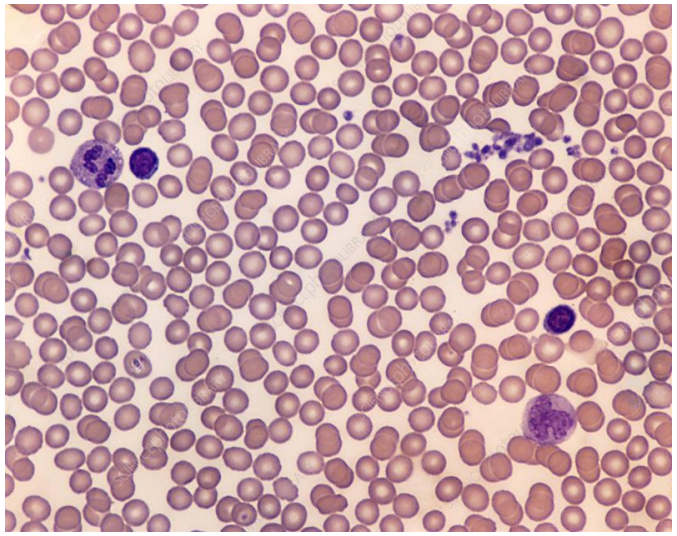
WORK UP OF ANEMIA

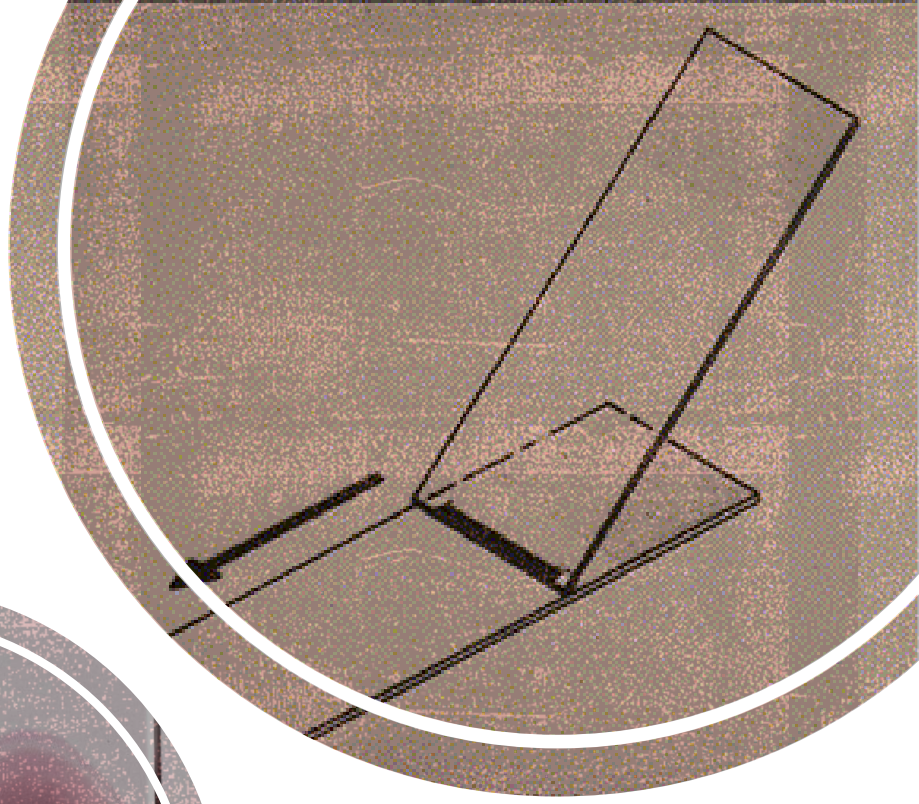
- Basic Lab Work Up
- Complete blood count (CBC) and differential
  - Hgb, Hct, platelets, WBC with differential
  - Differential means the percentages of different WBCs (neutrophils, lymphocytes, monocytes, eosinophils, basophils)
  - Red blood cell indices
- Reticulocyte count or percentage
- Peripheral smear
- Chemistry and liver function tests

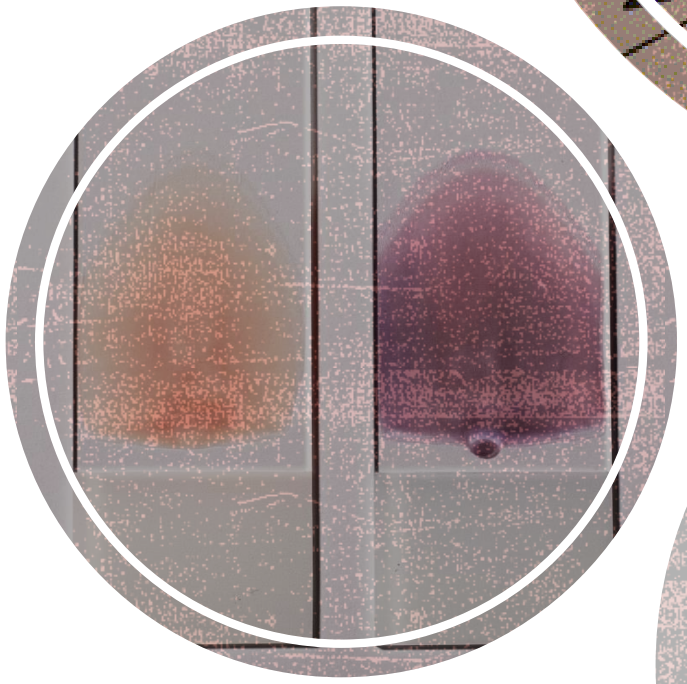
- **Based on the clinical history and the above counts, more may be ordered**

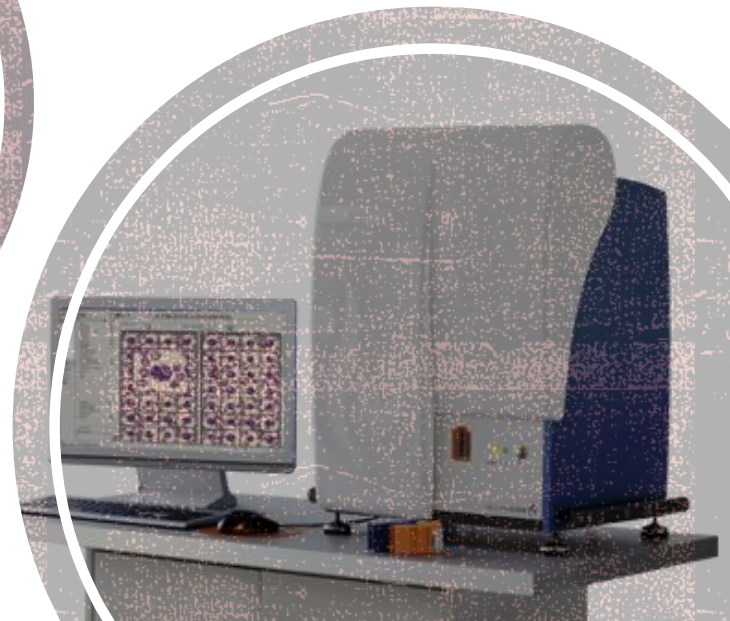
PERIPHERAL SMEAR

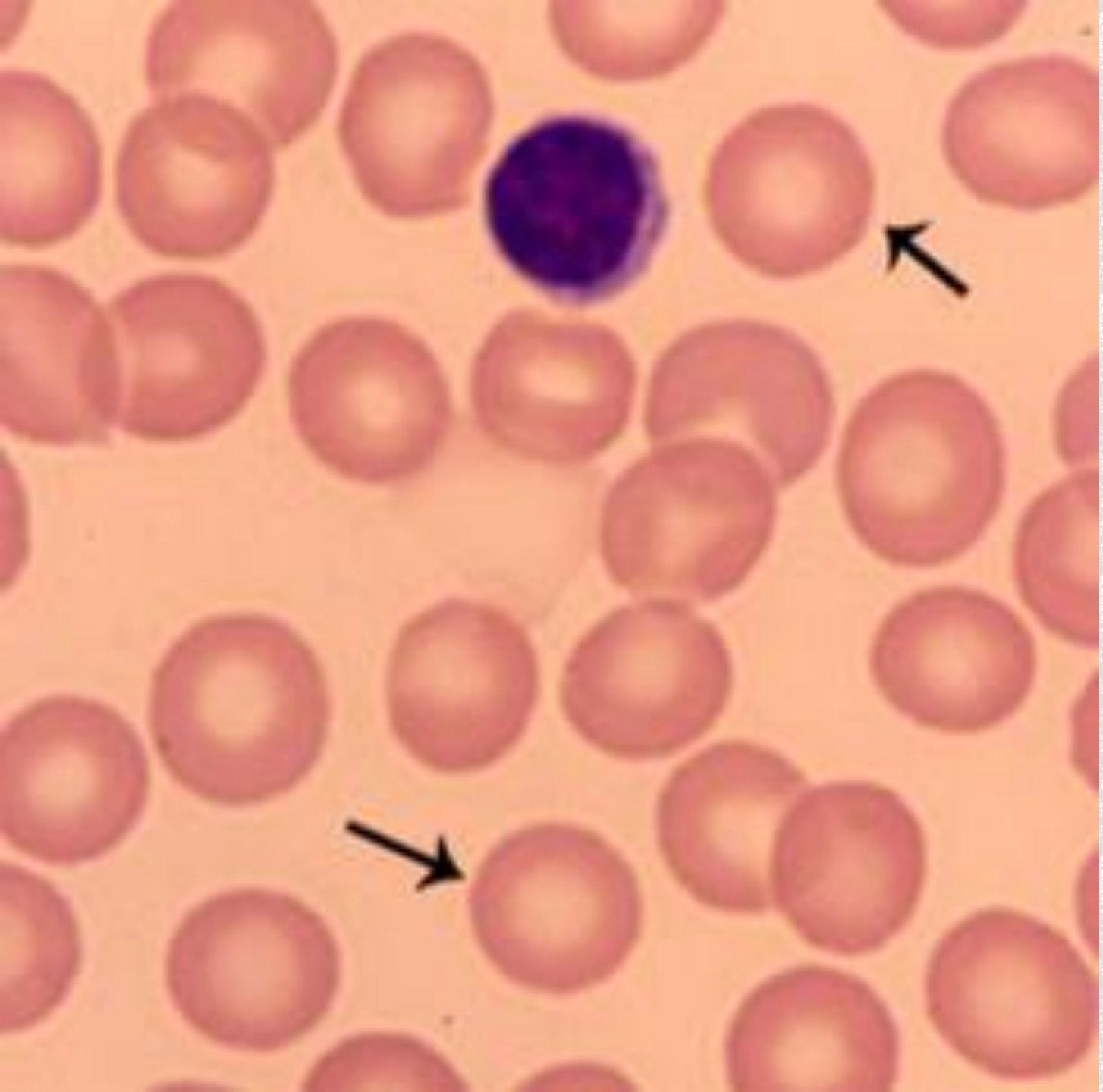
The secret to success!





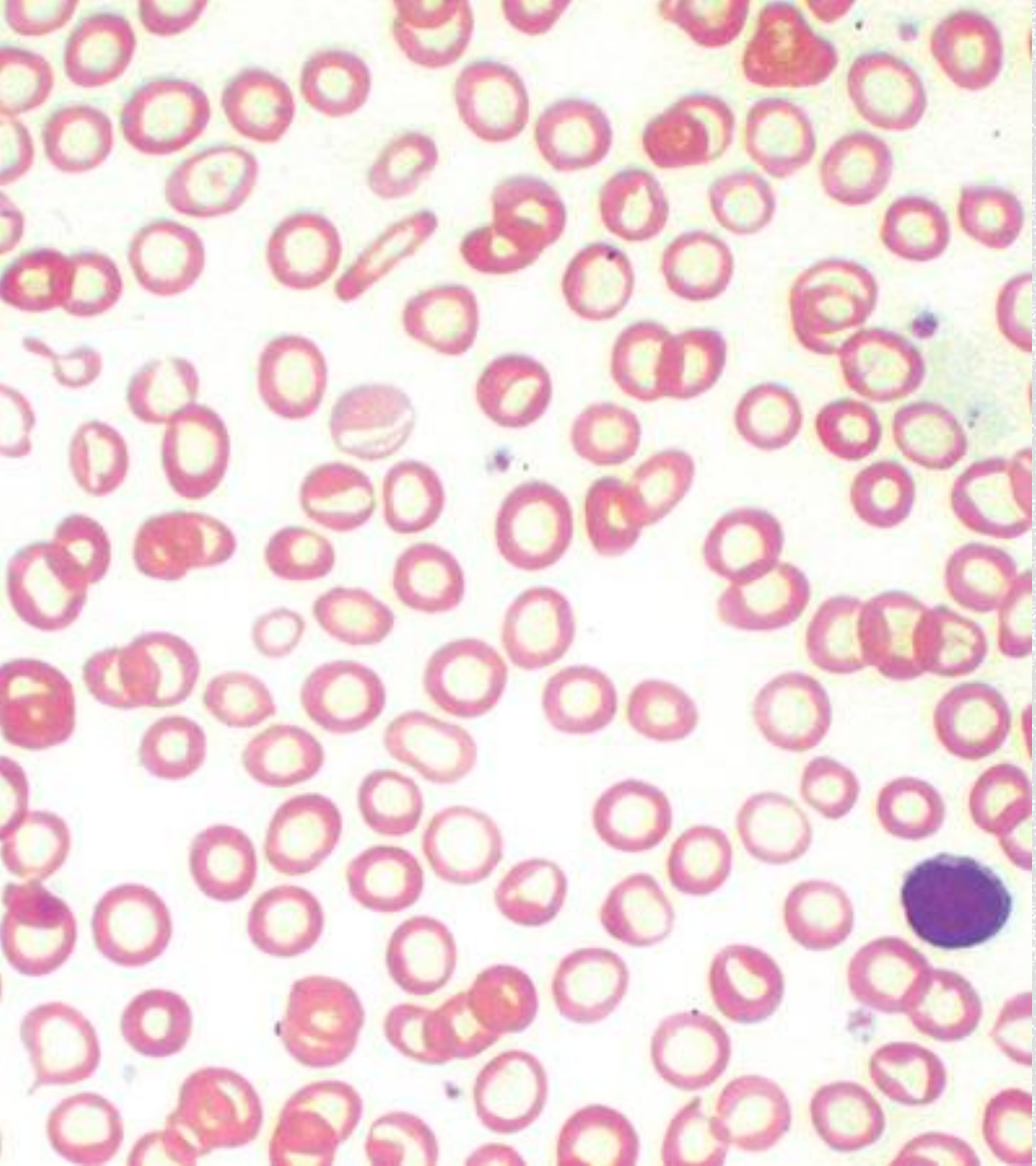






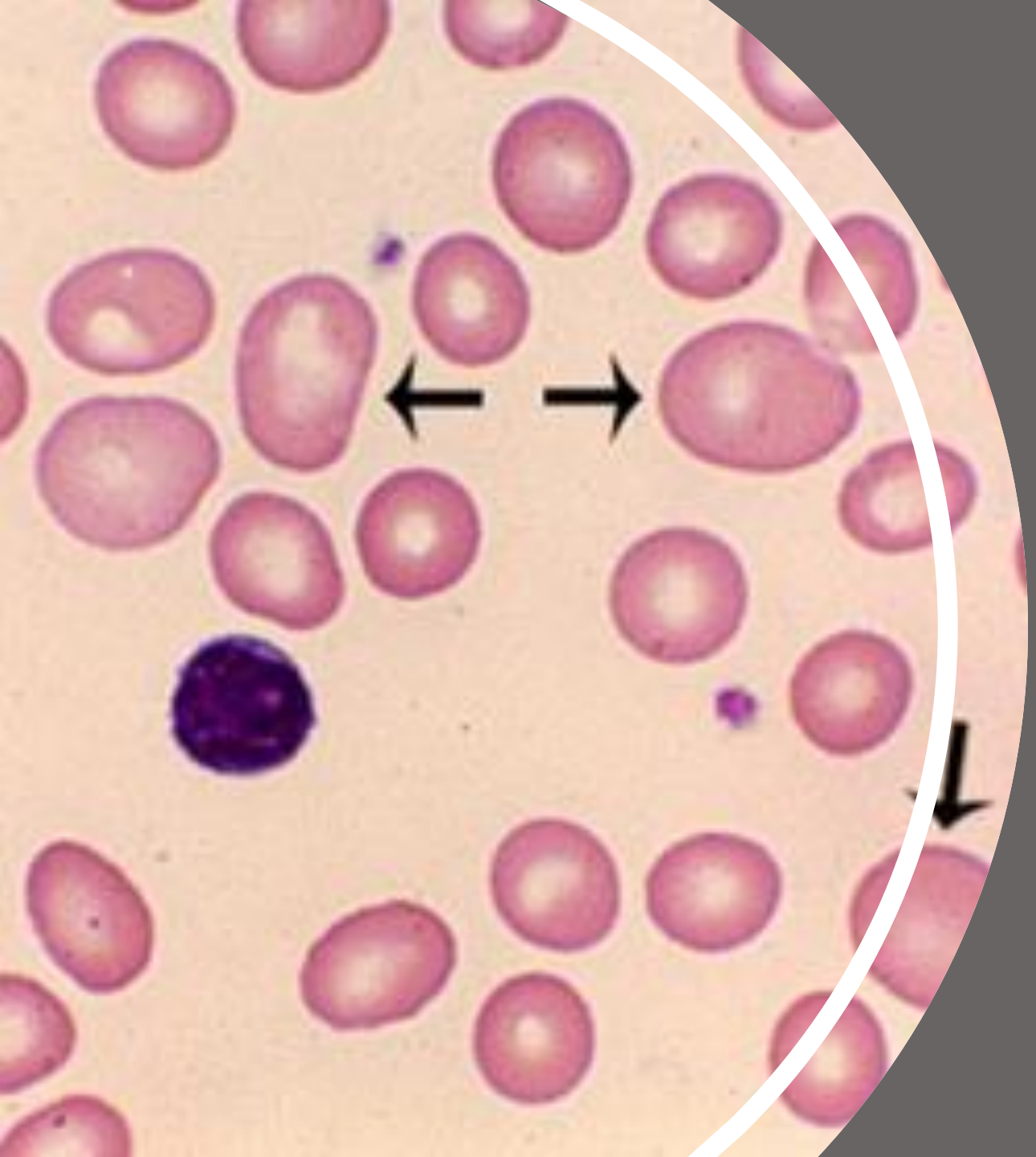
RED BLOOD CELL SIZE

Size of RBCs: Size of a small typical lymphocyte nucleus



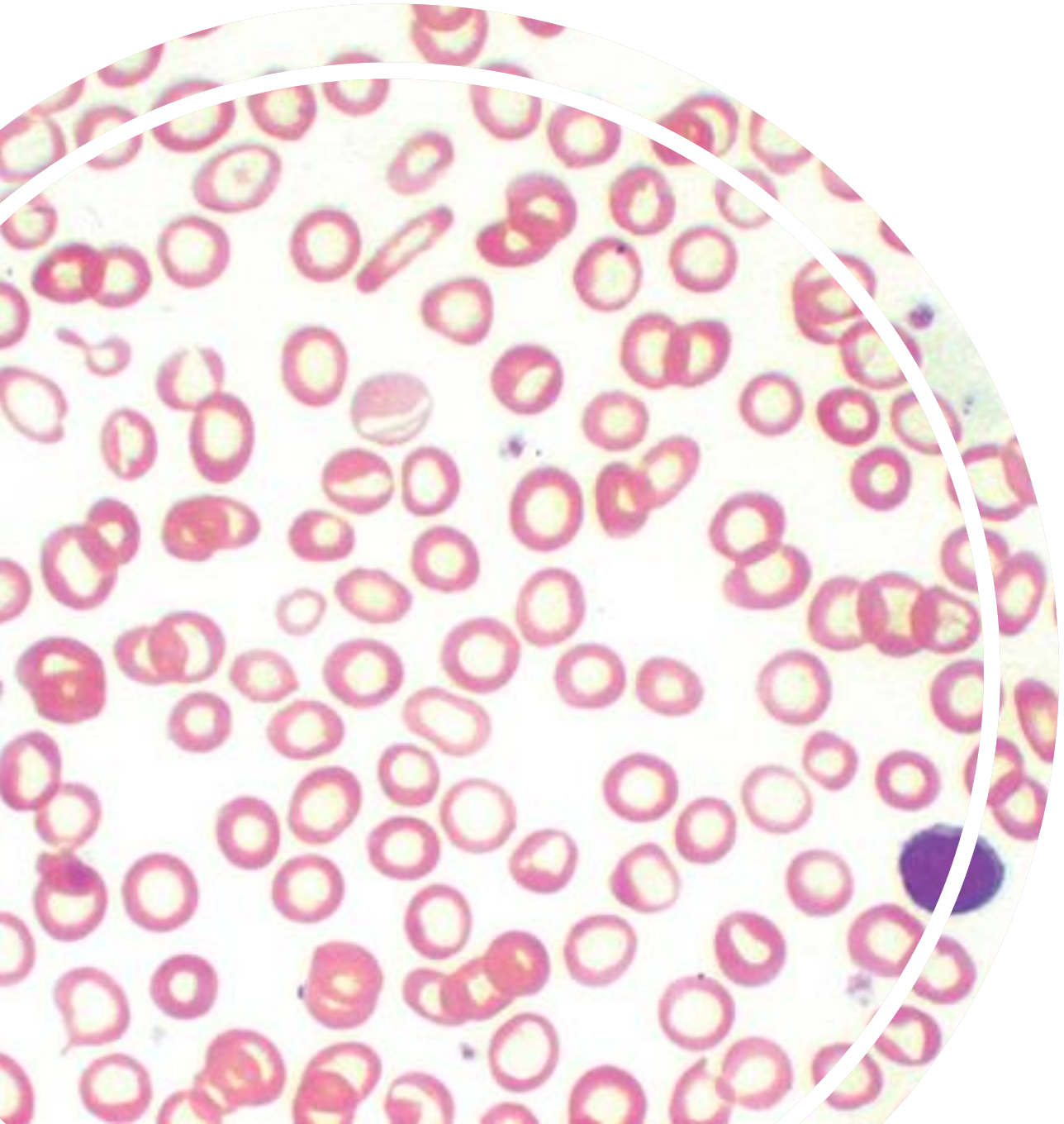
MICROCYTIC ANEMIA

- RBC size smaller than small lymphocytes



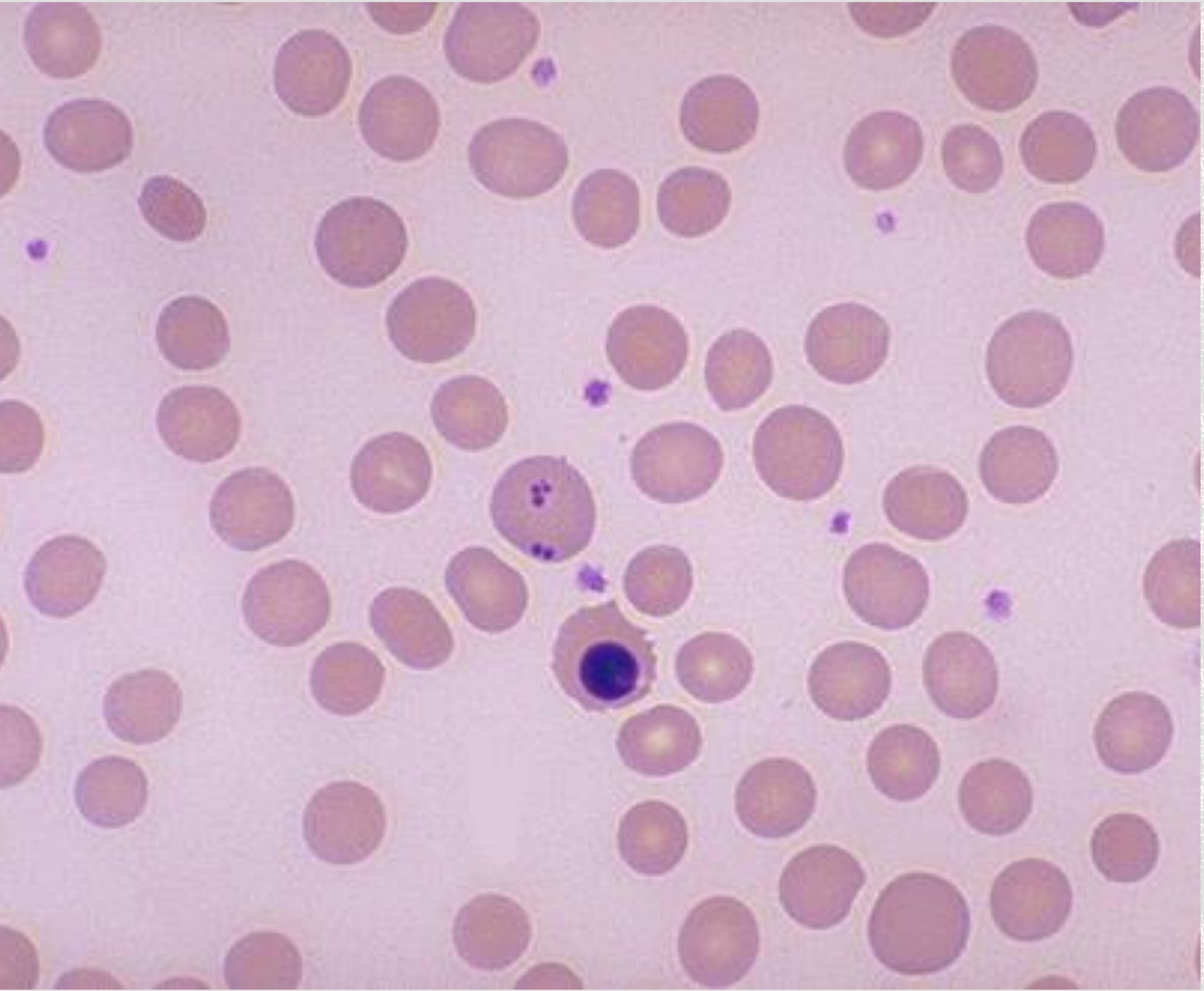
MACROCYTIC ANEMIA

- RBC size larger than small lymphocytes



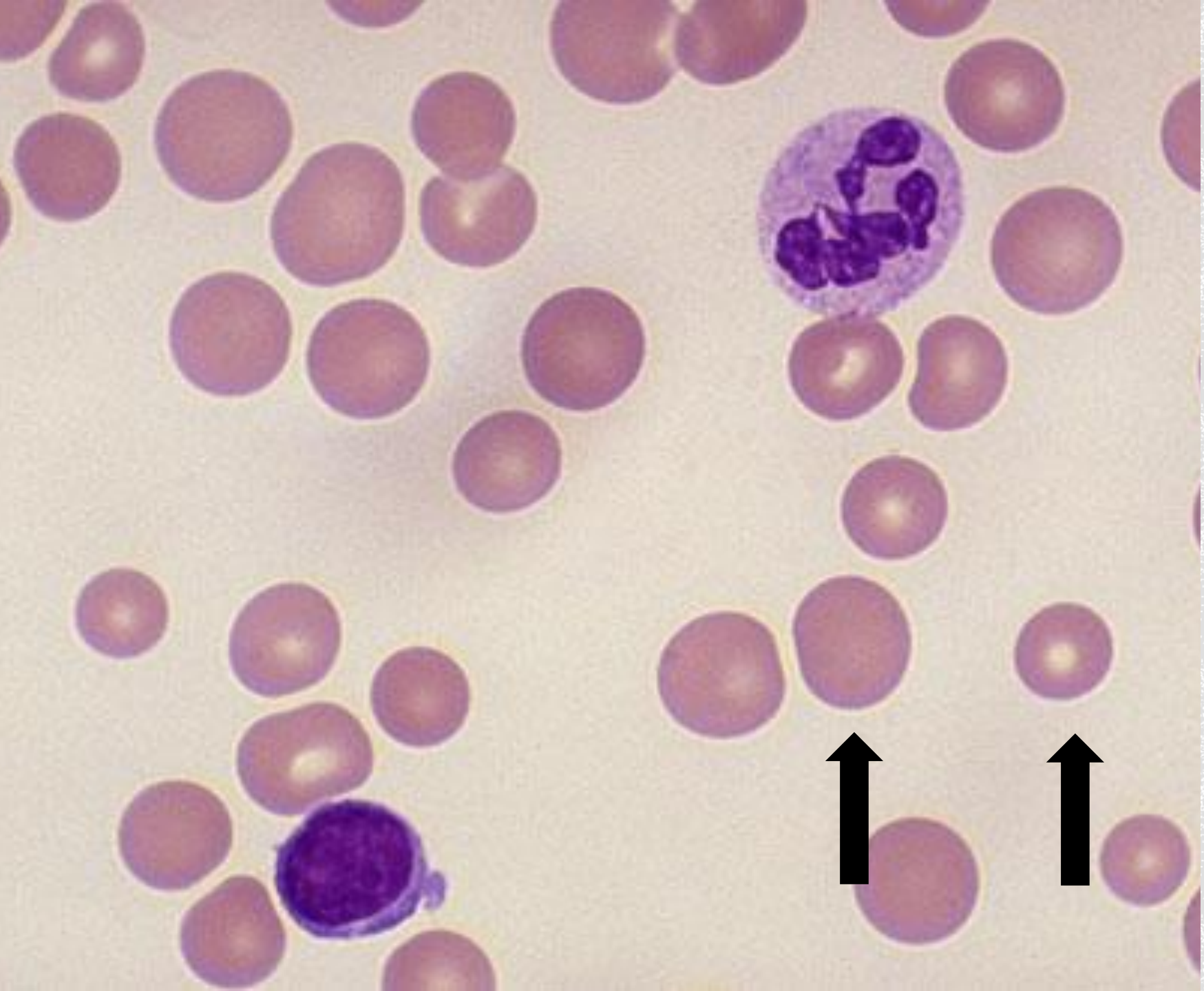
HYPOCHROMIC

- Pallor is > 1/3 of the diameter of the RBCs



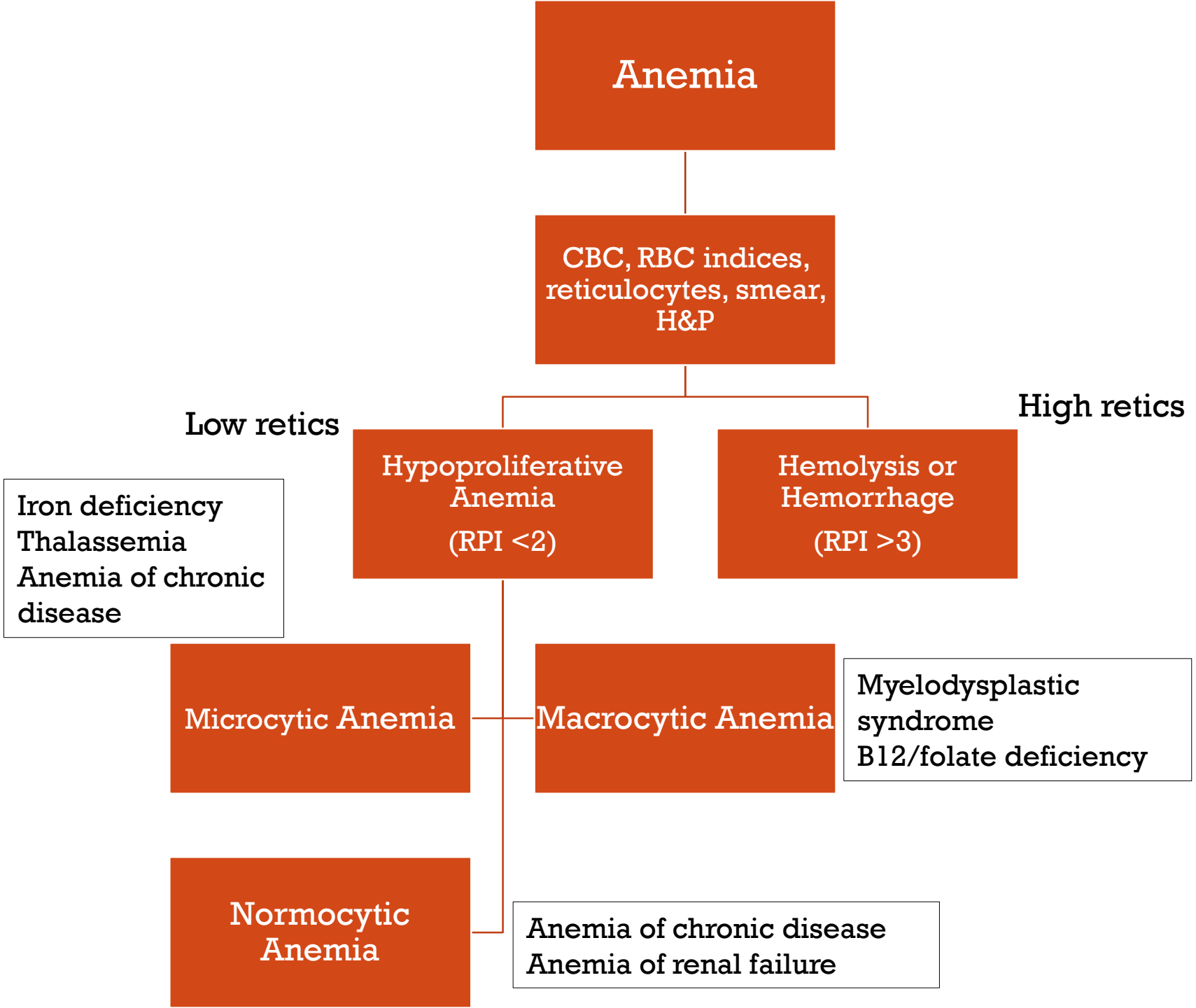
HYPERCHROMIC

- Pallor is < 1/3 diameter of the RBC



INCREASED RDW

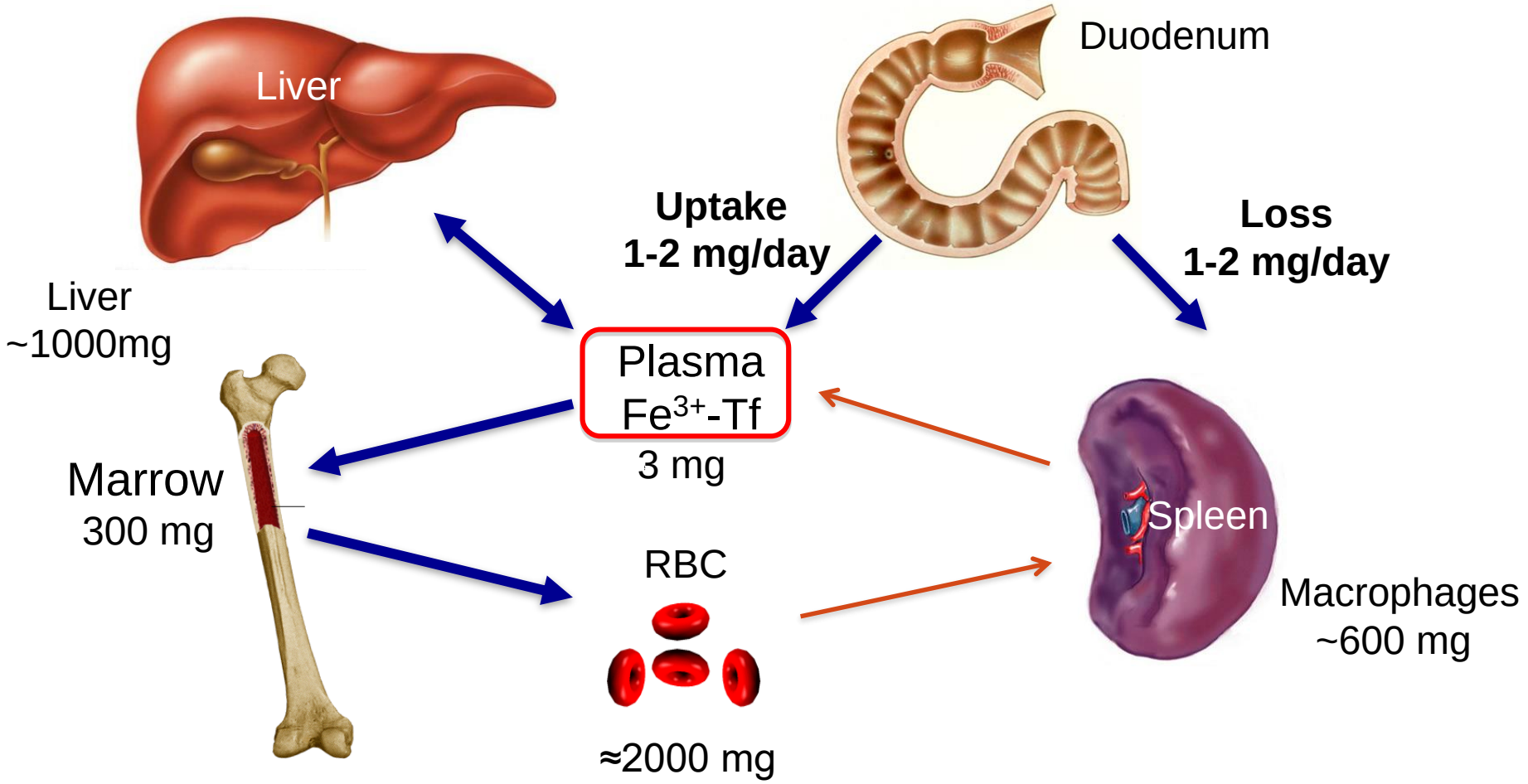
Increased variation is the size of the RBC diameter



APPROACH TO ANEMIA

Anemia is not a disease, but rather the expression of an underlying disorder or disease.

IRON STORAGE



IRON DEFICIENCY ANEMIA

- Iron is needed for heme, and so iron deficiency anemia leads to low Hgb production
- Most common anemia in U.S.
- Etiologies – most of the time, you have to lose blood!
  - GI losses (colon cancer, gastritis, diverticulosis)
  - Bleeding elsewhere (retroperitoneal)
  - Menstruation
  - Malabsorption (gastric bypass, Celiac disease, H. pylori)
  - Decreased intake: a LONG time… and hard to do since our foods are fortified
- Symptoms
  - Typical symptoms of anemia
  - Pica
  - Angular cheilitis
  - Restless legs

IRON DEFICIENCY ANEMIA

- Work up
  - CBC with RBC indices
    - Microcytic, hypochromic anemia
    - Thrombocytosis
  - Ferritin
  - Iron studies (TIBC, iron sat, iron level)
  - Soluble transferrin receptor (sTfR); sometimes sTfR/ferritin ratio
  - Work up for blood loss: MAINLY GI WORK UP!!

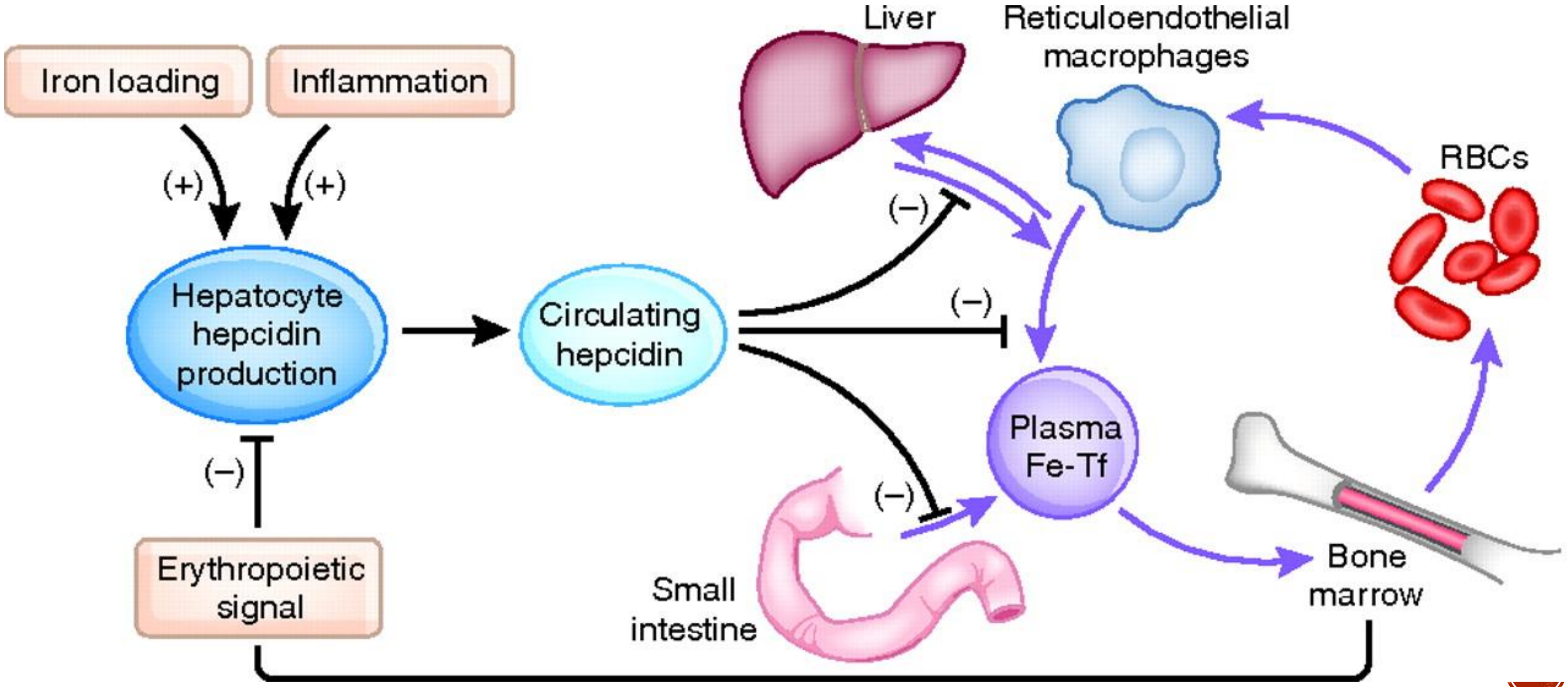
IRON DEFICIENCY ANEMIA

Lab	Use in iron deficiency anemia	Levels
MCV	Size of RBC	↓
Retics	Immature RBCs, used to assess marrow response to anemia	↓
Ferritin	Reflection of iron storage CAVEAT: acute phase reactant	↓
Iron level	Measure of iron in the blood CAVEAT: can reflect your last meal	↓
TIBC (Transferrin iron-binding capacity)	Measures the blood's capacity to bind iron with transferrin	↑
Transferrin saturation = iron saturation	Serum iron divided by the total iron-binding capacity; how much serum iron is bound	↓
Soluble transferrin receptor (sTfR)	Proteolytic cleaved extracellular domain of the transferrin receptor, released when there is iron deficiency CAVEAT: can be used as a (sTfR/ferritin ratio)	↑

IRON DEFICIENCY ANEMIA

- Treatment: Iron replacement
  - Oral:
    - Key is to take with *acidic* drinks/foods such as orange juice, Vitamin C since there is better absorption
    - Side effects are GI (GI upset, constipation)
  - Intravenous: For those with:
    - Poor compliance
    - Poor tolerance
    - Poor absorption (Crohn's, Celiac)
    - Need it more urgently
    - Ease

ANEMIA OF CHRONIC DISEASE/INFLAMMATION



http://cjasn.asnjournals.org/content/4/8/1384/F1.expansion

ANEMIA OF CHRONIC DISEASE/INFLAMMATION

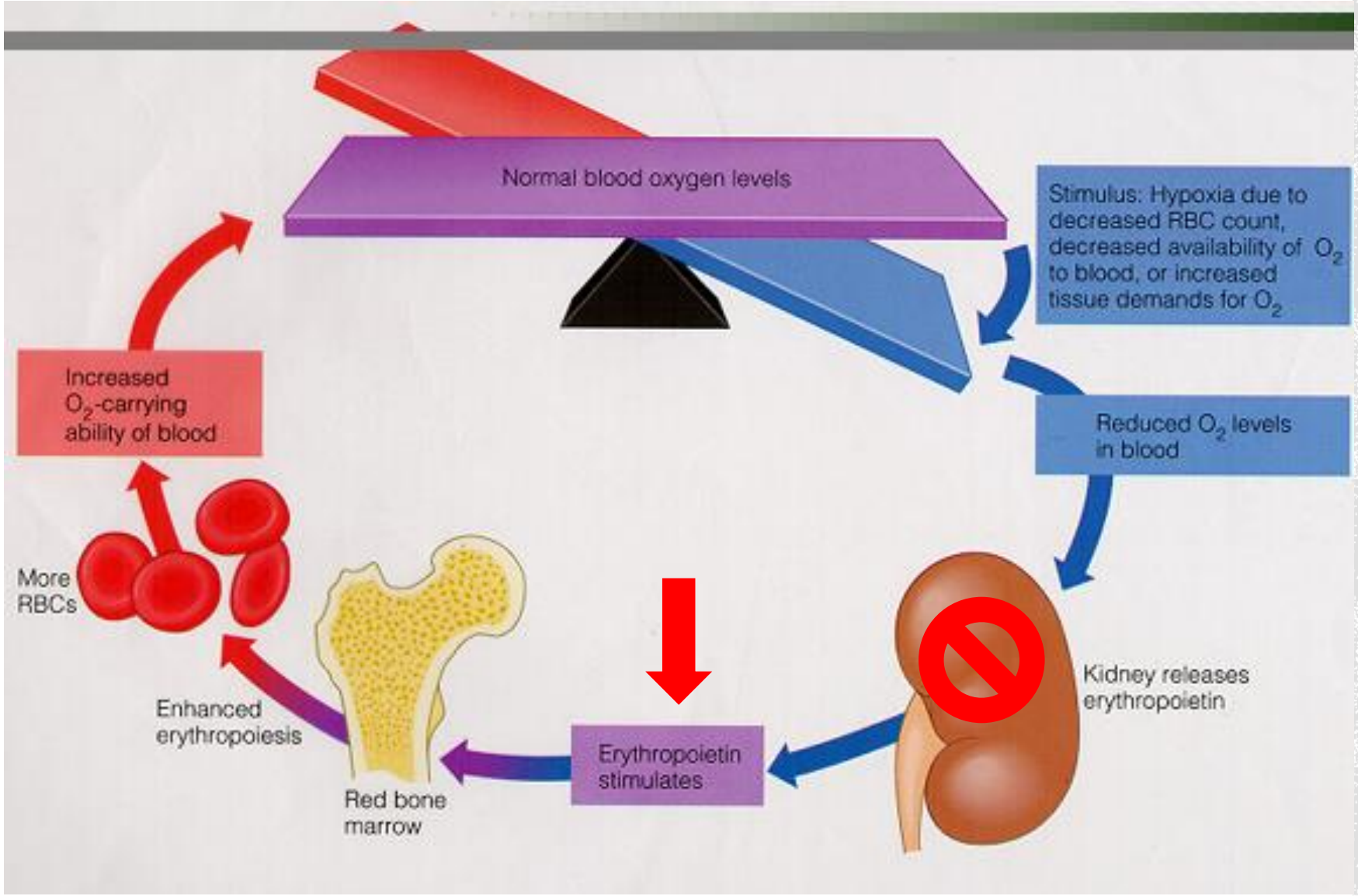
- Decreased RBC production:
  - Hepcidin induced reduction in PLASMA iron (but NOT total iron)
    - So sensed "like" iron deficiency anemia but total iron up
  - Inability to respond appropriately to erythropoietin
  - Decreased erythropoietin production

- Etiologies
  - Age
  - Chronic infection
    - Osteomyelitis, endocarditis, cellulitis
    - HIV
  - Malignancy
  - Collagen vascular diseases
    - Rheumatoid arthritis
  - Diabetes

ANEMIA OF CHRONIC DISEASE/INFLAMMATION

Lab	Anemia of Chronic Disease	Iron deficiency anemia
Hgb	↓	↓
MCV	Normal/↓	↓
Ferritin	↑	↓
Iron level	↓	↓
TIBC (Transferrin iron-binding capacity)	↓/Normal	↑
Transferrin saturation = iron saturation	↓	↓
sTfR/ferritin ratio	Normal	↑
Hepcidin (cannot be measured)	↑	↓

Anemia due to Renal Failure: Erythropoietin Deficiency



Renal failure → decreased production of erythropoietin

All patients on dialysis need replacement of erythropoietin

Labs:

- **Normocytic anemia**
- **Renal failure**
- **Inappropriate erythropoietin levels**

HEMOLYTIC ANEMIA

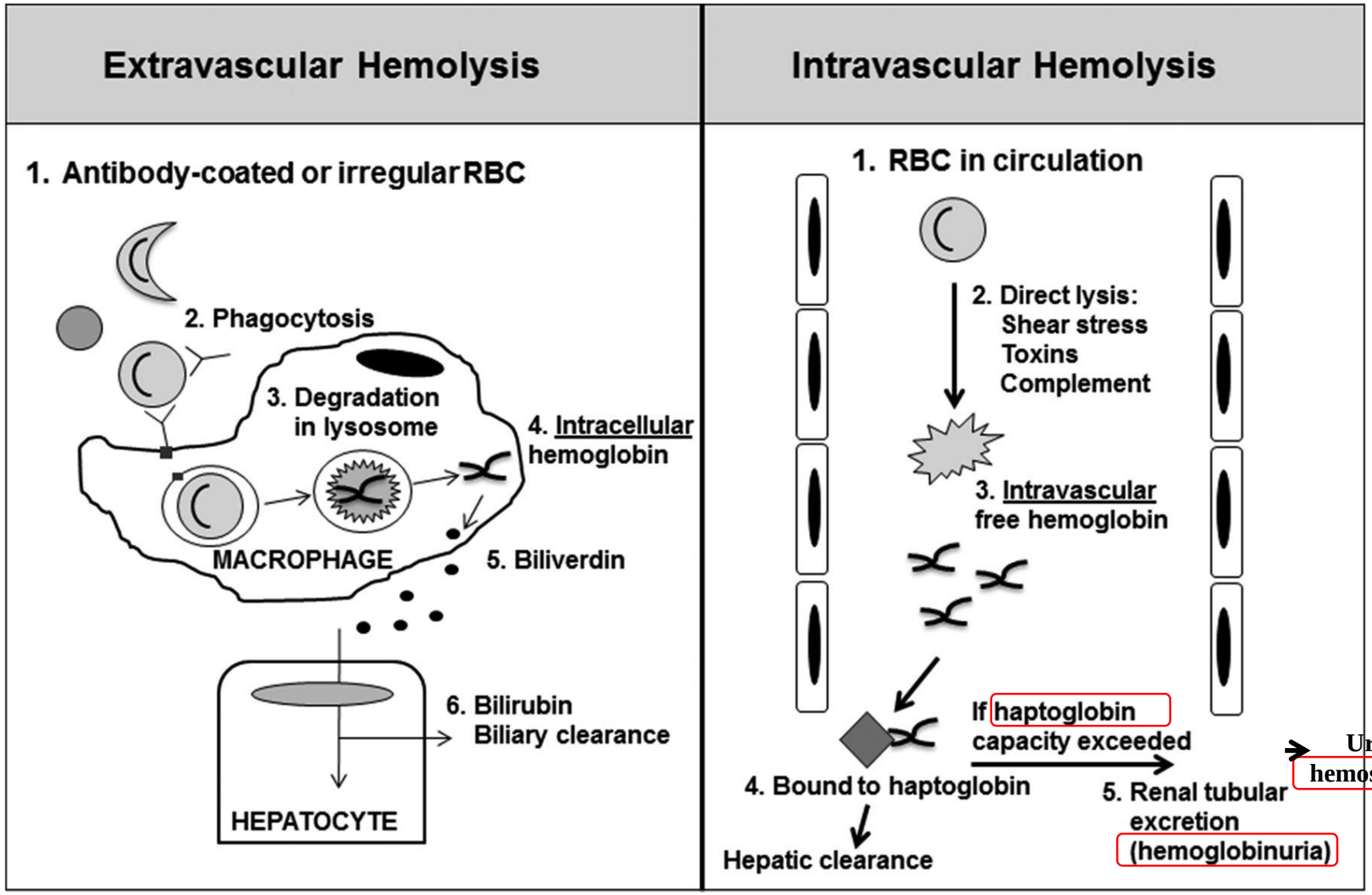
- Hemolysis = shortened RBC survival via destruction of RBC outside of the bone marrow

- General categories of hemolysis (but confusingly, many ways to categorize)

  - Extravascular: hemolysis OUTside of the vascular space; RBCs destroyed within the spleen or liver

  - Intravascular: hemolysis INside of the vascular space; RBCs destroyed within the circulation/blood vessels

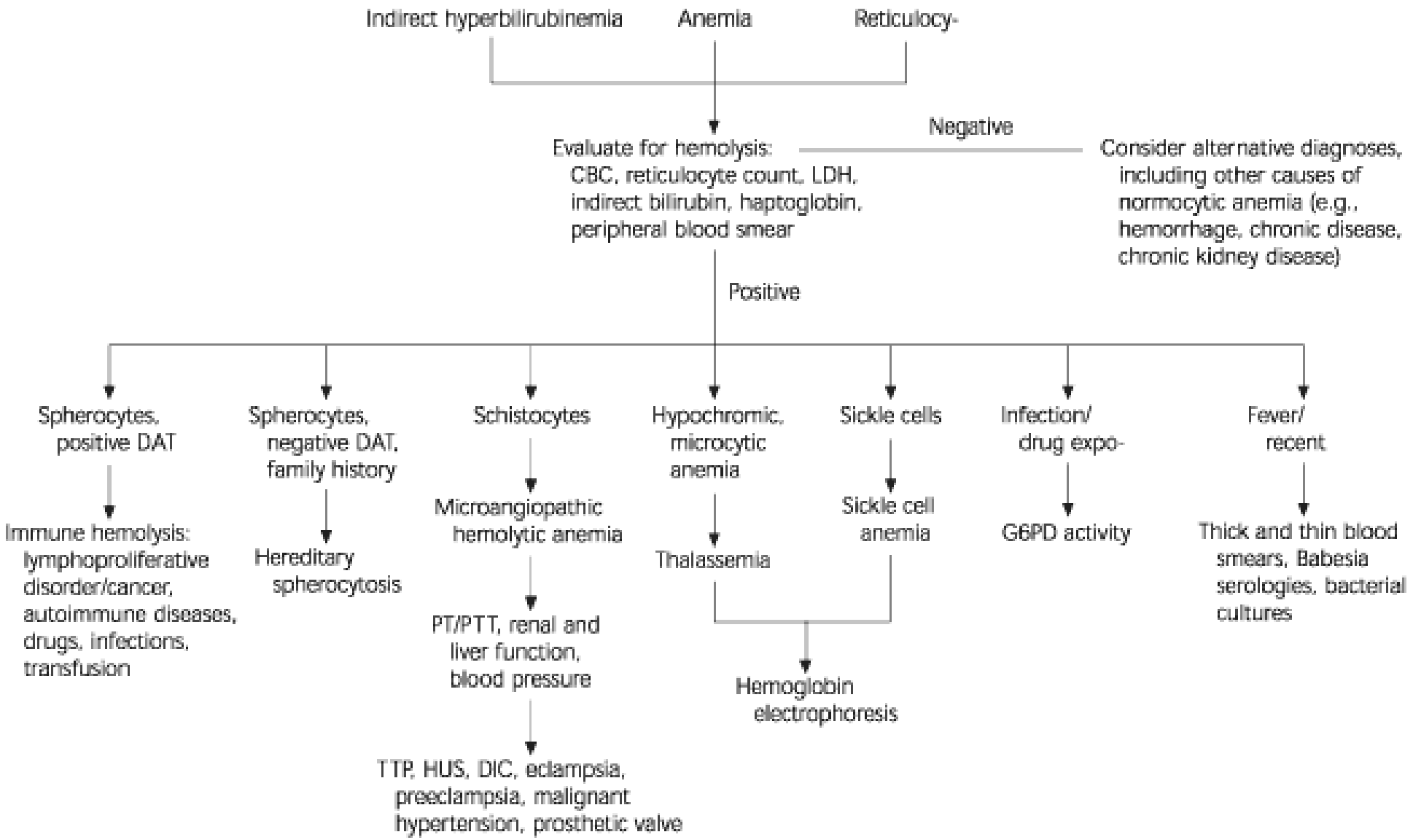
Toxins
Autoimmune
Hereditary spherocytosis
Infections
Hypersplenism



Sickle cell
MAHA
G6PD deficiency
Mechanical stress

(OTHER) CLASSIFICATION OF HEMOLYTIC ANEMIAS

	Intrinsic to the RBC	Extrinsic to the RBC
Hereditary/ Congenital	-Hemoglobinopathies -Enzymopathies -Membrane-Cytoskeletal Defects -Hereditary Spherocytosis	
Acquired	-Paroxysmal nocturnal hemoglobinuria (via complement system)	-Immune (autoimmune, drug-related) -Toxins -Infections -Mechanical -Hypersplenism


Indirect hyperbilirubinemia
Anemia
Reticulocy-
Evaluate for hemolysis: CBC, reticulocyte count, LDH, indirect bilirubin, haptoglobin, peripheral blood smear
Negative
Consider alternative diagnoses, including other causes of normocytic anemia (e.g., hemorrhage, chronic disease, chronic kidney disease)
Positive
Spherocytes, positive DAT
Immune hemolysis: lymphoproliferative disorder/cancer, autoimmune diseases, drugs, infections, transfusion
Spherocytes, negative DAT, family history
Hereditary spherocytosis
Schistocytes
Microangiopathic hemolytic anemia
PT/PTT, renal and liver function, blood pressure
TTP, HUS, DIC, eclampsia, preeclampsia, malignant hypertension, prosthetic valve
Hypochromic, microcytic anemia
Thalassemia
Sickle cells
Sickle cell anemia
Hemoglobin electrophoresis
Infection/ drug expo-
G6PD activity
Fever/ recent
Thick and thin blood smears, Babesia serologies, bacterial cultures

HOW TO DIAGNOSE HEMOLYTIC ANEMIA

- Symptoms/Signs

- Anemia
- Fatigue
- Pale
- Jaundice
- Dark urine
- Gallstones
- Enlarged spleen

- Family history is important in congenital/hereditary hemolytic anemias

WORK UP OF HEMOLYTIC ANEMIA

- **All hemolysis results in:**
  - ↑ **indirect bilirubin**
  - ↑ **lactate dehydrogenase (LDH)**
  - ↑ **reticulocyte count/index (RPI > 3)**
    - Reticulocyte index is critical to differentiate hypoproduction vs. increased destruction

- Caveats:
  - Indirect bilirubin can be elevated in various congenital and acquired disorders of the liver
  - Bilirubin and LDH also elevated with ineffective erythropoiesis (eg Folate/B12 deficiency)

- RBC on the peripheral smear can provides diagnosis

LABS TO EVALUATE HEMOLYSIS

	Intravascular	Extravascular
Reticulocyte Count	↑	↑
LDH	↑	↑
Indirect Bilirubin	↑	↑
Haptoglobin (reflection of hemoglobinemia)	Undetectable	↓
Urine hemosiderin	↑	-
Urine hemoglobin	↑	-
Direct antiglobulin test	-	+
Smear	Variable	Variable

Labs to Evaluate Intravascular Hemolysis

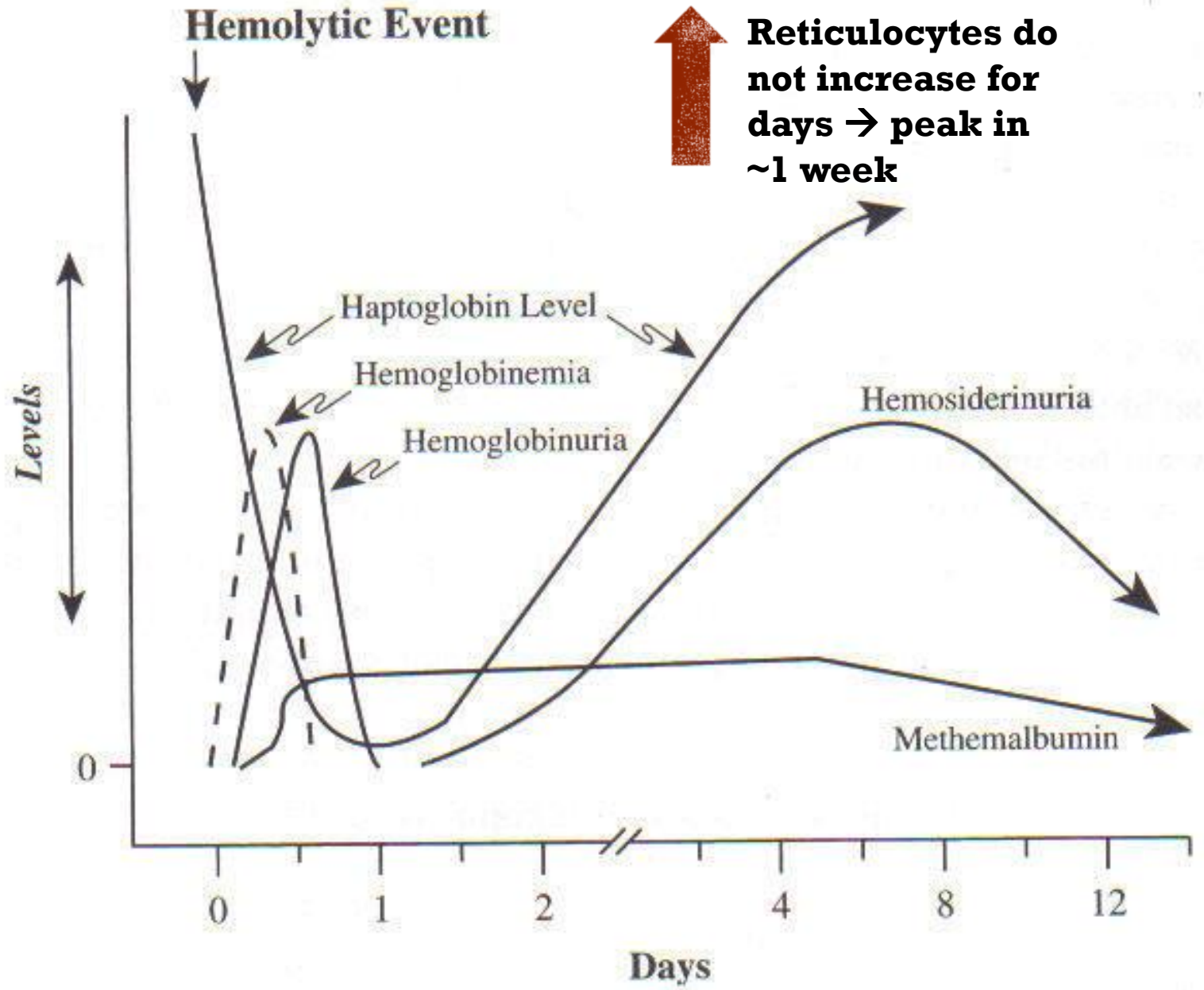


Fig 11.3. Hillman and Ault. Hematology in Clinical Practice 1998.

CONGENITAL/HEREDITARY HEMOLYTIC ANEMIA

- Three general categories
  - Membrane defects
  - Enzymatic defects
  - Hemoglobin defects

CONGENITAL/HEREDITARY MEMBRANE DISORDERS

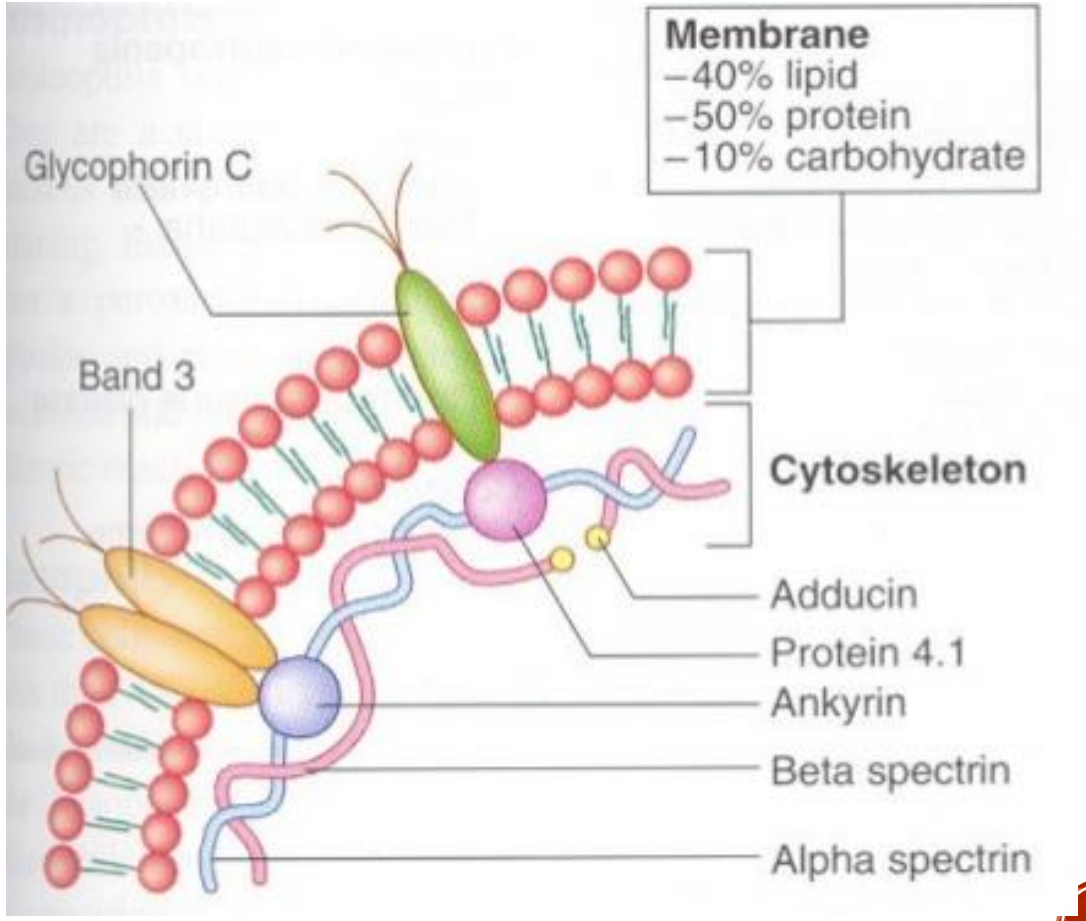
- Generalizations:
  - Autosomal dominant
  - Various mutations and variable severity
  - **Extravascular hemolysis (spleen, sometimes liver)**
  - Splenectomy often curative; partial splenectomies are common in children
  - Suffer (variably) from anemia, jaundice, splenomegaly, pigmented gallstones

- Main RBC membrane abnormalities
  - **Hereditary spherocytosis** (most common)
  - Hereditary elliptocytosis
  - Hereditary stomatocytosis

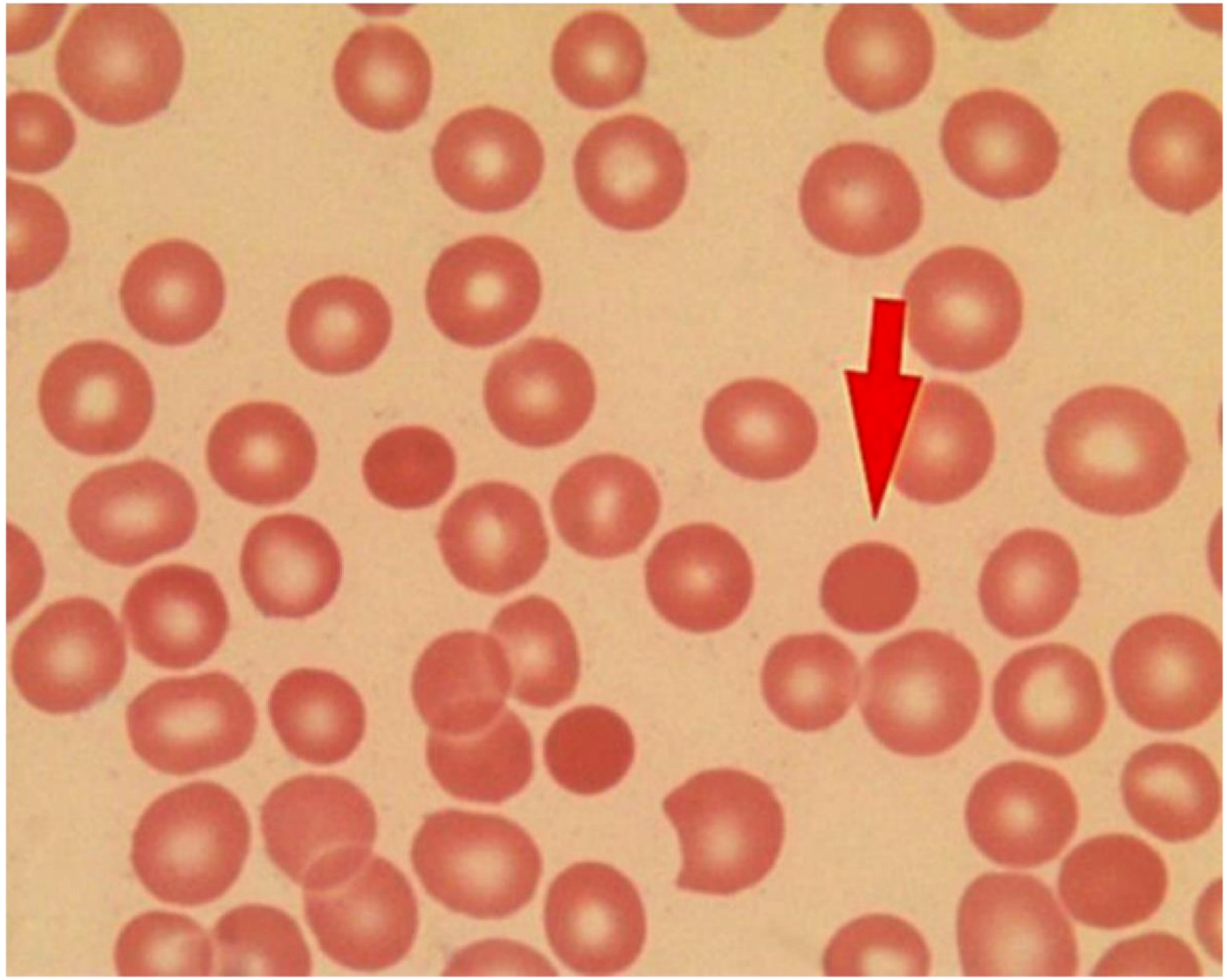
- **Usually detected by peripheral smear**

HEREDITARY SPHEROCYTOSIS

- Most common congenital hemolytic anemia
- 1/2500 in USA
- Deficiencies of the membrane proteins spectrin, ankyrin or band 3
- Cause loss of membrane
- Low surface to volume ratio results in spherical shape
- **Hemolysis occurs in the spleen due to the lack of deformability (increased spleen phagocytosis)**



SPHEROCYTES

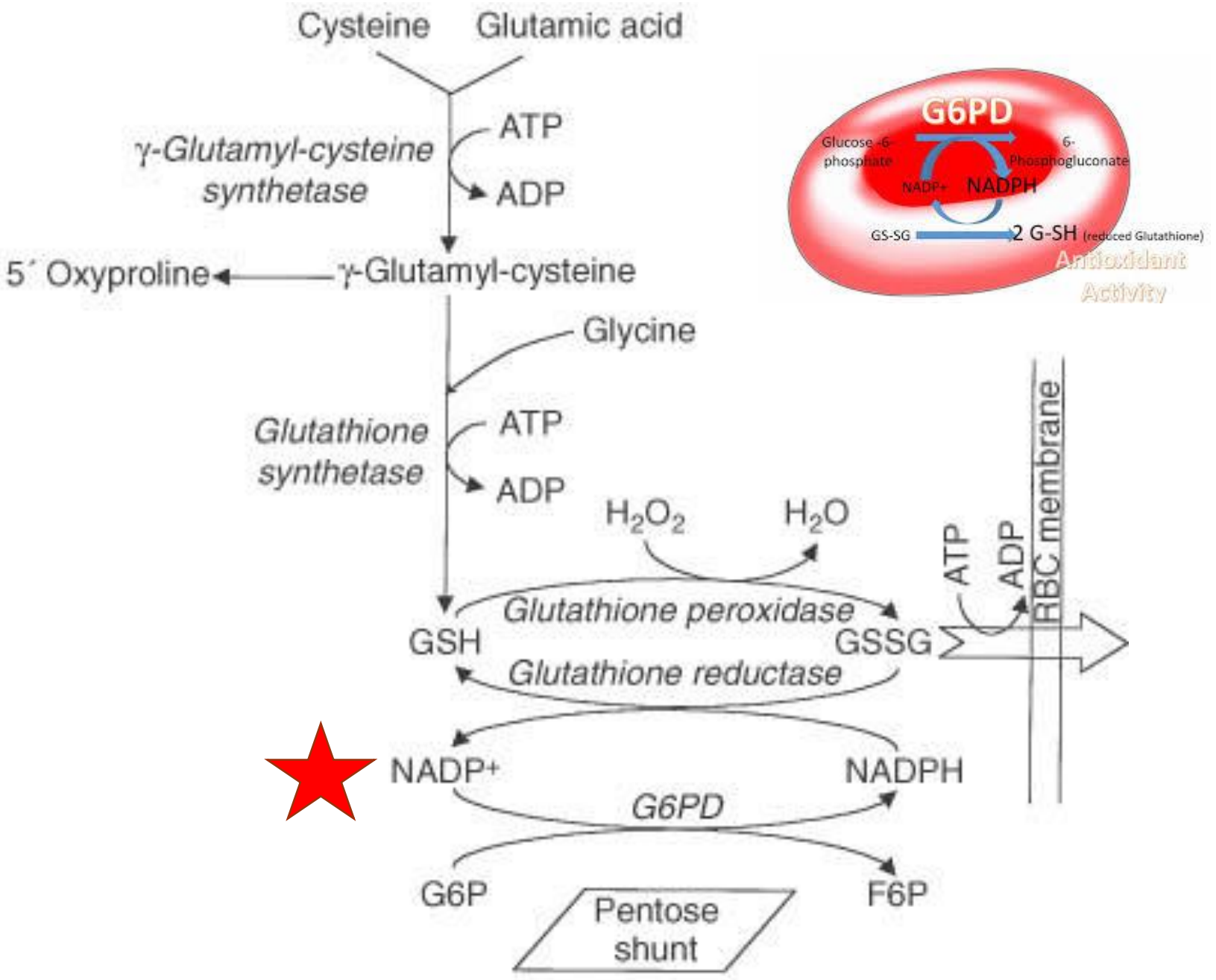


COMPLICATIONS DURING STRESS

- Hemolytic crises: usually with some infectious illness
  - Characterized by anemia, jaundice, increased splenomegaly, and increased reticulocytosis
  - Common in children

- Aplastic crises: usually after viral infection
  - Most commonly parvovirus B19 In patients with severe HS

- Megaloblastic crisis: usually with increased folate demands
  - Pregnancy, growing children, elderly



CONGENITAL/ HEREDITARY RBC ENZYMOPATHIES

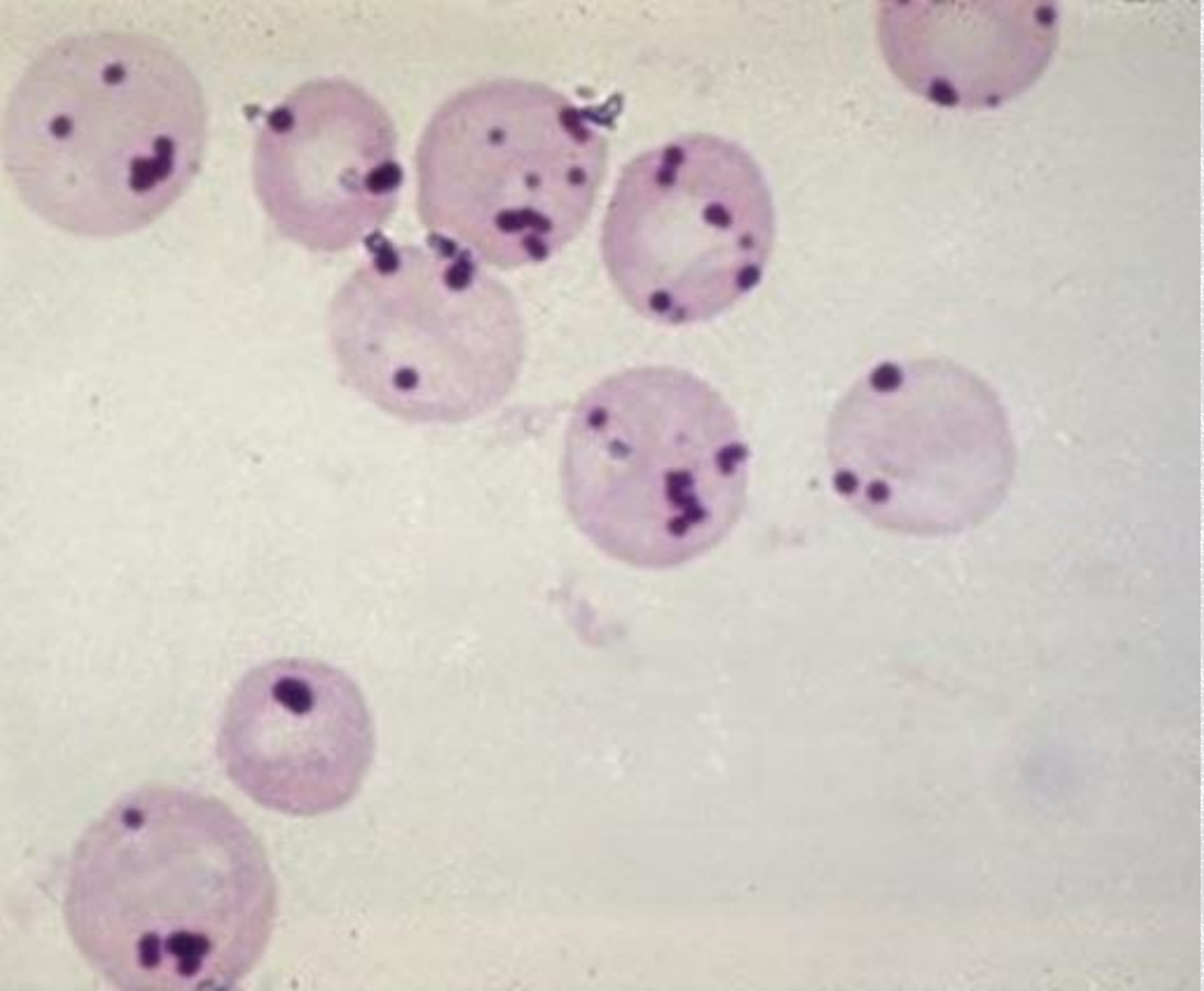
Glucose 6-Phosphate Dehydrogenase Deficiency

(G6PD deficiency)

https://www.drawittoknowit.com/pop-quizzes/biochemistry/patients-with-pyruvate-kinase-deficiency-exhibit-hemolytic-anemia-because

HEMOLYTIC ANEMIA FROM G6PD DEFICIENCY

- Common: Most common in **Africans** and those of **Mediterranean** origin (so ethnicity matters!)
- **X-linked** (men >>>> women)
- Present variably: acute events only or chronic low grade hemolysis
- **Oxidative stress** from drugs, infections, foods (fava beans) and acidosis precipitates attacks of hemolysis
- Oxidized hemoglobin precipitates as **Heinz bodies;** the spleen "pits"cells resulting in **"bite" cells**



HEINZ BODIES

(Supervital Stain)

CONGENITAL HEMOGLOBIN DEFECTS

- **Thalassemia**
  - **Alpha**
  - **Beta**
- **Sickle cell**
- Methemoglobinemia
- Unstable Hb variants

ACQUIRED HEMOLYTIC ANEMIAS

- The most common cause of hemolytic anemias
- Can be intravascular or extravascular; most are due to an extrinsic causes

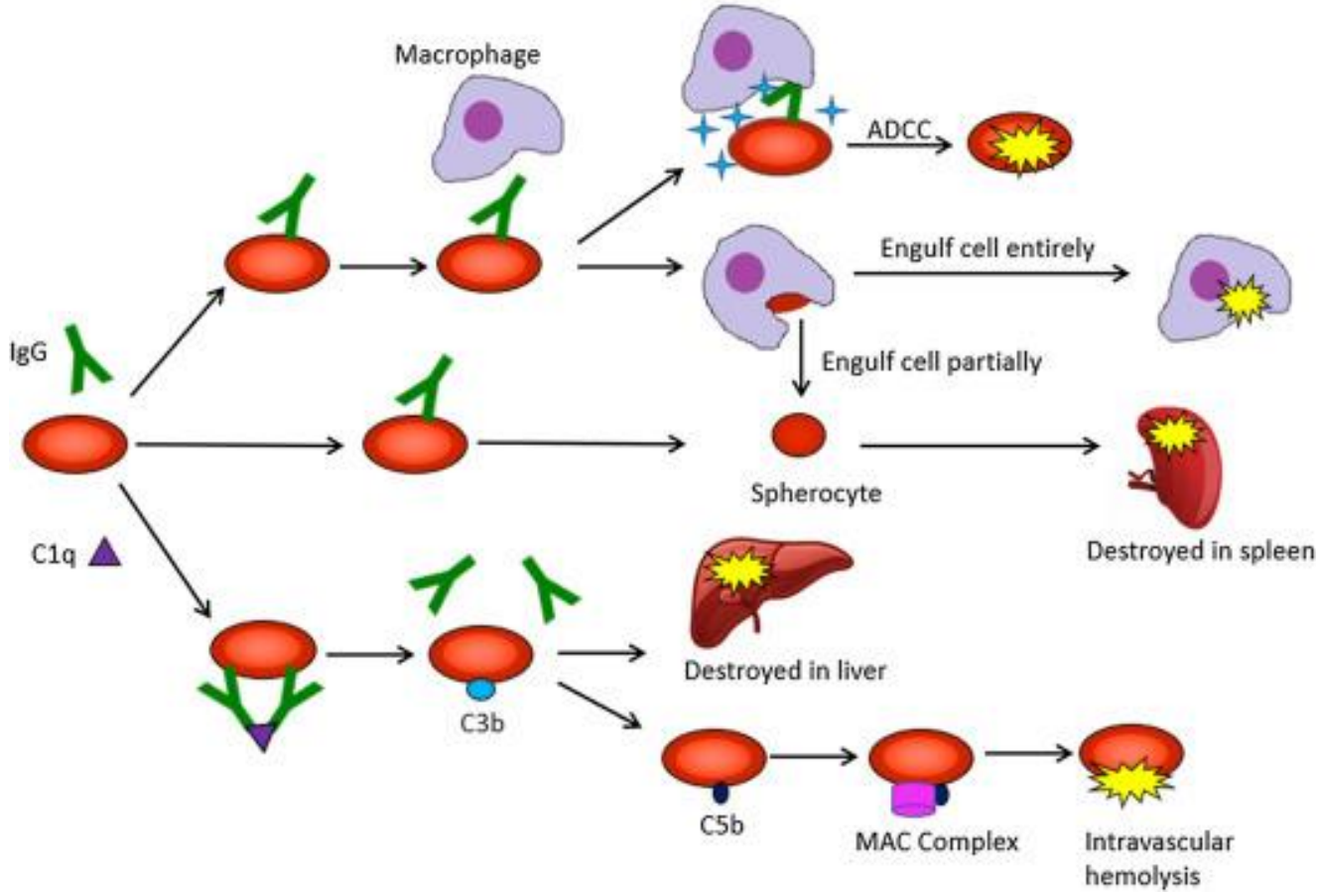
ACQUIRED HEMOLYTIC ANEMIAS

- Immune-related
- Infections (e.g. malaria)
- External agents such as venoms, chemicals, burns, drugs
- Fragmentation/Traumatic Hemolysis
  - Mechanical valve
  - Microangiopathic: destruction caused by factors in the small blood vessels
- Other, rare, causes: hypophosphatemia, paroxysmal nocturnal hemoglobinuria (PNH), spur cell anemia

IMMUNE-MEDIATED HEMOLYTIC ANEMIAS

- **Antibody or complement binding to antigens on the RBC surface → hemolysis**

- Subtypes include:
  - Warm autoimmune (IgG, complement)
  - Cold autoimmune (IgM)
  - Transfusion-related (ABO mismatching)



https://www.sciencedirect.com/science/article/pii/B9780323675093000032

WARM AUTOIMMUNE HEMOLYTIC ANEMIA

- **IgG** Auto-Antibodies preferentially reacts with the RBCs at body temperature (warm)

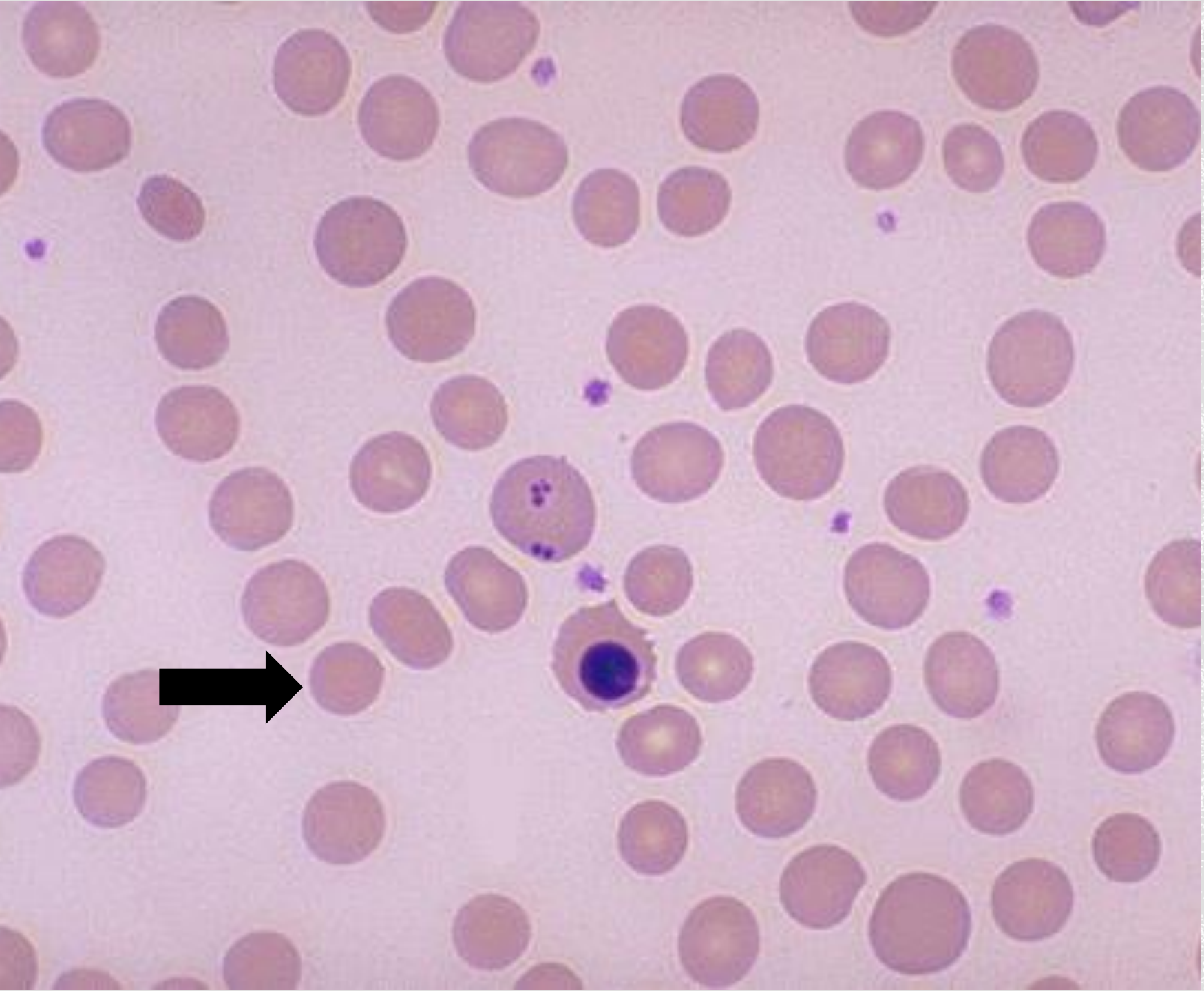
- Can be idiopathic or secondary to:
  - Drugs
  - Autoimmune disease
  - Malignancies

- Complications during stress

WARM AUTOIMMUNE HEMOLYTIC ANEMIA

- Work up:
  - Blood smear (**spherocytes**)
  - Positive direct antiglobulin test (DAT)/**Coombs test –IgG positive**

- **Treatments** include suppression of the immune system and/or treatment of the underlying disorder
  - **Steroids, Rituximab, immunosuppression**



SPHEROCYTES

Warm autoimmune hemolytic anemia

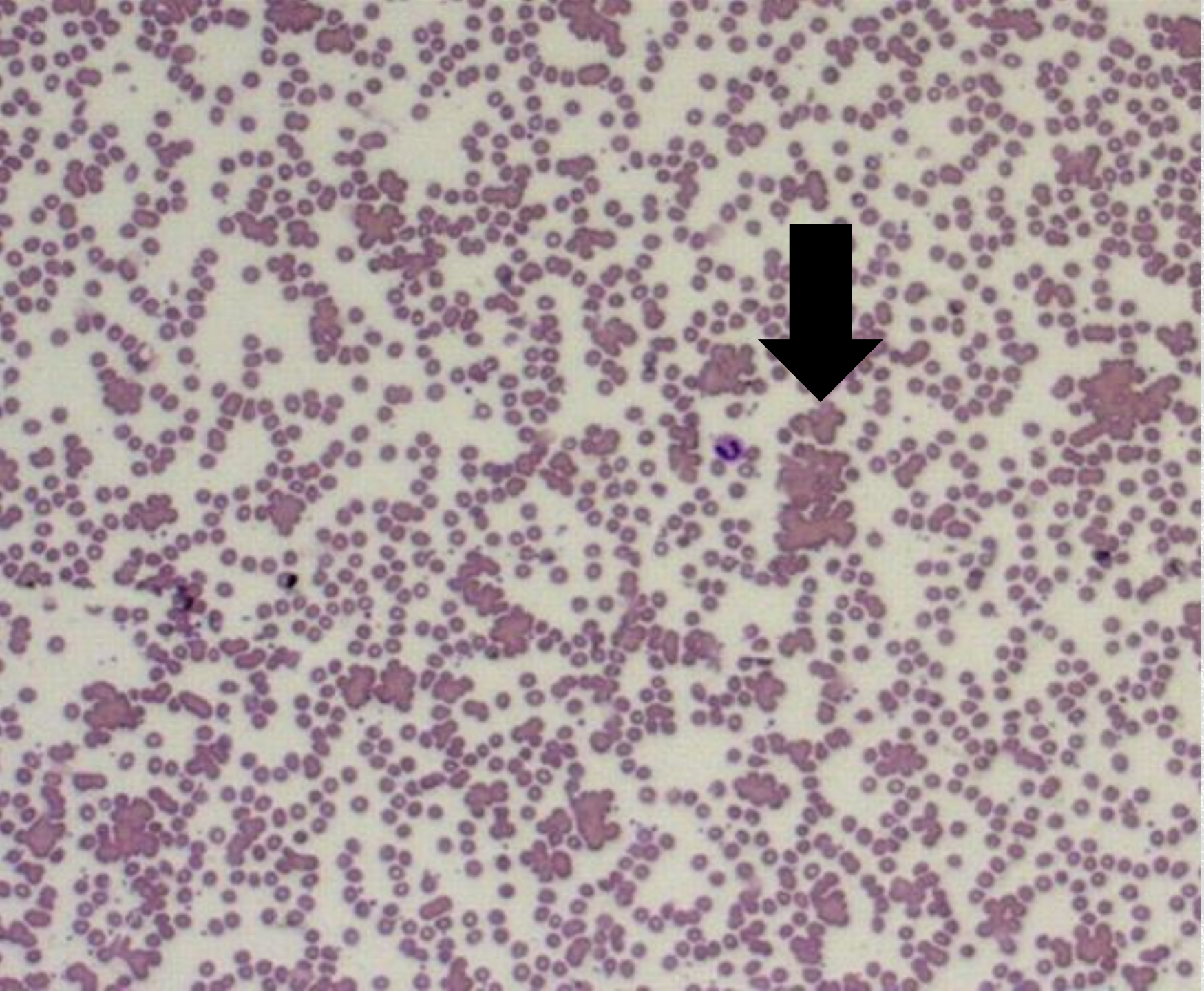
COLD AGGLUTININ HEMOLYTIC ANEMIA

- Auto-Antibodies preferentially reacts with the RBCs at colder temperature (4 degrees)
  - **IgM** temporarily bind to the RBC membrane → activates complement → deposits complement factor C3 on the cell surface.
  - C3-coated RBCs are cleared by the macrophages of the liver

- Secondary to:
  - Infectious such as Mycoplasma pneumonia, EBV
  - Lymphoproliferative disorders

- Work up includes a blood smear (agglutination) and positive DAT/**Coombs for C3** (versus IgG in *warm* AIHA)

- Treatments: suppression of the immune system and/or treatment of the underlying disorder



AGGLUTINATION

Cold autoimmune hemolytic anemia

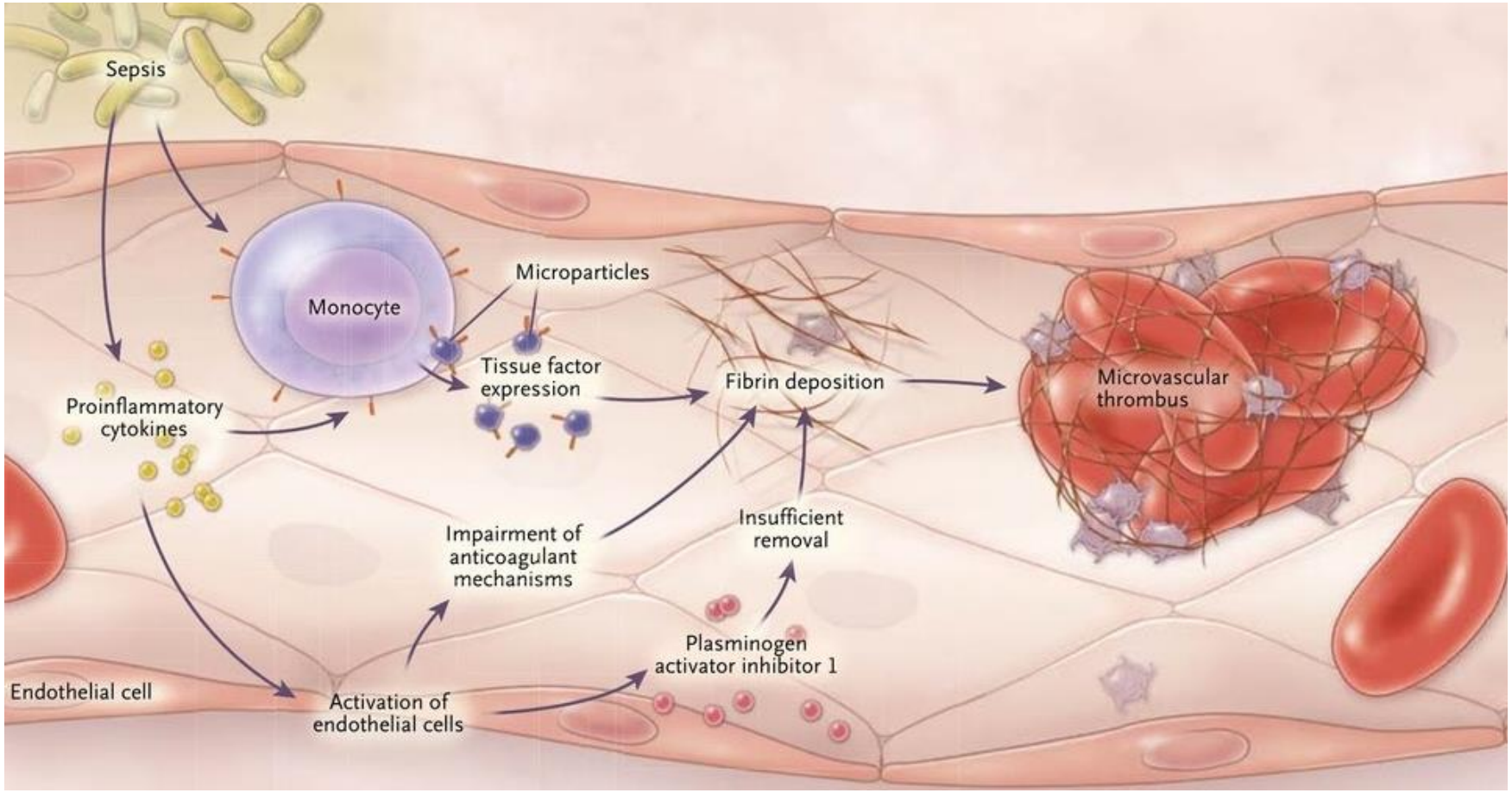
FRAGMENTATION HEMOLYSIS

- Microangiopathy

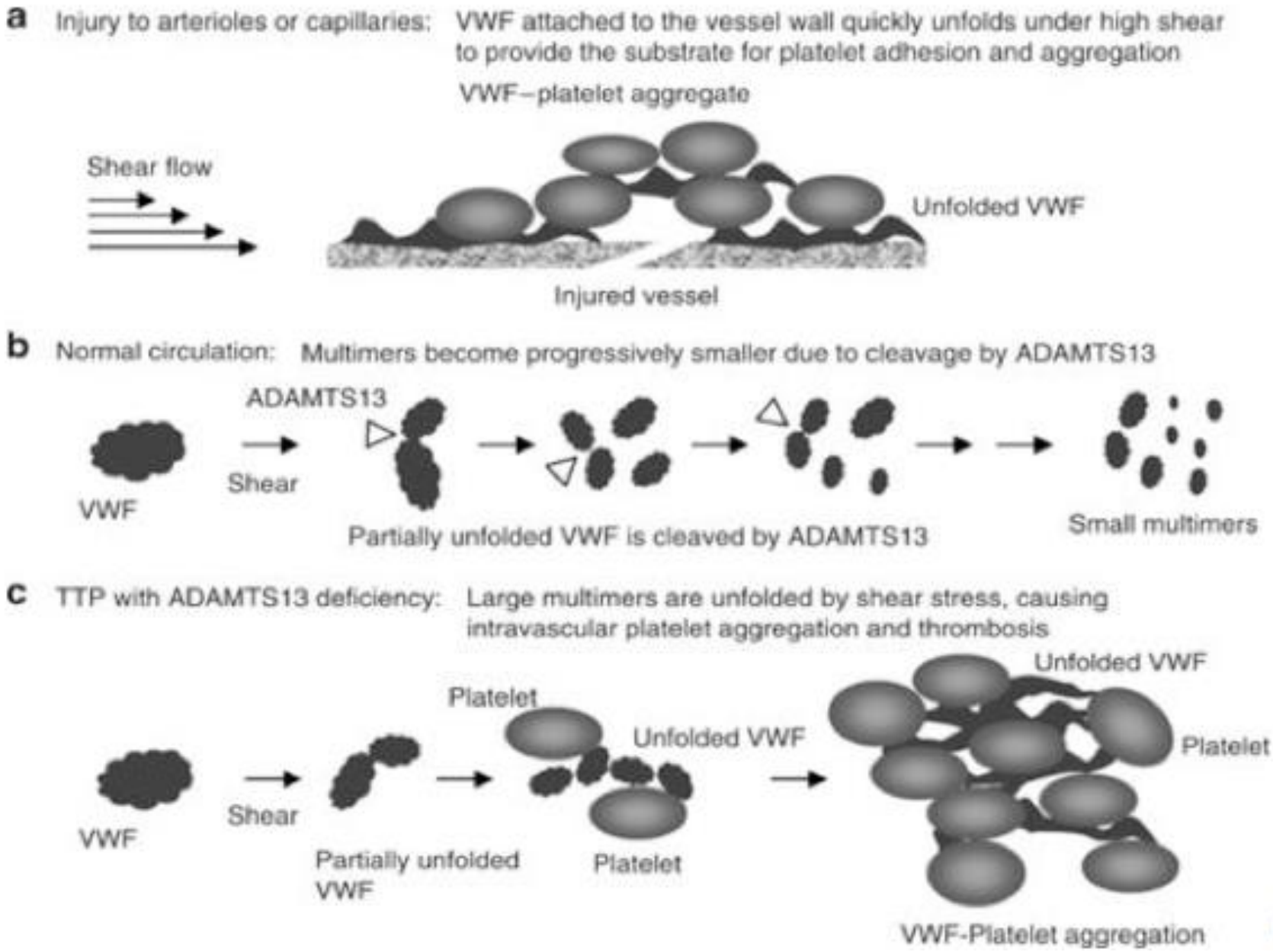
- Mechanical trauma fragments RBCs

- Categories
  - Damaged microvasculature (injured endothelium) causes microangiopathic hemolytic anemia
    - **Disseminated intravascular coagulation (DIC)**
      - **Infections, sepsis, burns, malignancy**
    - **Thrombotic thrombocytopenic purpura (TTP)**
    - **Hemolytic uremic syndrome (HUS)**
    - Vasculitis
    - Malignant hypertension
  - Damaged heart valves, left ventricular assist devices causing shearing of RBCs

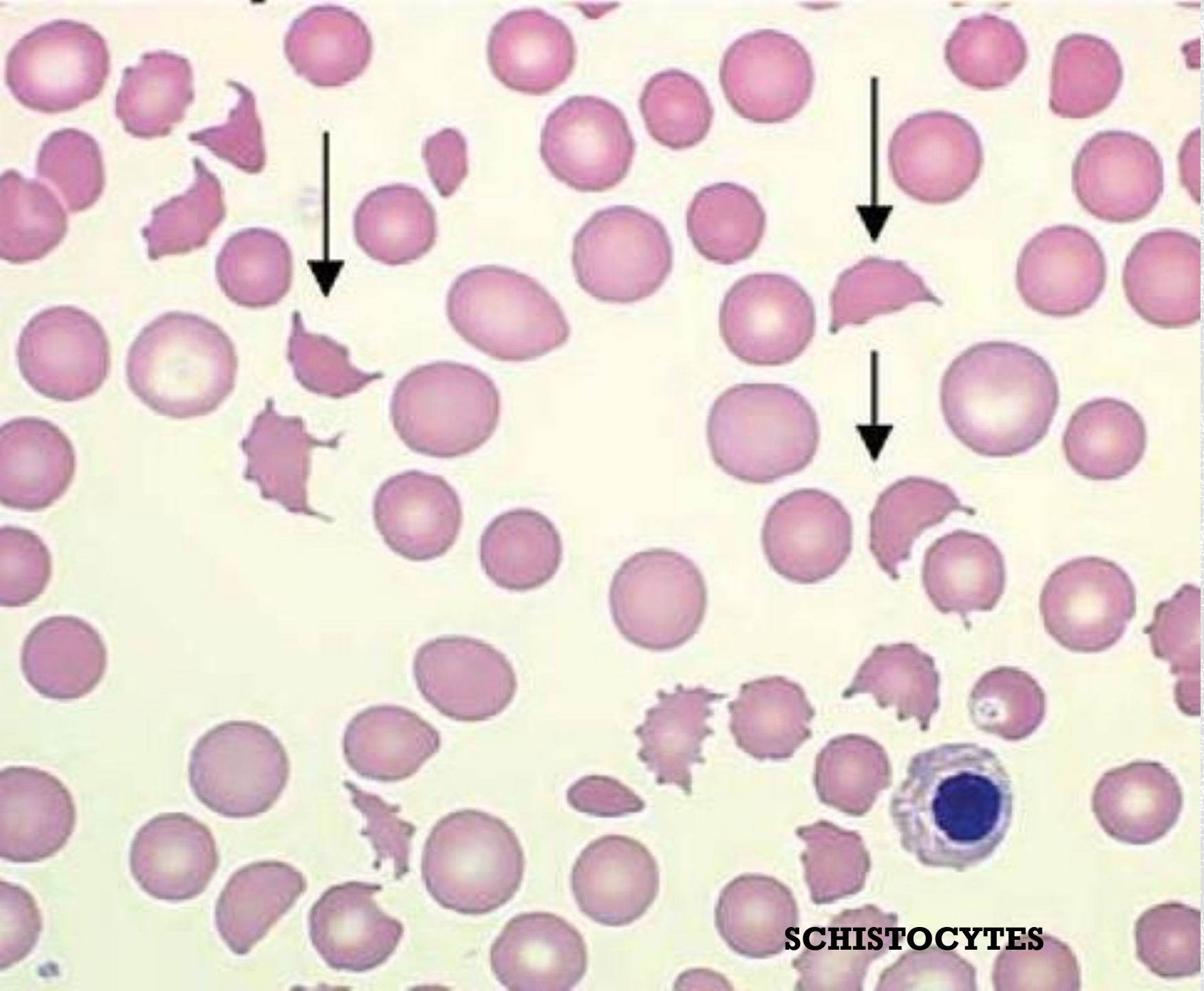
DISSEMINATED INTRAVASCULAR COAGULATION



THROMBOTIC THROMBO-CYTOPENIC PURPURA (TTP)



SCHISTOCYTES



SCHISTOCYTES

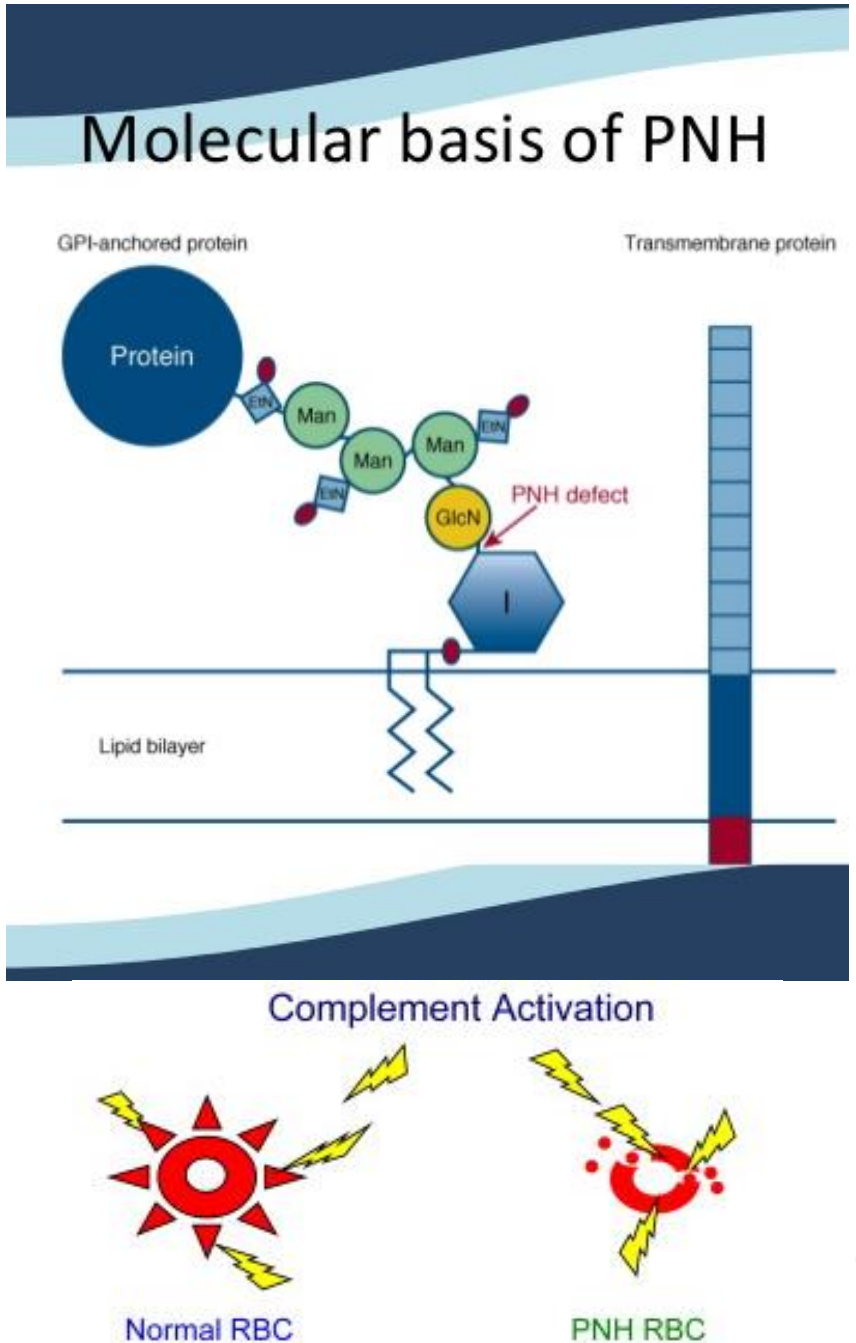
DIFFERENCES BETWEEN DIC AND TTP

Variables/ Lab Parameters	DIC	TTP
Pathogenesis	Overactivation of the coagulation pathway/Thrombin excess	ADAMTS13 deficiency (which help cleave VWF)
Clinical Picture	Underlying systemic illness: Sepsis, infection, malignancy	Idiopathic (usually)
Smear	Schistocytes Decreased platelets	Schistocytes Decreased platelets
PT/PTT	Prolonged	Normal
Fibrinogen	Decreased	Normal

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

- Acquired genetic mutation in a hematopoietic stem cell

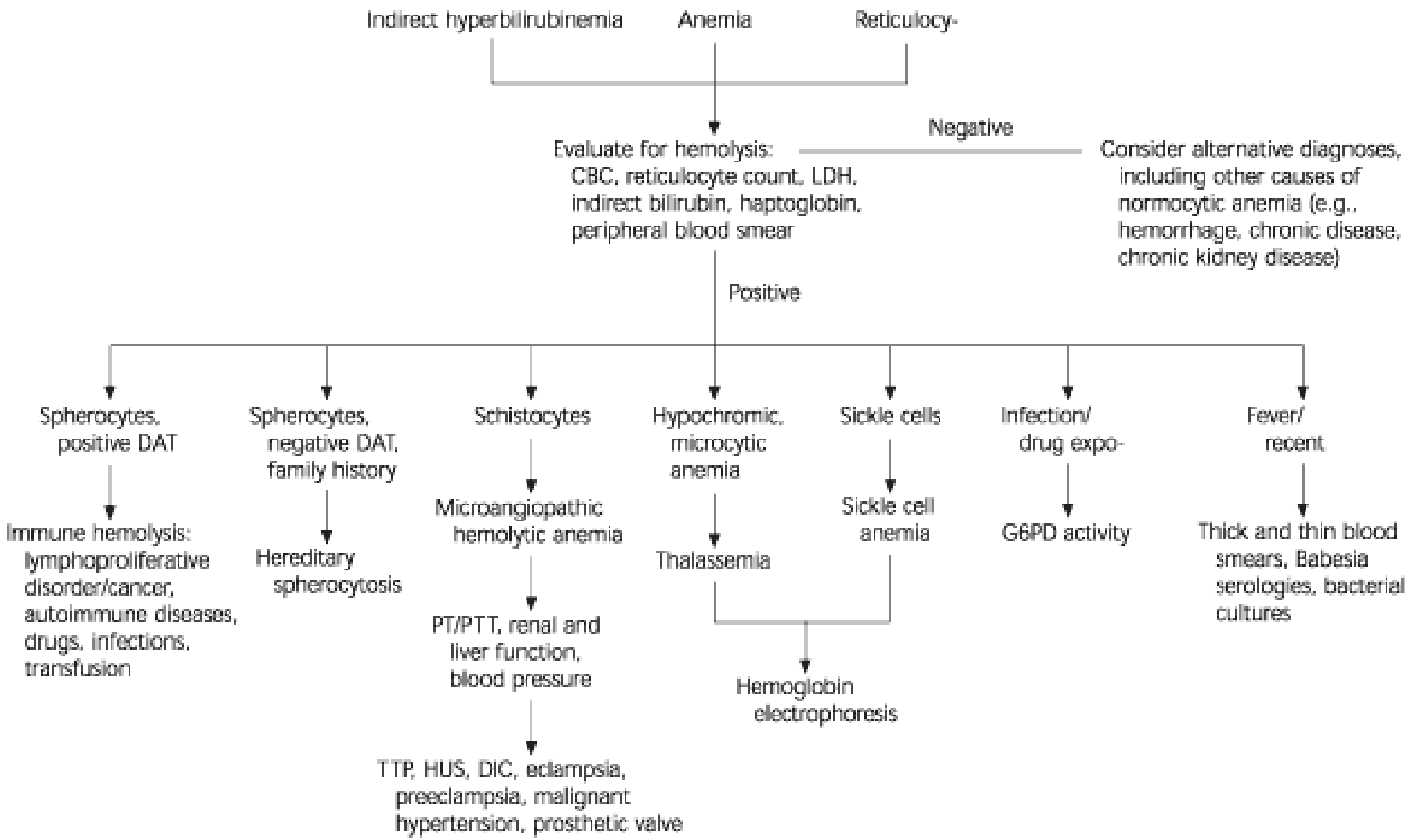
- Mutation occurs in *PIGA* gene (**p**hosphatidyl**i**nositol **g**lycan anchor biosynthesis, class **A**) gene - responsible for the first step in the synthesis of glycosylphosphatidylinosiol (GPI) anchor that attaches a subset of proteins to the cell surface



PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

- Hemolytic anemia
- Classic symptoms are dark urine at night
- Can be associated with:
  - **Thrombosis**
  - Other marrow bone marrow failure syndromes
- Diagnosis
  - Anemia with intravascular hemolysis (and some degree extravascular)
  - Urine for hemoglobin or hemosiderin
  - Flow cytometry (a special process that can identify specific cell surface markers) looking for ABSENT normal GPI-linked proteins

ALGORITHM FOR HEMOLYTIC ANEMIA



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