HAEMATOLOGY

- Study of blood & its components
- Multiple cellular & acellular elements
- Window of rest of body

- Delivery of nutrients
  - Oxygen
  - Food
- Removal of wastes
  - Carbon dioxide
  - Nitrogenous wastes
  - Cellular toxins
- Protection *versus* invading microorganisms
- Coagulation

- Red Blood Cells/Oxygen & CO_2 transport
- White Blood Cells/Protection *versus* microorganisms
- Coagulation/platelets/Maintenance of vascular integrity

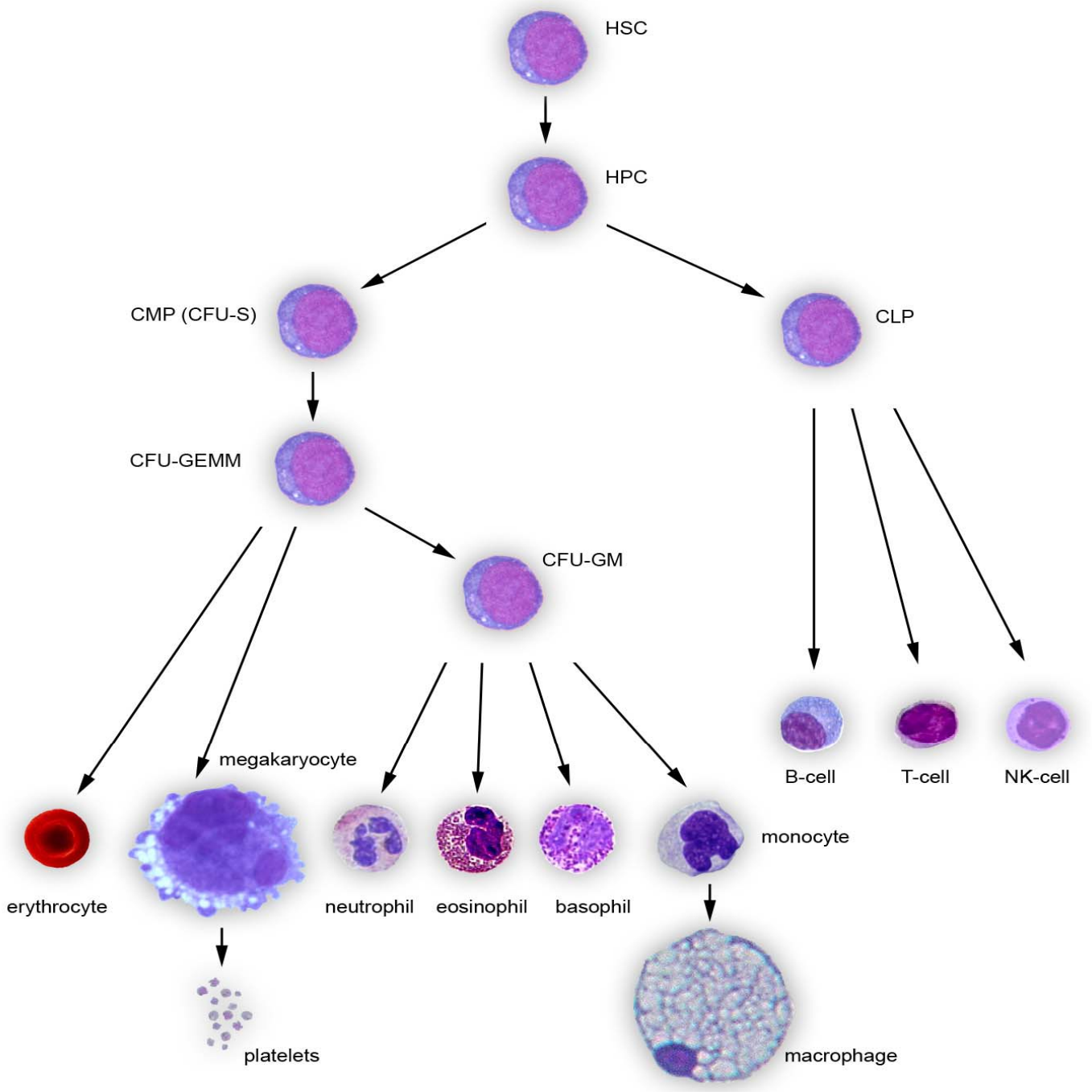
Haematopoiesis

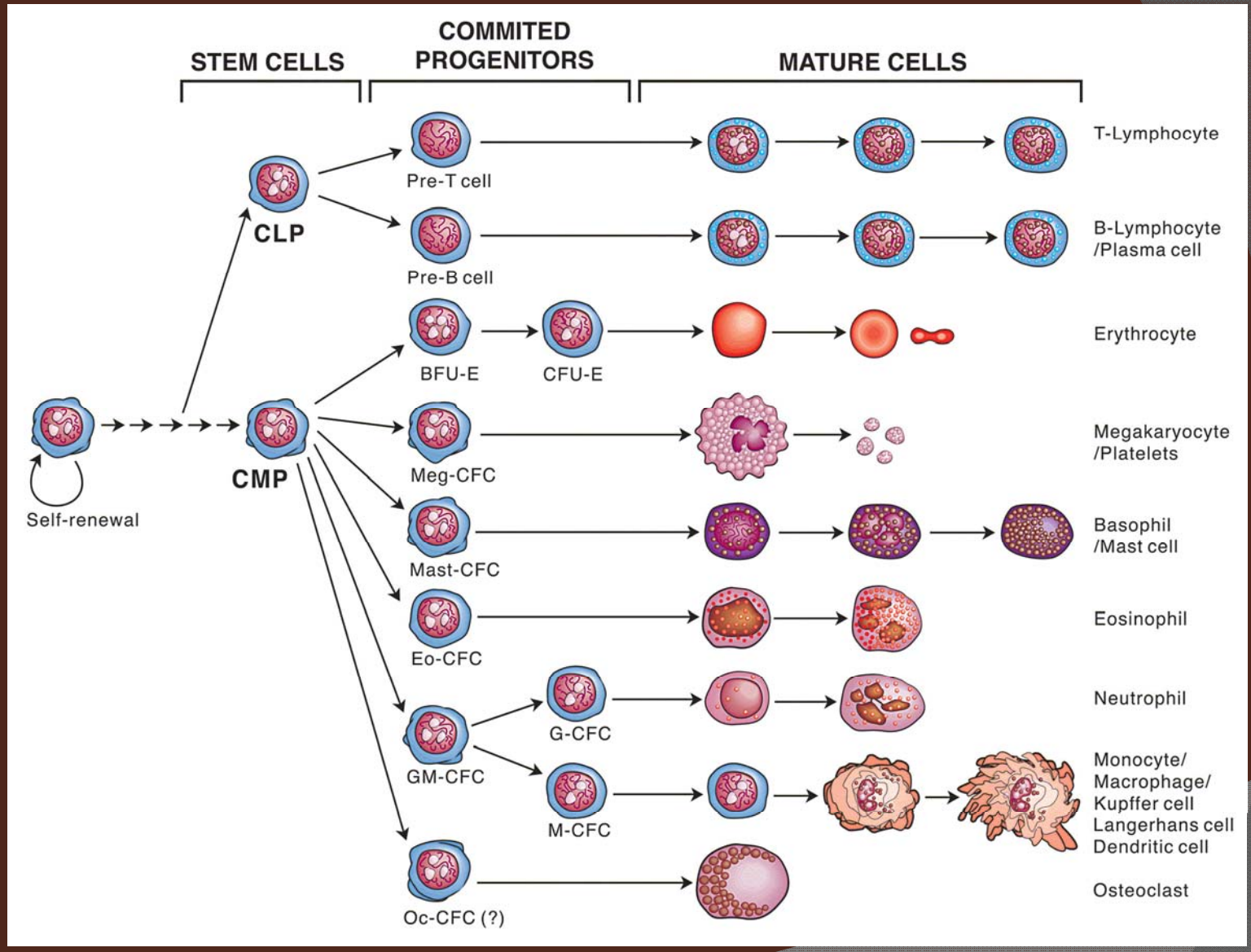
The embryonic hematopoietic organs	*The adult hematopoietic organs*
The yolk sac (6-10 weeks of gestation)	The bone marrow - is the exclusive site of postnatal hematopoiesis (myelopoiesis and lymphopoiesis) under normal circumstances.
The fetal liver and the spleen (10 weeks – second trimester)	
The bone marrow (from 4 month)	

- Red marrow(BIRTH TO PUBERTY)
- Yellow marrow

Haematopoiesis

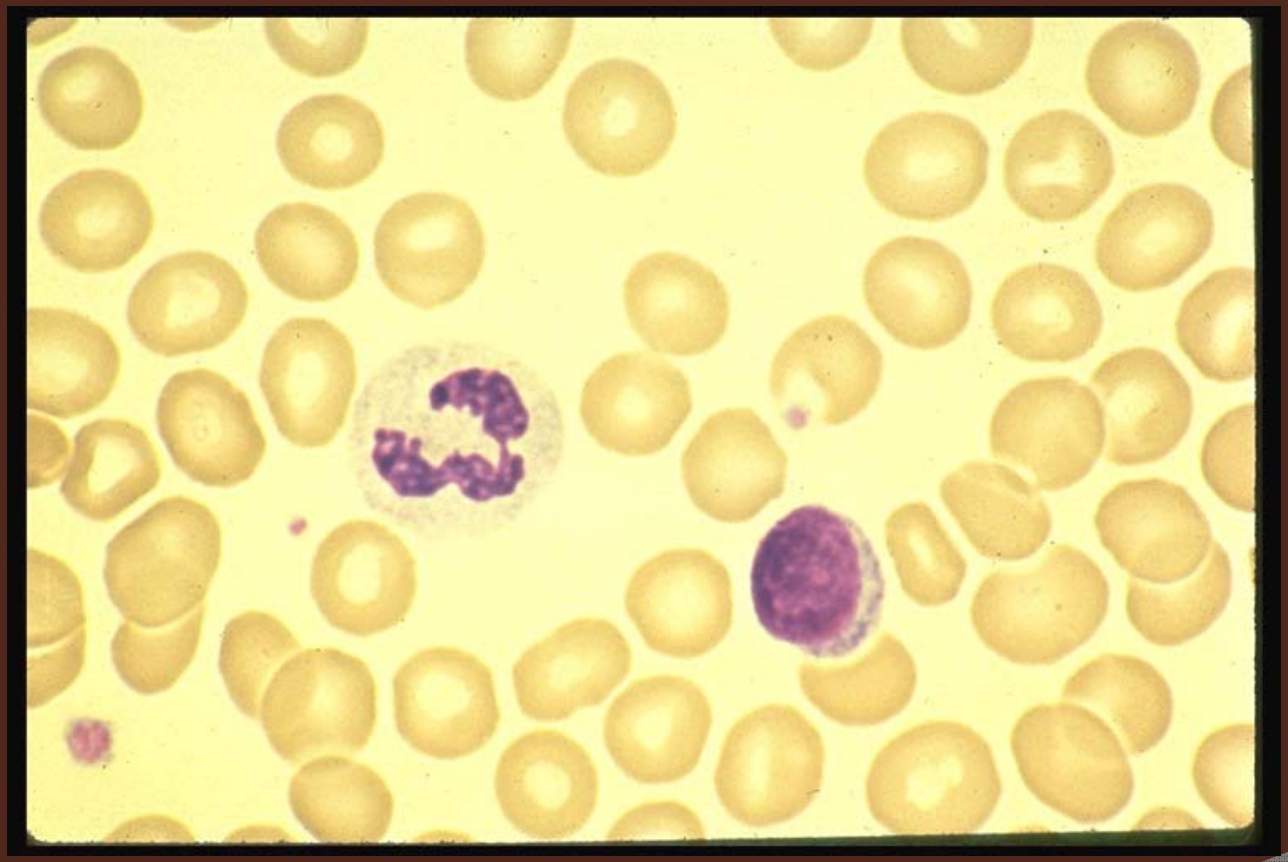
- In humans, occurs in bone marrow exclusively
- All cellular elements derived from pluripotent stem cell (PPSC)
- PPSC retains ability to both replicate itself and differentiate
- Types of differentiation determined by the influence of various cytokines


HSC
HPC
CMP (CFU-S)
CLP
CFU-GEMM
CFU-GM
B-cell
T-cell
NK-cell
megakaryocyte
erythrocyte
neutrophil
eosinophil
basophil
monocyte
platelets
macrophage


STEM CELLS
COMMITED PROGENITORS
MATURE CELLS
Self-renewal
CLP
CMP
Pre-T cell
Pre-B cell
BFU-E
CFU-E
Meg-CFC
Mast-CFC
Eo-CFC
G-CFC
GM-CFC
M-CFC
Oc-CFC (?)
T-Lymphocyte
B-Lymphocyte /Plasma cell
Erythrocyte
Megakaryocyte /Platelets
Basophil /Mast cell
Eosinophil
Neutrophil
Monocyte/ Macrophage/ Kupffer cell Langerhans cell Dendritic cell
Osteoclast

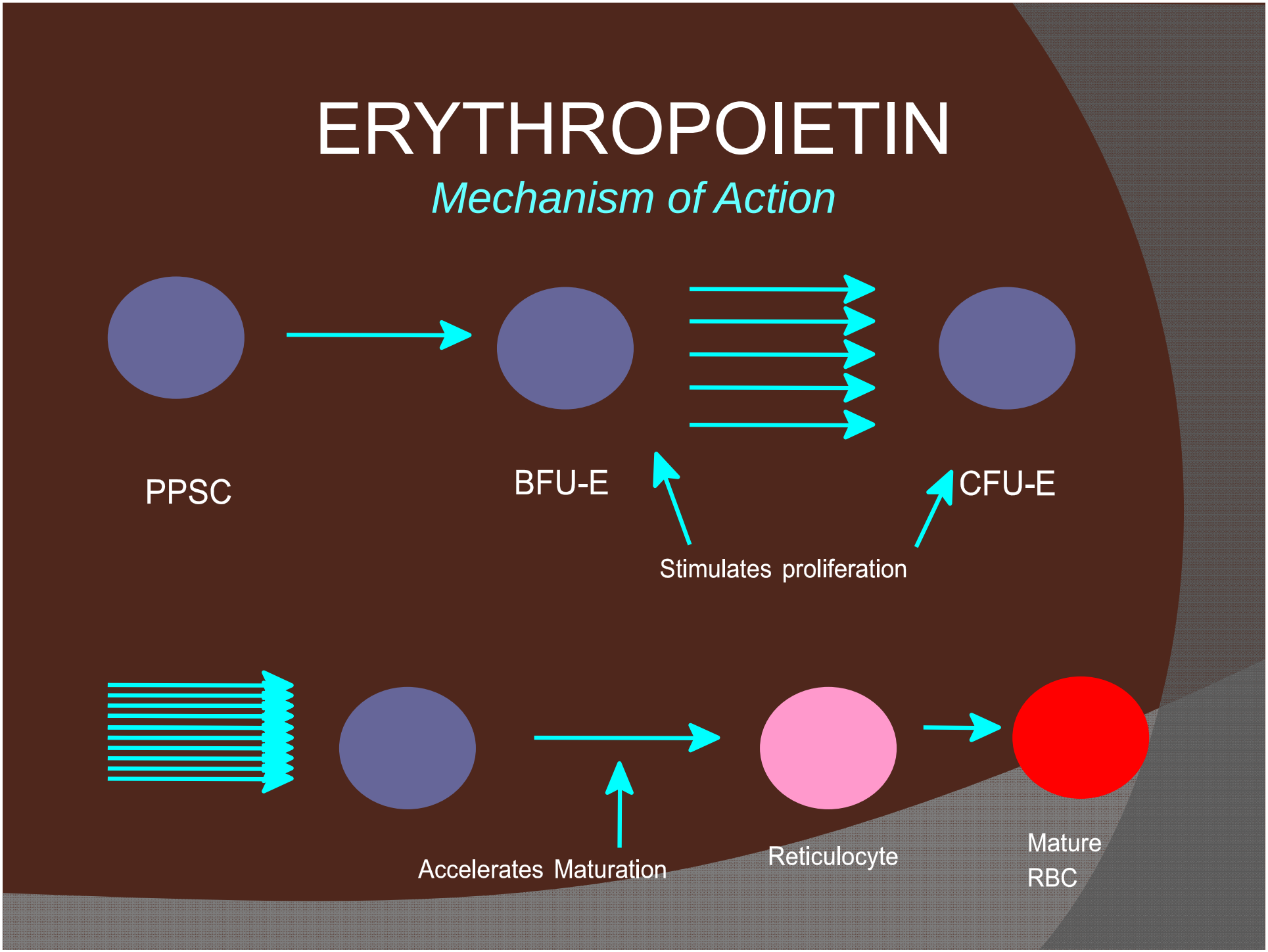
Erythrocytes

- Normal - Anucleate, highly flexible biconcave discs, 80-100 femtoliters in volume
- Flexibility essential for passage through capillaries
- Major roles - Carriers of oxygen to & carbon dioxide away from cells



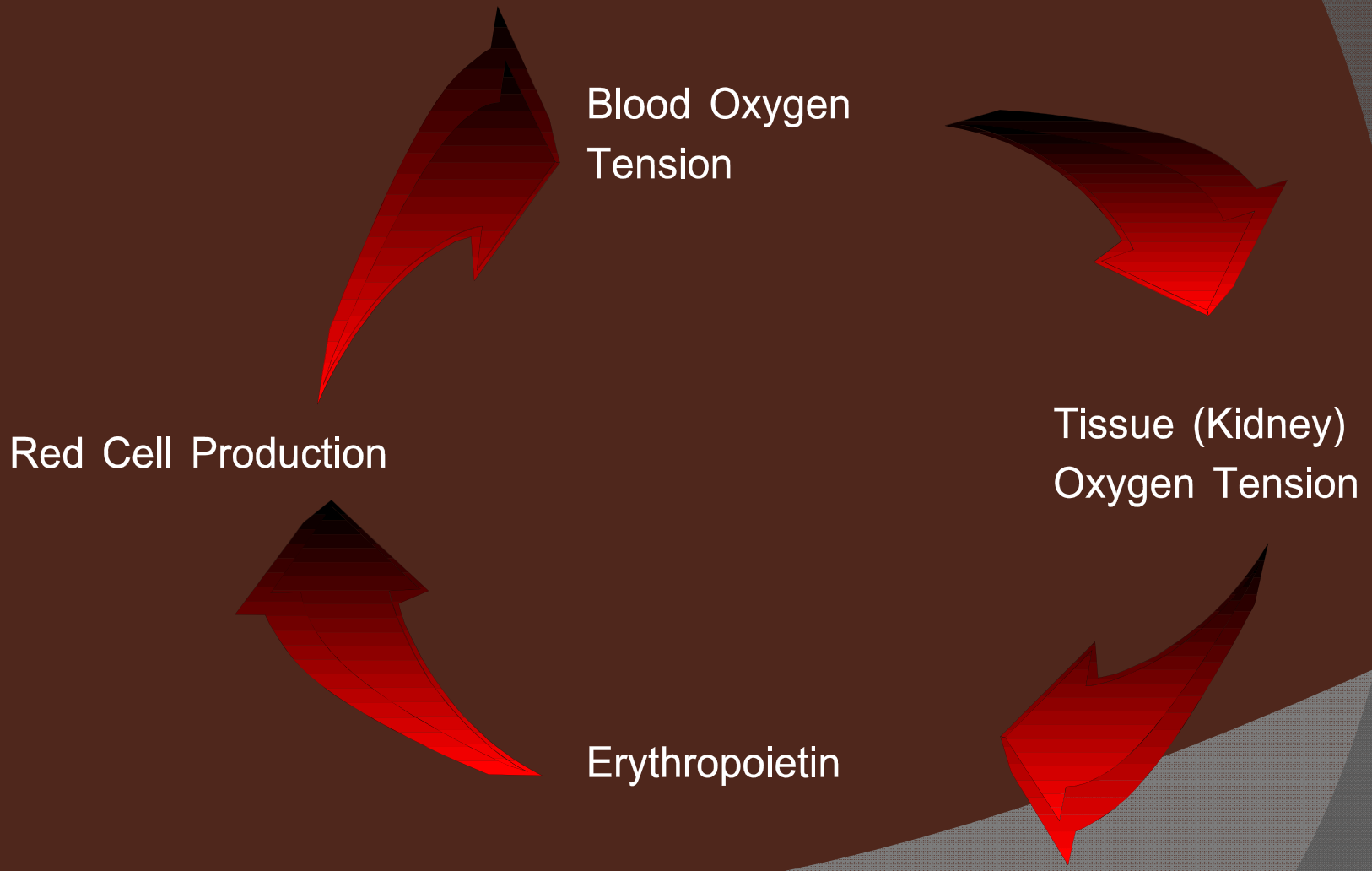
Erythropoietin

- Cytokine - Produced in the kidney
- Necessary for erythroid proliferation and differentiation
- Absence results in apoptosis (programmed cell death) of erythroid committed cells
- Anemia of renal failure 2° to lack of EPO


ERYTHROPOIETIN
Mechanism of Action
PPSC
BFU-E
CFU-E
Stimulates proliferation
Accelerates Maturation
Reticulocyte
Mature RBC

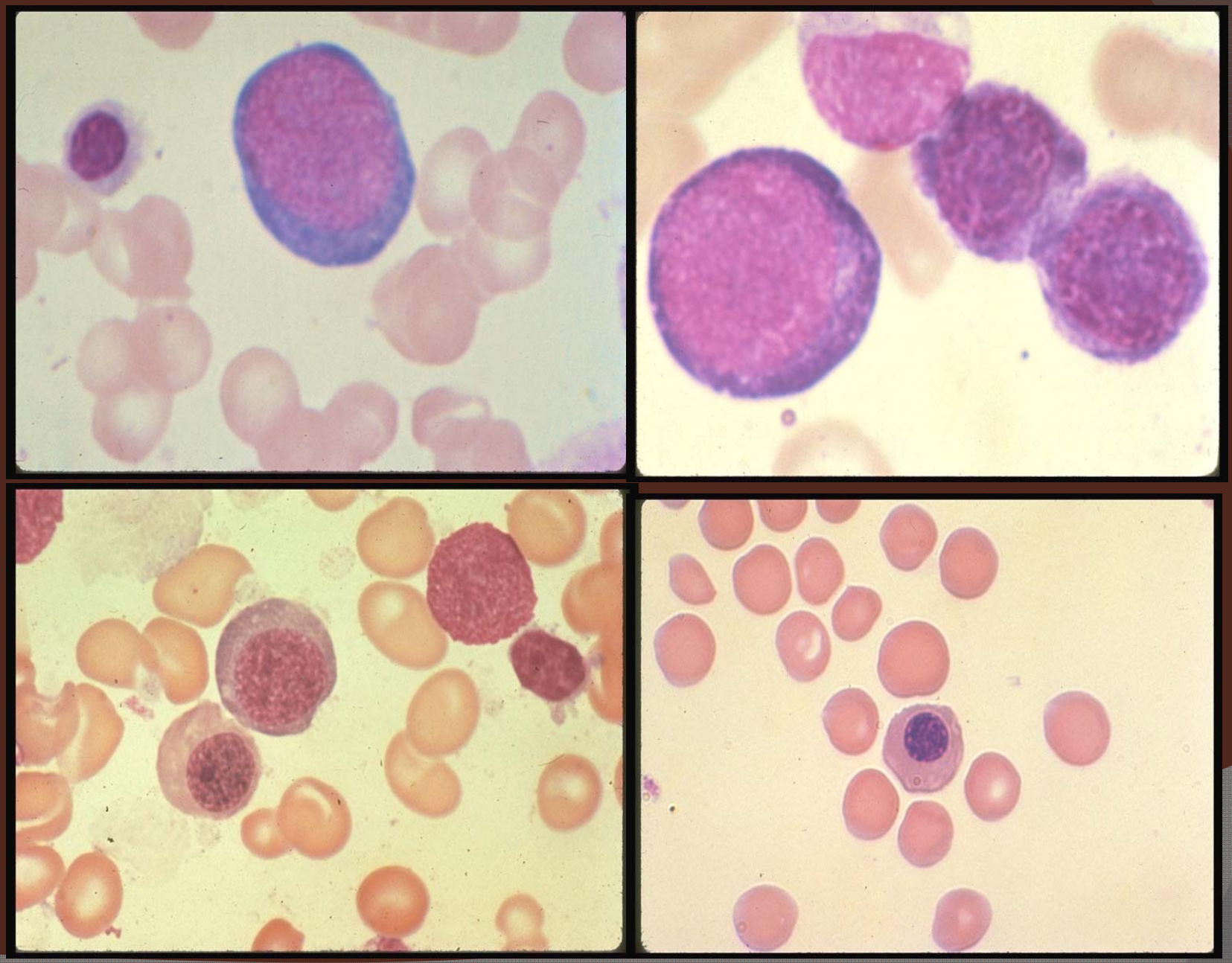
ERYTHROPOIETIN

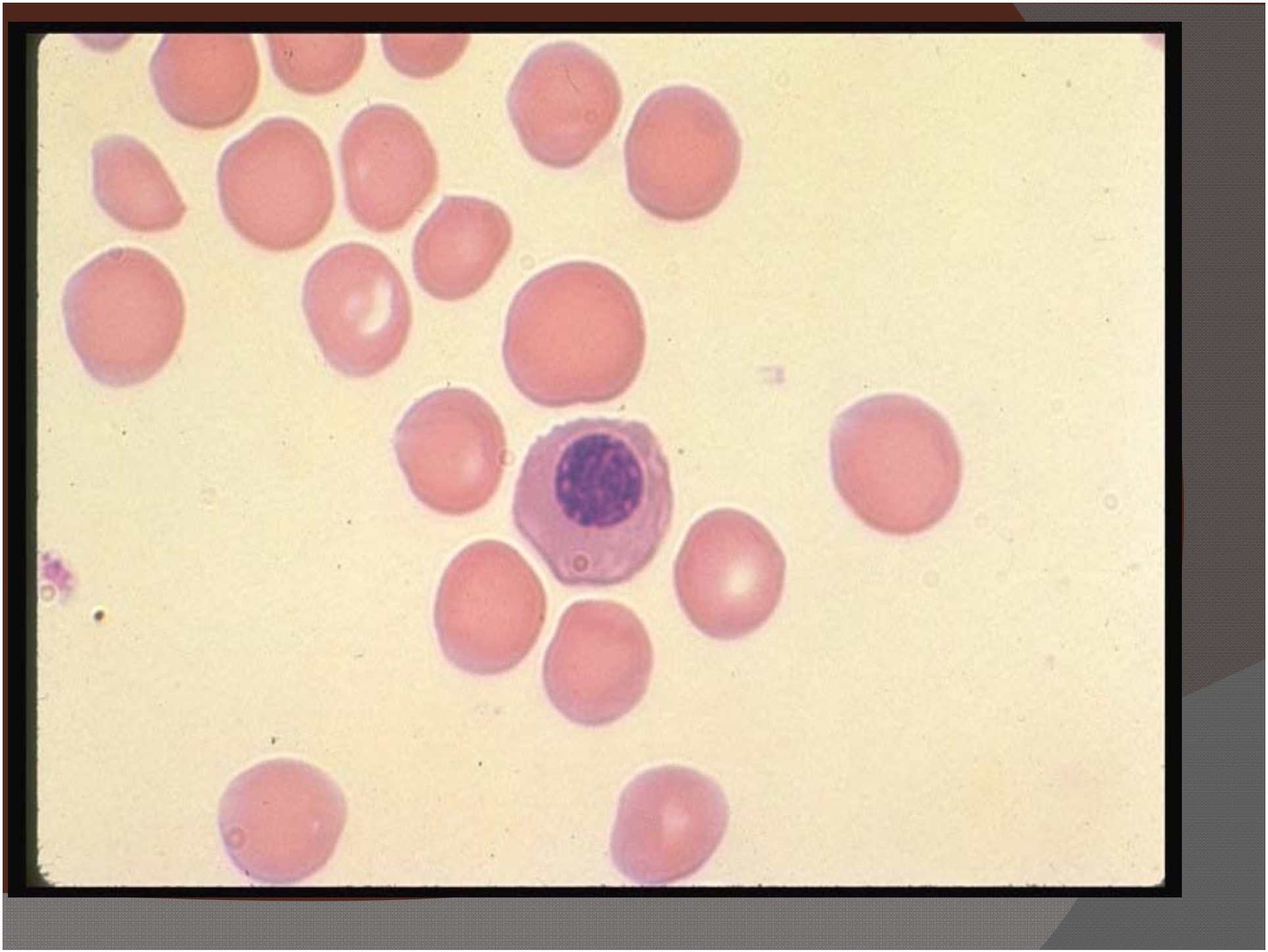
Regulation of Production

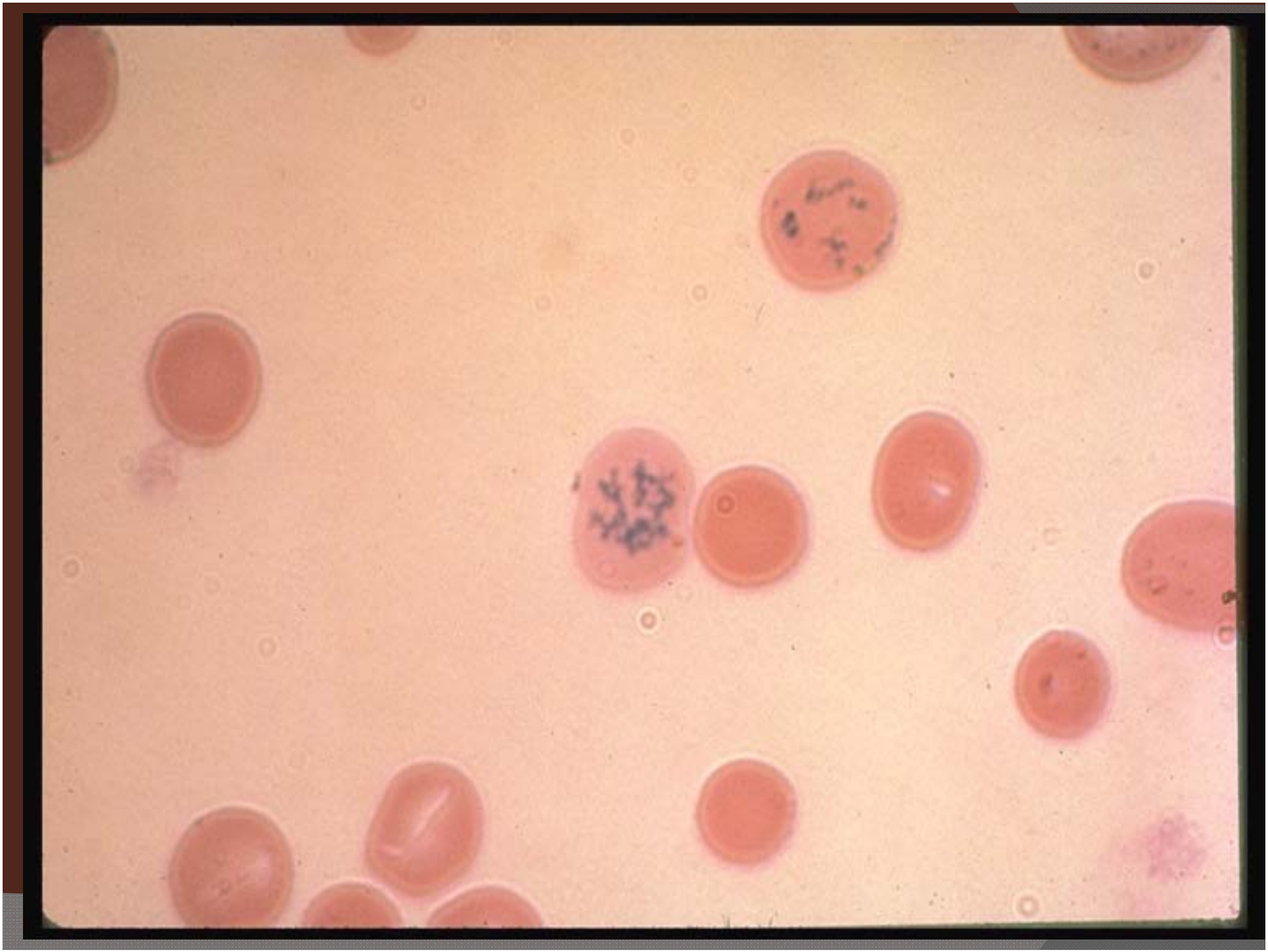


RBC Precursors

- Pronormoblast
- Basophilic normoblast/Early
- Polychromatophilic Normoblast/Intermed
- Orthrochromatophilic Normoblast/late
- Reticulocyte
- Mature Red Blood Cell
- 5-7 days from Pronormoblast to Reticulocyte







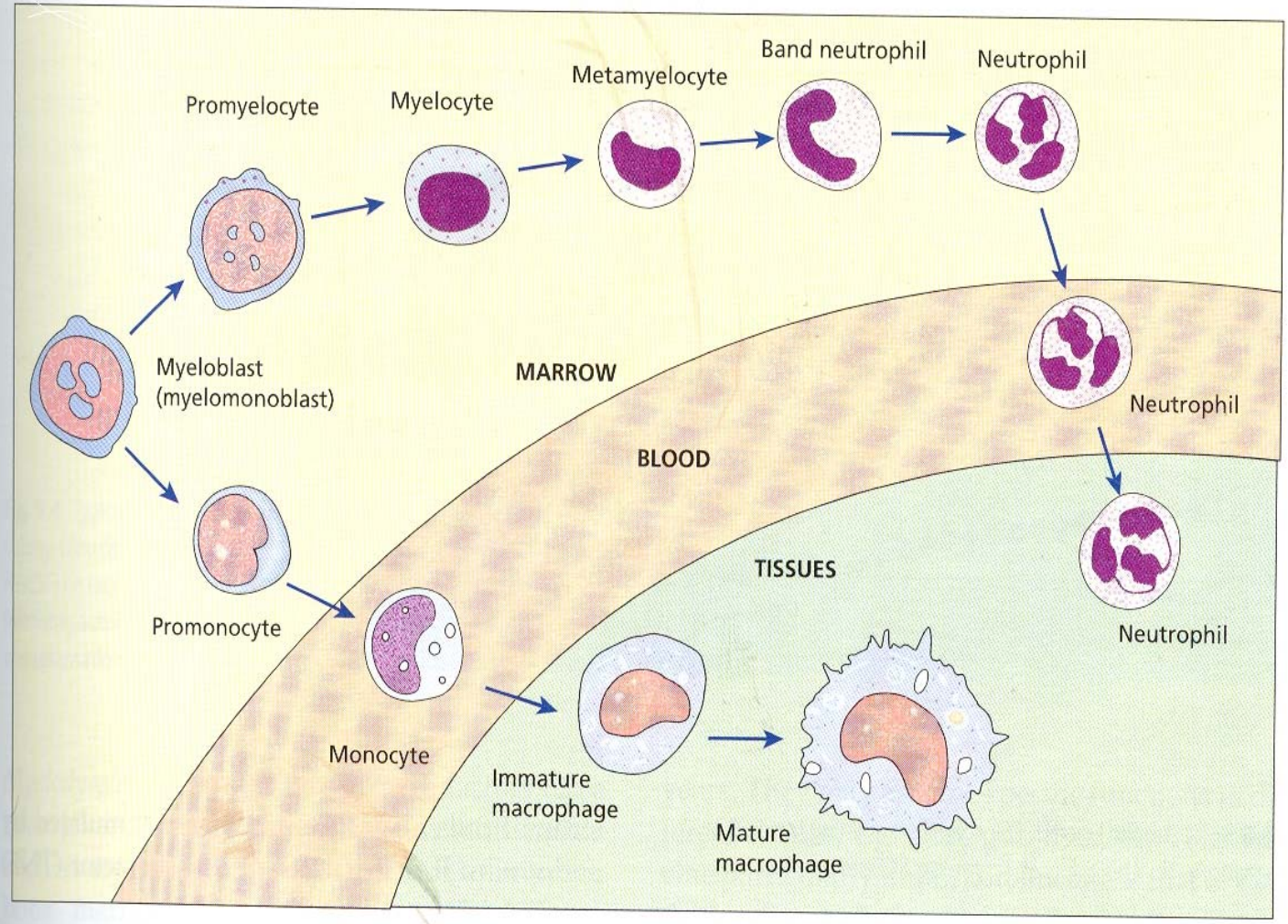


Fig. 9.2 The formation of the neutrophil and monocyte phagocytes. Eosinophils and basophils are also formed in the marrow in a process similar to that for neutrophils.

Leukocytes –White blood cells (WBC) heterogeneous population of blood nucleated cells

- **Granulocytes:**

  Neutrophils

  eosinophils

  basophils

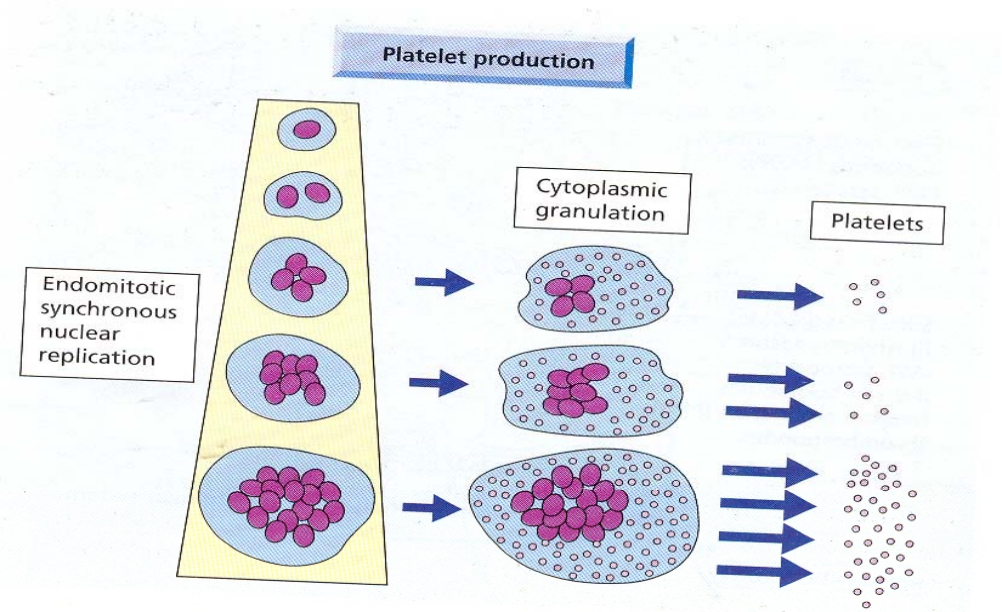
  Agranulocytes:

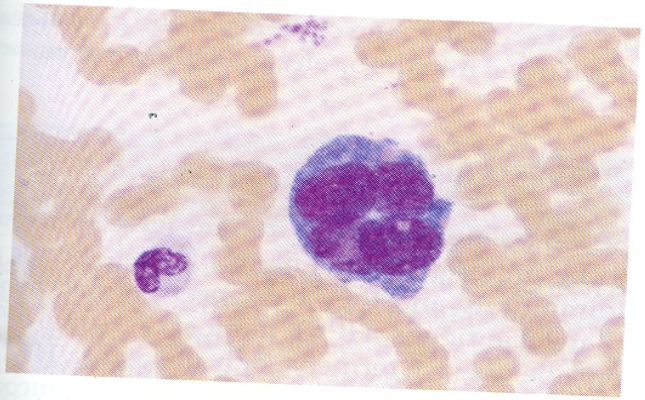
  Monocytes

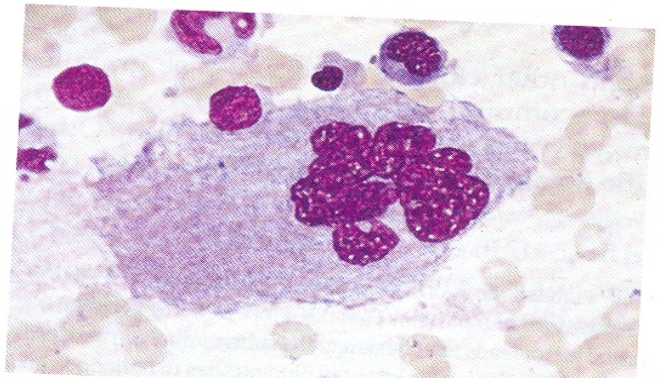
  Lymphocytes

Different WBC count:

	Neutrophil	Eos.	Bas.	Lym.	Mon.
	40-75%	1-6%	0-1%	20-45%	2-8%


Platelet production
Endomitotic synchronous nuclear replication
Cytoplasmic granulation
Platelets





Platelets (Thrombocytes)

- Very small anuclear cells (parts of megakaryocyte cytoplasm) containing molecules required for hemostasis
- The life spans – 8 days
- Pl. count150,0 00– 450,000/µl

THROMBOCYTOPENIA
I-100,000-- < 150,000
II-50,000-<100,000
III-25,000- < 50,000
IV- < 25,000

THROMBOCYTOSIS (>4.5 LAKHS)
- REACTIVE
- ESSENTIAL

Disorders of erythrocytes

- Anaemia
- Erythrocytosis

Red Blood Cells

Normal Values

RBC Parameters	Normal Values
Hematocrit	
	35-47%
Females	40-52%
Males	
Hemoglobin	
	12.0-16.0 gm/dl
Females	13.5-17.5 gm/dl
Males	
MCV	80-100 fl
Reticulocyte Count	0.2-2.0%

Definition

- Anaemia has been defined as a reduction in one or more of the major red blood cell (RBC) measurements:
- hemoglobin concentration
- hematocrit
- RBC count

Definition-contd.

- **Hemoglobin concentration (Hb**) measures the concentration of the major oxygen-carrying pigment in whole blood.
- **Hematocrit (Hct)** is the percent of a sample of whole blood occupied by intact red blood cells.
- **RBC count** is the number of red blood cells contained in a specified volume of whole blood.

WHO criteria for diagnosis of anaemia

Hb concentration > 2 SD below mean for population

Age & Sex Group	Hb (g/dl)
Children 6 months-6yrs	<11
Children 6 -14yrs	<12
Adults(males)	<13
Adults(females,non-pregnant)	<12
Adults(females, pregnant)	<11

Increasing evidence that African American children and adults have lower hemoglobins, not due to a difference in iron status.

- Hb cutoff for Anaemia can be adjusted downward by 0.3g/dL or 1%.

Grading of Anaemia

- Mild Anaemia <10.0 g/dl
- Moderate Anaemia 7-9 g/dl
- Severe Anaemia <6 g/dl
- Very Severe Anaemia <5g/dl

Classification of Anaemia

- Pathophysiological Classification
- Morphological Classification
- Functional Classification

Pathophysiological Causes Of ANEMIA

- **Decreased RBC production**
  - Lack of nutrients, such as iron, B12, or folate. This can be due to dietary lack, malabsorption (eg, pernicious anemia, sprue), or blood loss (iron deficiency)
  - Bone marrow disorders (eg, aplastic anemia, pure RBC aplasia, myelodysplasia, tumor infiltration)
  - Bone marrow suppression (eg, drugs, chemotherapy, irradiation).

Pathophysiological Causes Of ANEMIA-contd.

- Low levels of hormones which stimulate RBC production, such as EPO (eg, chronic renal failure), thyroid hormone (eg, hypothyroidism), and androgens (eg, hypogonadism).

- Chronic disease/inflammation, associated with infectious, inflammatory, or malignant disorders, is characterized by reduced availability of iron.

  decreased absorption from the gastrointestinal tract

  decreased release from macrophages

  a relative reduction in erythropoietin levels, and a mild reduction in RBC lifespan.

Pathophysiological Causes Of ANEMIA-contd.

- **Blood loss**
  - Obvious bleeding (eg, trauma, melena, hematemesis, menometrorrhagia)
  - Occult bleeding (eg, slowly bleeding ulcer or carcinoma
  - Induced bleeding (eg hemodialysis losses, excessive blood donation)
- **Increased RBC destruction**
  - Inherited hemolytic anemias (eg, hereditary spherocytosis, sickle cell disease, thalassemia major)
  - Acquired hemolytic anemias (eg, Coombs'-positive autoimmune hemolytic anemia, malaria)

Two main approaches that are not mutually exclusive:

1. Biologic or kinetic approach

2. Morphological approach

Anaemia – First Test

RETICULOCYTE COUNT %

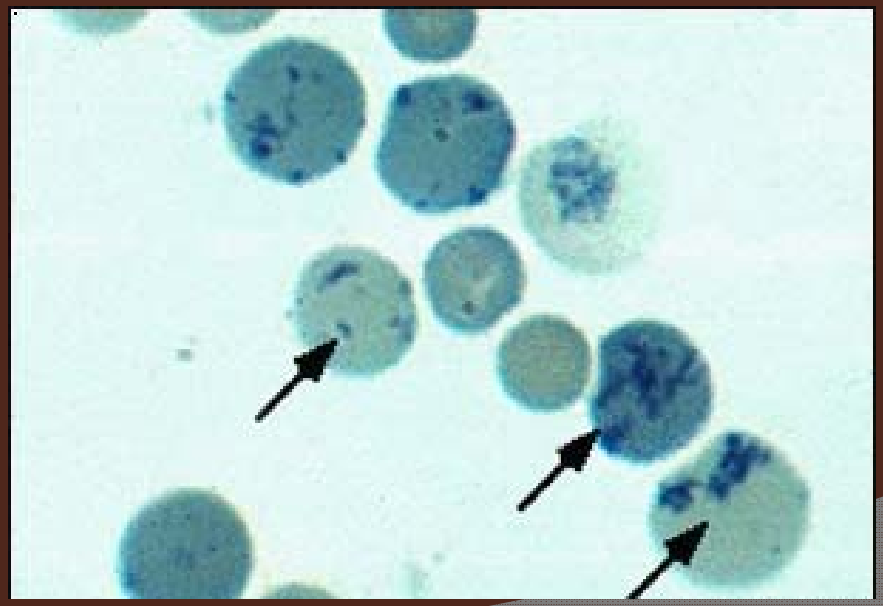
- ‘RBC to be’ or Apprentice RBC
- Fragments of nuclear material
- RNA strands which stain blue

Normal

Less than 2%

Reticulocyte count

- Retic count = % immature RBC
- Normal 0.5-2% (for non-anemic)



Reticulocyte Production Index

- Correction for left shift – Retic life span is increased in blood
- RPI = % Retic X Hct/45 X 1/CF

Hct	Correction factor (CF)
40-45	1.0
35-39	1.5
25-34	2.0
15-24	2.5

- Normal RPI = 1 (for non-anemic pt)
- RPI < 2 : hypoproliferative
- RPI >=2 : hyperproliferative

Reticulocyte Production Index

For example the RPI is calculated as follows

Reticulocyte count 9%

Hb 8.5

Hct 25

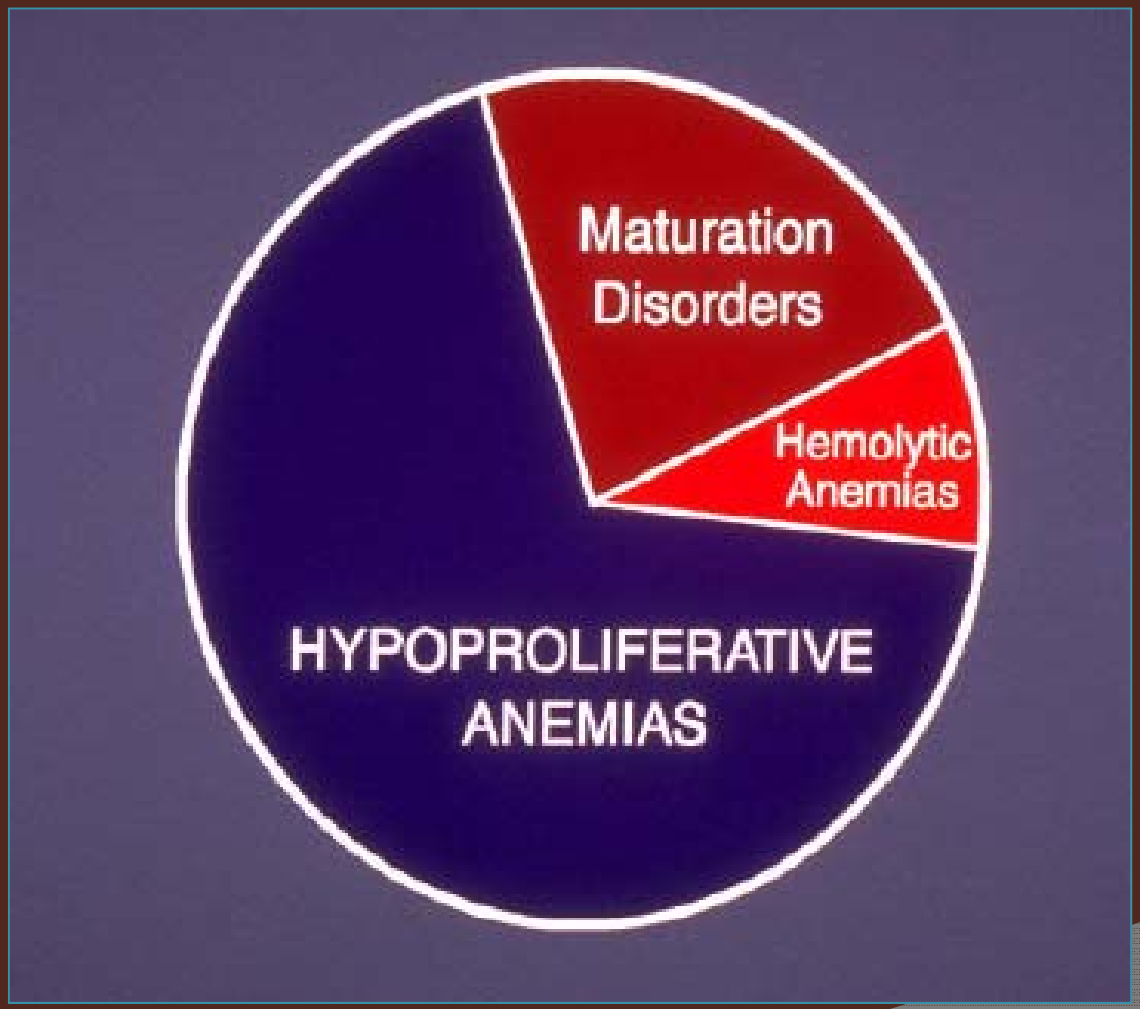
1. Correction for Anaemia

   = 9 x (8.5 ÷ 15) = 9 x 0.6 = 5.4 %

2. 5.4 ÷ 2 = 2.7

3. Thus, the RPI is 2.7

Types of Anaemia



Red Blood Cell Indices

- **MCV** :PCV/RBC(80- 96fl)
  Volume occupied by a single RBC

- **MCH** : Hb/RBC – (27-32pg/l)
  Average Hb in RBC

- **MCHC** : Hb/PCV (30-36 g/dl)
  Measure of the concentration of hemoglobin in packed volume of RBCs
  Decrease in MCHC is known as Hypochromic anemia
  Normal is known as Normochromic anemia

Anemia Workup – 3rd Test
Red cell Distribution Width – RDW

- Index of variation in RBC size
- Measure of anisocytosis (cells of many sizes)
- Normal RDW = uniform cell sizes
- Normal is 11.5-14.5%
- High in iron deficiency
- Normal in thalassemia minor (uniformly small)

Morphological Causes Of ANEMIA

Normocytic
[MCV 80-100fl]

- AOCD
- Mixed deficiencies
- Renal failure
- PRCA

Microcytic [MCV <80fl]

- Iron deficiency
- Thal. trait
- AOCD 30-40%)
- Sideroblastic anemias
- Lead poisoning

Macrocytic
[MCV>100fl]

- B12, Folate def
- Drugs (chemo.; Azathioprine, MTx, Pyrimethamine)
- Endocrinopathy
- MDS

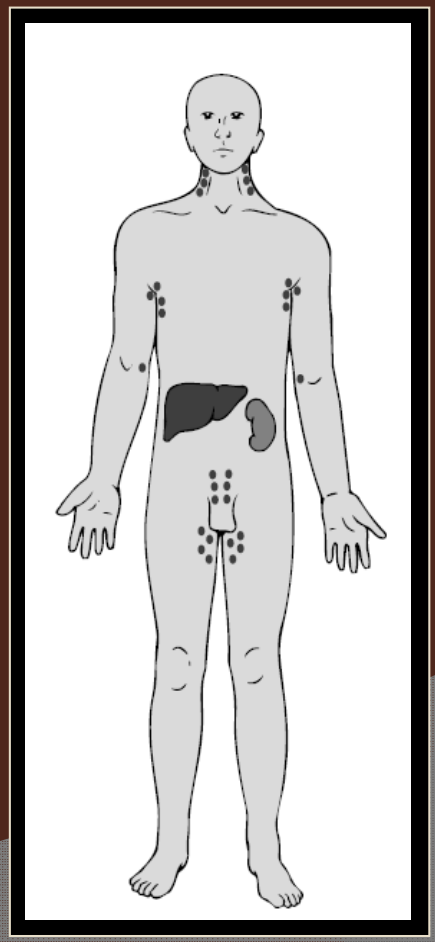
EVALUATION OF THE PATIENT contd.

Symptoms

- Easy fatiguability
- Lassitude
- Muscle cramps
- Postural dizziness
- Lethargy
- Syncope
- Persistent hypotension, shock, and death (in severe cases)

Clinical Signs to be looked for

- Skin / mucosal pallor,
- Palmar creases
- Bald tongue, Glossitis
- Mouth ulcers, Ang .stomatitis
- Koilonychia
- Jaundice, Purpura
- Lymphadenopathy
- Hepato-splenomegaly
- Bony Pain
- Sternal tenderness
- Gum hypertrophy.
- Knuckle hyperpigmentation
- Breathlessness
- Tachycardia, CHF
- Bleeding, Occult Blood



Laboratory Tests in Anemia Diagnosis

- I Complete blood count (CBC)
  - A. Red blood cell parameters
    - 1.R.B.C. count
    - 2.. Hemoglobin
    - 3.. Hematocrit
  - B. Red blood cell indices
    - 1. Mean cell volume (MCV)
    - 2. Mean cell hemoglobin (MCH)
    - 3. Mean cell hemoglobin concentration (MCHC)
    - 4. Red cell distribution width (RDW)
  - C. White blood cell count
    - 1. Cell differential
    - 2. Nuclear segmentation of neutrophils
  - D. Platelet count
  - E. Cell morphology
    - 1. Cell size,SHAPE
    - . Anisocytosis
    - Poikilocytosis.
    - 2.Hemoglobin content
    - 3. Polychromasia
- II. Reticulocyte count
- III. Iron supply studies
  - A. Serum iron
  - B. Total iron-binding capacity
  - C. Serum ferritin, marrow iron stain
- IV. Tests for hemolysis
  - Urine & Plasma Hb
  - DCT & ICT
  - PNH
  - Osmotic fragility
  - Hb. Electrophoresis
  - Tests for sickling
- V. Marrow examination
  - A. Aspirate
    - 1. M/E ratio
    - 2. Cell morphology
    - 3. Iron stain
  - B. Biopsy
    - 1. Cellularity
    - 2. Morphology

- Microcytic Hypochromic Anaemias

Iron Deficiency Anaemia

Causes

- Blood Loss
  - Gastrointestinal Tract
  - Menstrual Blood Loss
  - Urinary Blood Loss (Rare)
  - Blood in Sputum (Rarer)
- Increased Iron Utilization
  - Pregnancy
  - Infancy
  - Adolescence
  - Polycythemia Vera
- Malabsorption
  - Tropical Sprue

- Gastrectomy
- Chronic atrophic gastritis

- Dietary inadequacy (almost never sole cause)
- Combinations of above

CLINICAL FEATURES

- Specific Signs in IDA
  - Koilonychia
  - Brittle nails
  - Atrophy of tongue
  - Angular stomatitis
  - Brittle hair
  - Plumer Vinson Synd–dysphagia and glossitis

Iron Balance

Minimal loss 1 – 2mg/d

Total body iron of 2 g– females
4g---males

Erythropoietic iron requirement only 20mg/d

Important homeostatic mechanisms prevent excessive iron absorption in duodenum and regulate rate of iron release from RES

freeIron is toxic to human cells and essential for pathogens

Total body iron content is about 2 gm for women

6 gm for men.

- Approximately 80% of functional body iron is found in

  hemoglobin, myoglobin and iron-containing enzymes (e.g., catalase and cytochromes).

- The iron storage pool 15% to 20% of total body iron

  hemosiderin and ferritin-bound iron

- Iron is transported in the plasma by an iron-binding protein called transferrin.
- In normal persons, transferrin is about 33% saturated with iron, yielding

serum iron levels --120 µg/dL in men

--100 µg/dL in women.

Thus, the total iron-binding capacity of serum is in the range of 300 µg/dL to 350 µg/dL.

Cellular Iron Homeostasis

Concerned with each cells requirements for iron

Systemic Iron Homeostasis

Concerned with the body's need for iron

Cellular Iron Homeostasis

Concerned with each cells requirements for iron

Systemic Iron Homeostasis

Concerned with the body's need for iron

Overview of iron abs.

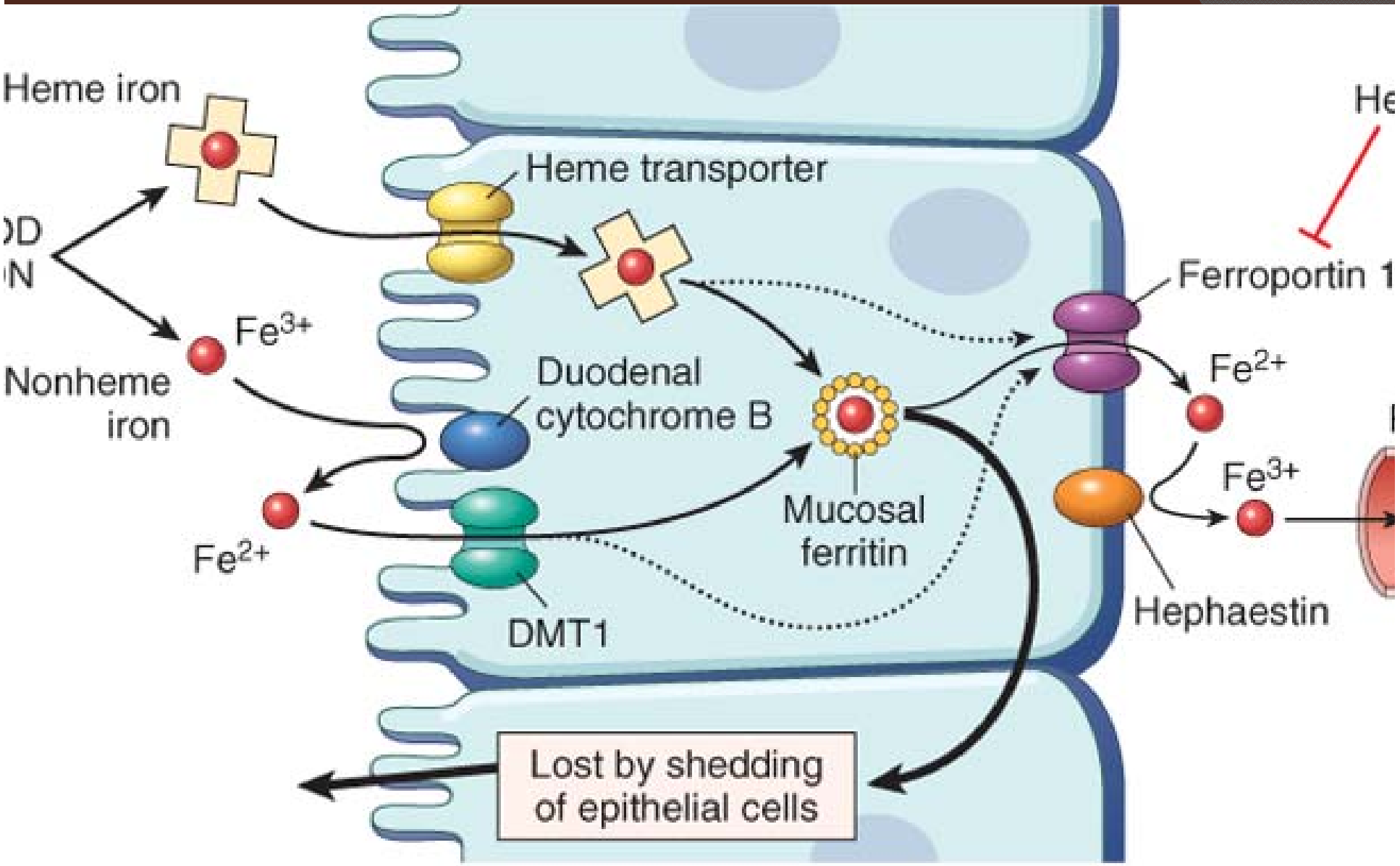
Iron Absorption (into enterocyte)

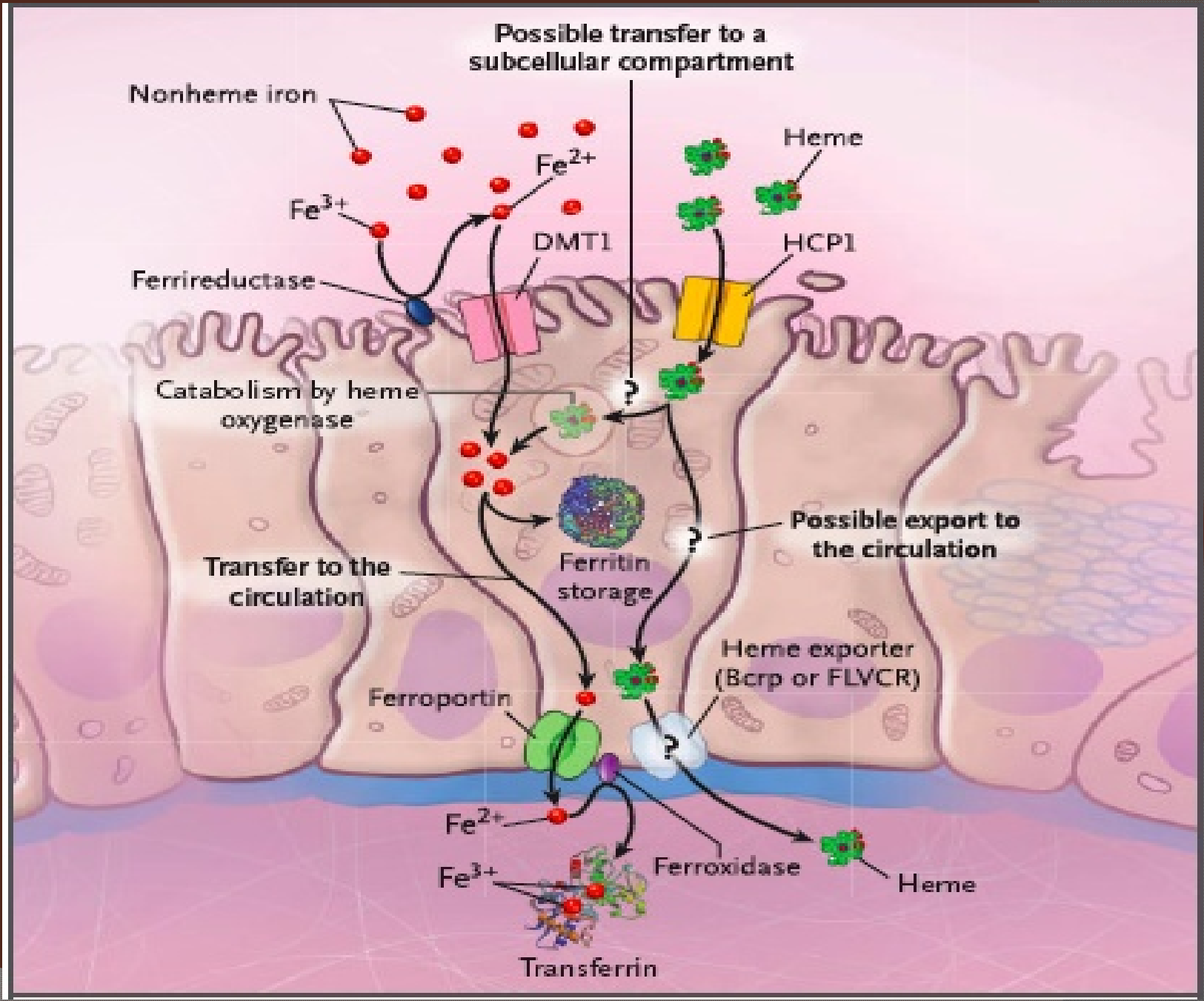
Luminal surface

- Dietary free iron (Fe3+) is reduced to Fe2+
- Occurs at brush border by duodenal ferric reductase (Dcytb)

Transluminal transport

- DMT1 (divalent metal transporter 1)
- Dietary haem iron via transporter and released from haem or absorbed into the circulation.




Possible transfer to a subcellular compartment
Nonheme iron
Fe^{2+}
Fe^{3+}
Heme
DMT1
HCP1
Ferrireductase
Catabolism by heme oxygenase
?
Ferritin storage
Possible export to the circulation
Transfer to the circulation
Heme exporter (Bcrp or FLVCR)
Ferroportin
Fe^{2+}
Fe^{3+}
Ferroxidase
Heme
Transferrin

Iron Absorption (out of enterocyte)

- Shed
- Basolateral absorption via ferroportin or haeme transporter.
- Hephaestin facilitates enterocyte iron release

Ferroportin

- Present on the basolateral membrane of enterocytes
- Present on macrophages and other RES cells
- Present on hepatocytes

Iron Tansport

Via transferrin

Iron Storage (Hepatic - major site)

Hepatic uptake of transferrin bound Fe via classic transferrin receptor TfR1 (& homologous TfR2)

Hepatocytes are storage reservoir for iron

Taking up dietary iron from portal blood

Releasing iron into the circulation via ferroportin in times of increased demand

Iron Utilisation

Erythropoiesis for haem synth / general cellular respiration via TfRs on erythroid precursors and other cells

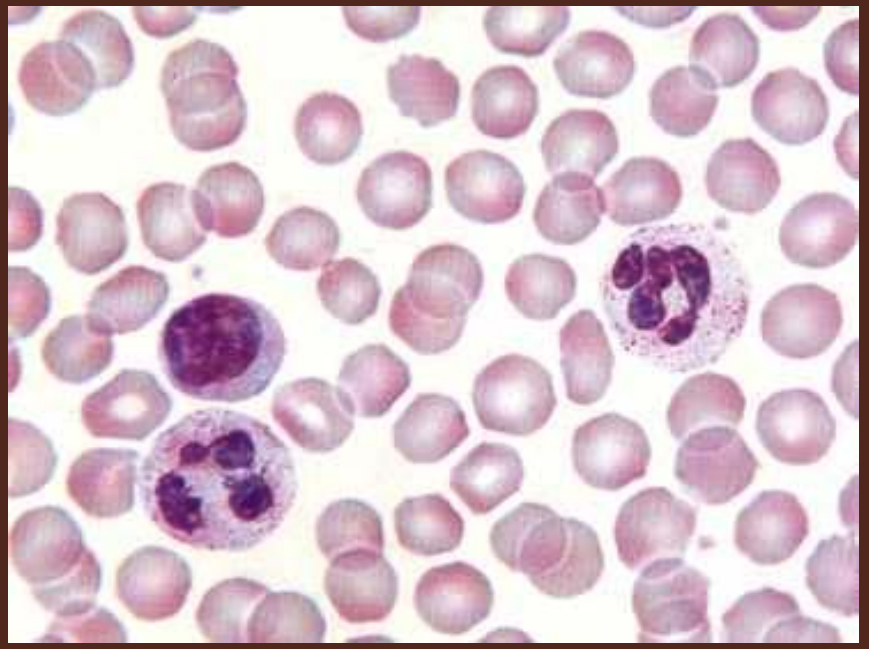
Hepcidin

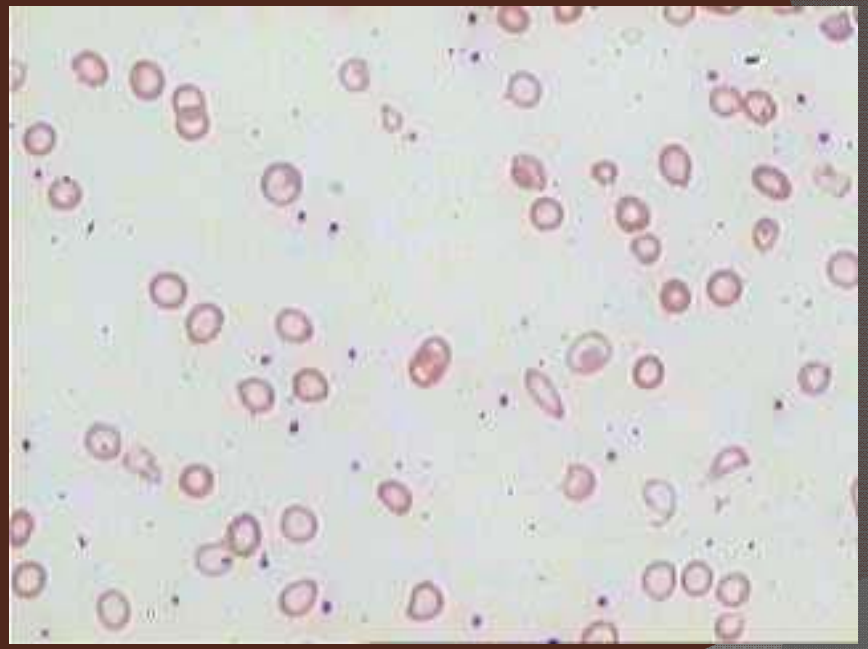
- 25 aa peptide. Identified 2000
- Antimicrobial activity. **Hep**atic bacterio**cid**al prote**in**
- Master iron regulatory hormone
- Cellular targets of hepcidin are villous enterocyte, RE macrophage and hepatocyte
- Factors regulating intestinal iron absorption also regulate the expression of hepcidin
  - Decreased iron stores
  - Increased erythropoietic activity
  - Anaemia
  - Hypoxia

Intestinal iron absorption varies inversely with liver hepcidin expression

Hepcidin decreases the functional activity of ferroportin by directly binding to it and causing it to be internalised from the cell surface and deregulated

- Decreases basolateral iron transfer and thus dietary iron absorption
- Decrease in iron export by hepatocyte and macrophage and a resultant increase in stored iron





Iron Studies

- Bone marrow aspirate

- Lab studies
  - Ferritin
  - Serum Iron
  - Total Iron Binding Capacity
  - Transferrin Saturation

Lab diagnosis

- Low transferrin Saturation (Fe/TIBC ratio)
  - ↓ Fe (not reliable)
  - ↑ **TIBC**
- Fe/TIBC (% saturation) <15%
- Smear:
  - hypochromic and microcytic (low MCV) RBCs,
  - platelet count is often elevated
- ↓ Ferritin: a measure of total body iron stores, but also an acute phase reactant
- **<15μg/l = Fe deficiency, >150 μg/l = Not Fe deficiency**
  15-150 μg/l = ? BM bx: absent Fe stores
  - Gold standard
- Therapeutic Trial of Oral Iron

Response to therapy (cont'd)

- Approx. 30 days
  - Increased Hemoglobin
- 2 to 3 months
  - Repletion of iron stores
- Treat for a total of at least 3 months

Treatment failure

- Poor compliance
  - 10% GI side effects
  - Poor taste
- Ongoing blood loss
- Malabsorption
- Wrong diagnosis
  - Thalassemia minor
  - AOCD
  - Ass.folate or B12 deficiency
  - Sideroblastic Anaemia

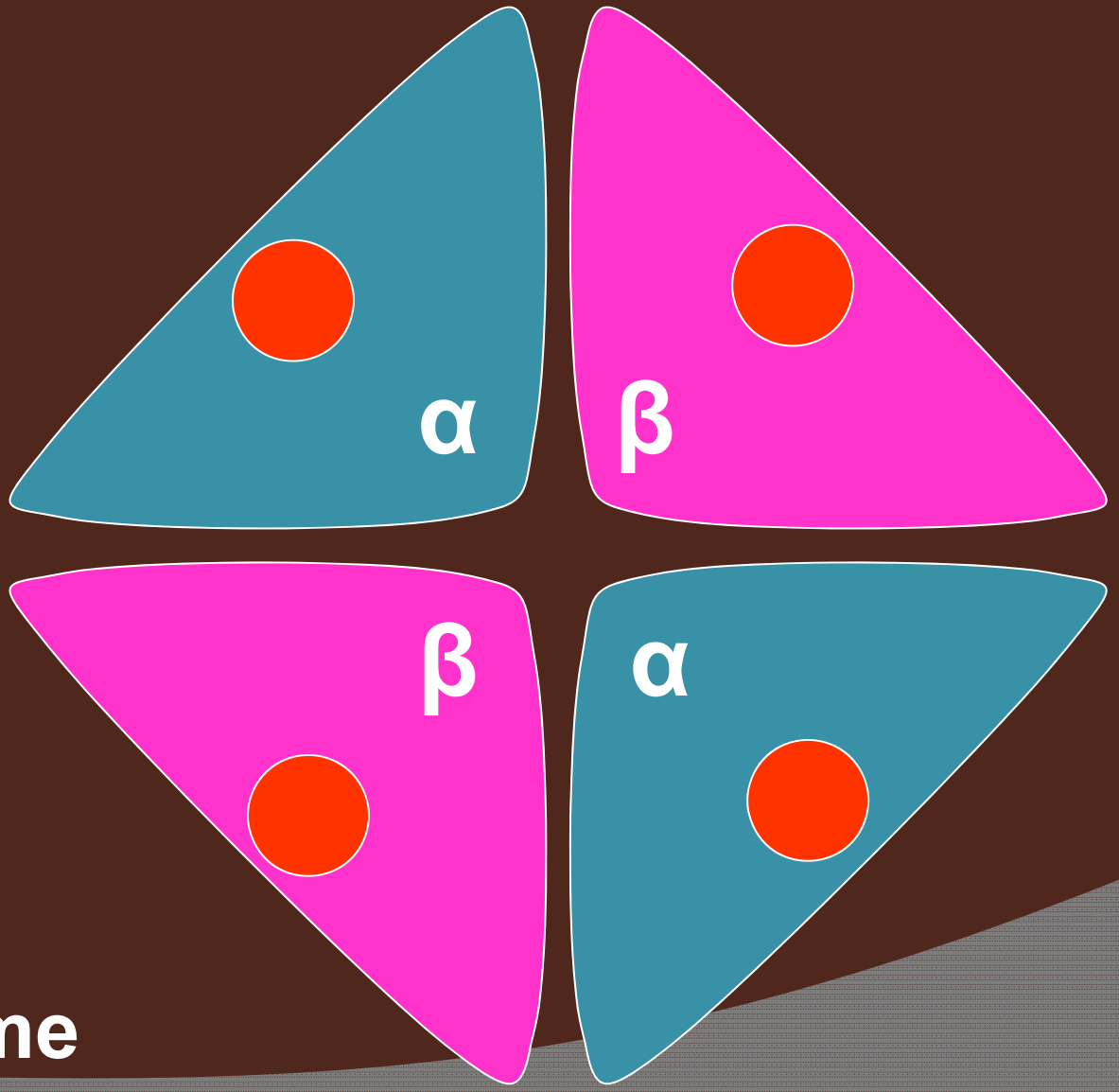
Thalassemia :

- Haemoglobinopathies
- Hereditary disorders that can result in moderate to severe anemia
  - ✓ Basic defect is *reduced production* of selected globin chains
  - ✓ Imbalance of globin chain synthesis leads to depression of hemoglobin production and precipitation of excess globin (toxic)
  - ✓ “Ineffective erythropoiesis”
  - ✓ haemolysis

Demographics: Thalassemia

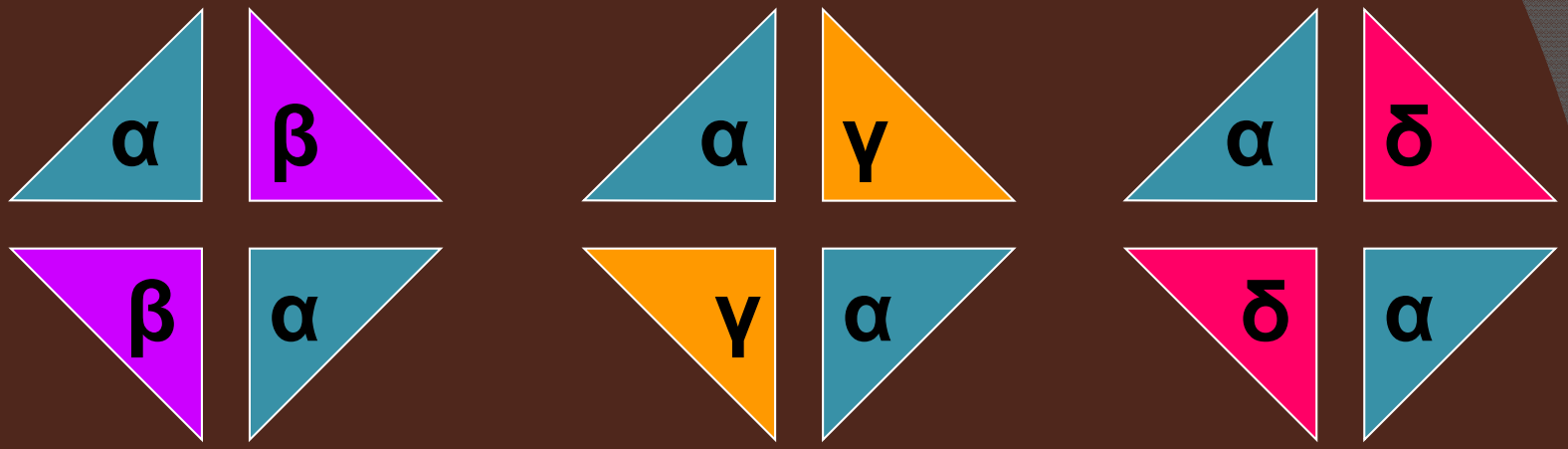
- Found most frequently in the Mediterranean, Africa, Western and Southeast Asia, India and Burma
- Distribution parallels that of Plasmodium falciparum

Hemoglobin structure



heme

Hemoglobins in normal adults



HbA	HbF	HbA_2
98%	~1%	<3.5%

Symbolism
Alpha Thalassemia

- Greek letter used to designate globin chain:

α

Symbolism
Alpha Thalassemia

/ : Indicates division between genes inherited from both parents:

$\alpha\alpha/\alpha\alpha$

- Each chromosome 16 carries 2 genes. Therefore the total complement of α genes in an individual is 4

Classification & Terminology

Alpha Thalassemia

- Normal $\alpha\alpha/\alpha\alpha$
- Silent carrier $-\ \alpha/\alpha\alpha$
- Minor $-\alpha/-\alpha$

  $--/\alpha\alpha$
- Hb H disease $--/-\alpha$
- Barts hydrops fetalis $--/--$

α Thalassemia

- Hb H
- 3 gene deletion
- β^4 tetramer
- Associated with --/-α thalassemia

α Thalassemia

- Hb Barts & hydrops fetalis
  - 4 gene deletion
  - Barts is a γ^4 tetramer
  - Associated with --/--
  - Lethal
  - High concentrations are capable of sickling

α Thalassemia

- Hb H
- 3 gene deletion
- β^4 tetramer
- Associated with --/-α thalassemia

α Thalassemia

- 2 Gene deletion
  - Microcytosis
  - Mild anemia
- 1 Gene deletion
  - Normal blood picture

Alpha thalassemia

αα/αα	Normal
αα/α-	Mild microcytosis
αα/- - α-/α-	Mild microcytosis
α-/- -	Hemoglobin H disease
- -/- -	Hemoglobin Barts – Hydrops Fetalis

Symbolism
Other Thalassemia

- Greek letter used to designate globin chain:

β

Symbolism
Other Thalassemia

$^{+}$: Indicates diminished, but some production of globin chain by gene:

β^{+}

Symbolism
Other Thalassemia

- ^{0} :Indicates no production of globin chain by gene:

β^0

Symbolism
Other Thalassemia

Superscript ^{T} denotes nonfunctioning gene:

$$\alpha^{T}$$

Classification & Terminology

Beta Thalassemia

- Normal β/β
- Minor β/β^0
  β/β^+
- Intermedia β^0/β^+
- Major β^0/β^0
  β^+/β^+

Molecular basis

- Most of the mutations in β-thalassemia fall into one of three molecular subtypes:

----The promoter region controls the initiation and rate of transcription. Some mutations lie within promoter regions and typically lead to reduced globin gene transcription. Because some β-globin is synthesized, such alleles are designated β+

-

- Mutations in the translator region

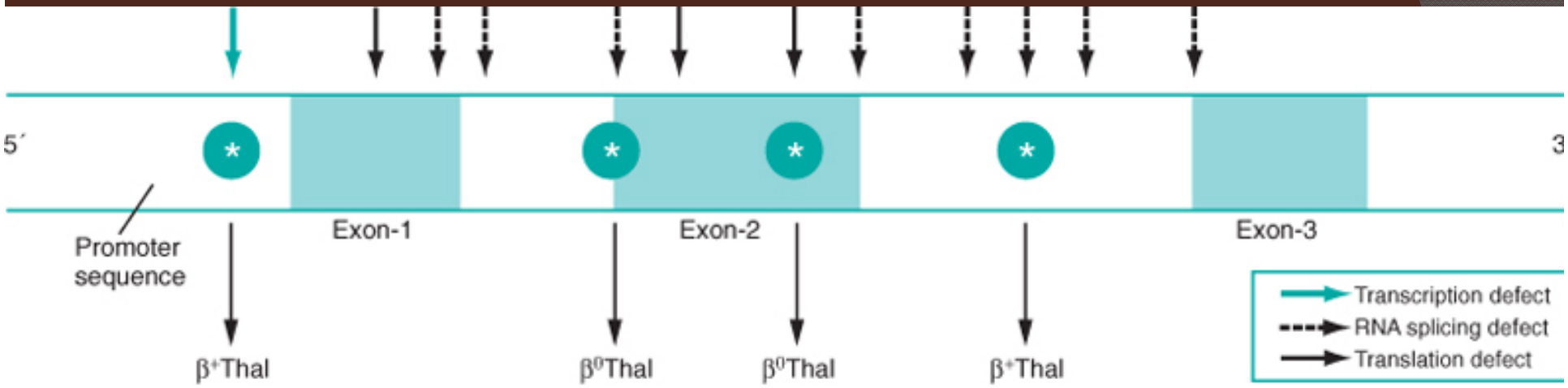
in some cases a single-nucleotide change in one of the exons leads to the formation of a termination, or "stop" codon, which interrupts translation of β-globin messenger RNA (mRNA) and completely prevents the synthesis of β-globin. Such alleles are designated designated β0.Mutations

Mutations at splicing sites that lead to aberrant mRNA processing are the most common cause ofthalassemia.

Most of these affect introns, but some have been located within exons.

mutation alters the normal splice junctions, splicing does not occur, and all of the mRNA formed is abnormal. Unspliced mRNA is degraded within the nucleus, and no β-globin is made.

However, some mutations affect the introns at locations away from the normal intron-exon splice junction. These mutations create new sites that are substrates for the action of splicing enzymes at abnormal locations-within an intron mutations can create either β0 or β+ alleles.



Thalassemias

- Beta Thalassaemia Minor (trait)
  - Asymptomatic
  - Mild/absent anemia
  - Decreased MCV/MCH
  - Differentiated from IDA by lab investigations
  - Hb electrophoresis shows
    - Increased α2
    - may/may not increased Hb F

Thalassemias

- Beta Thalassaemia Intermediate
  - Symptomatic
  - Moderate anemia (7-10g/dl)
  - May/may not require transfusion
  - Splenomegaly
  - Bone deformities
  - Leg ulcers
  - Gallstones

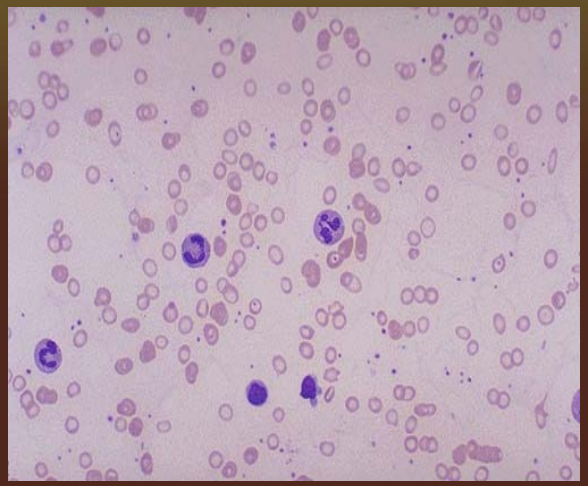
Thalassemias

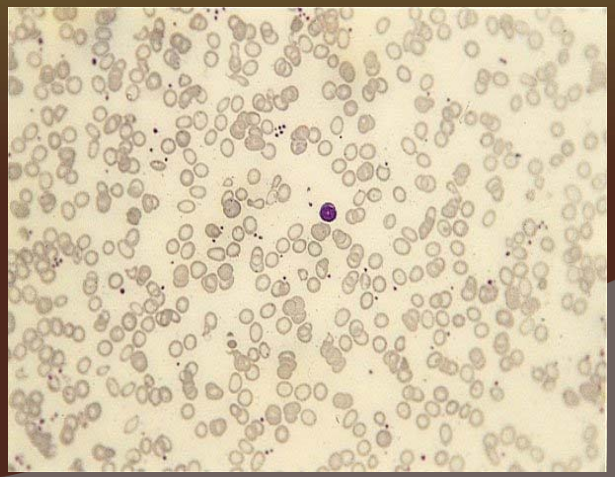
- Beta Thalassaemia Intermediate
  - Symptomatic
  - Moderate anemia (7-10g/dl)
  - May/may not require transfusion
  - Splenomegaly
  - Bone deformities
  - Leg ulcers
  - Gallstones

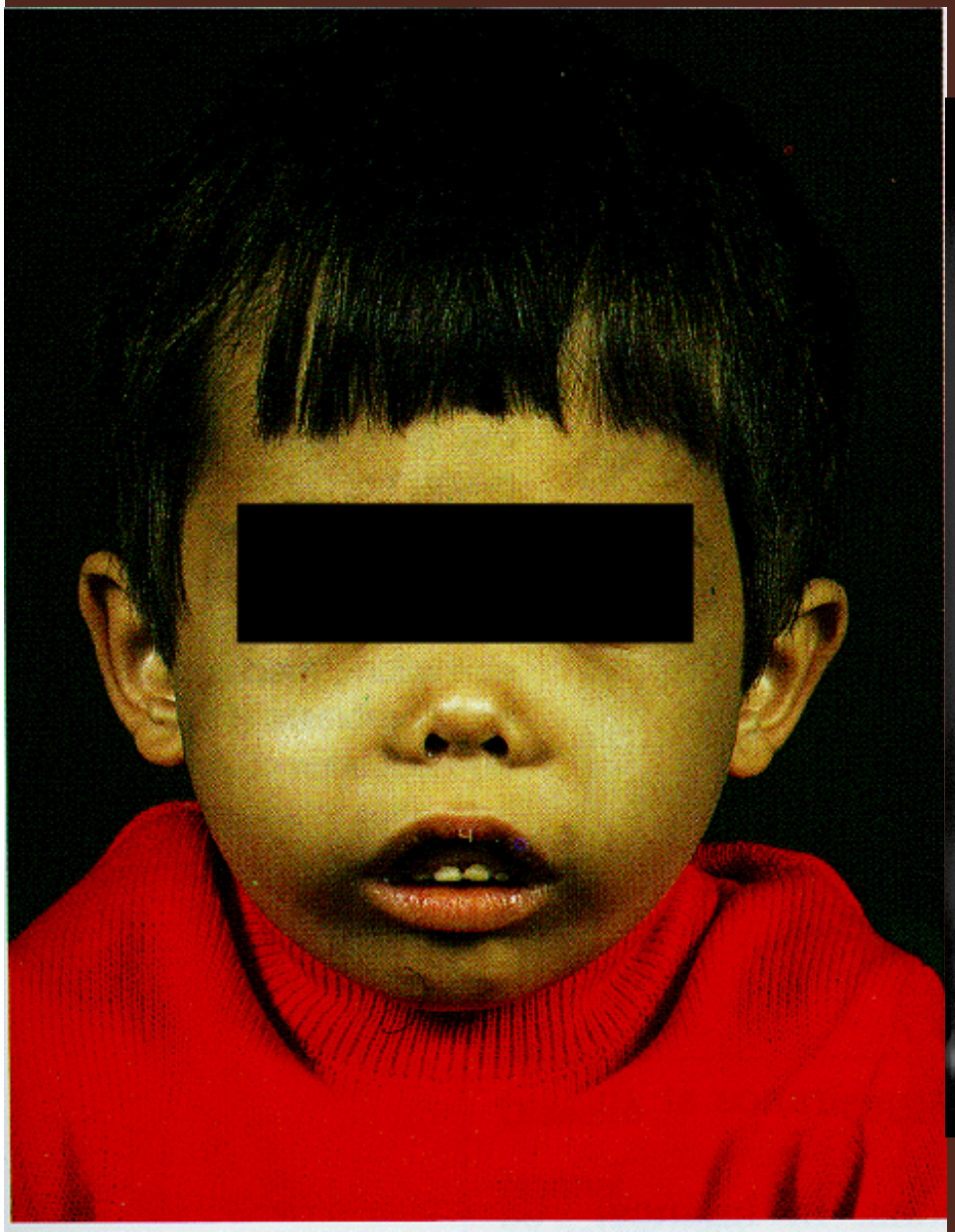
Thalassemias

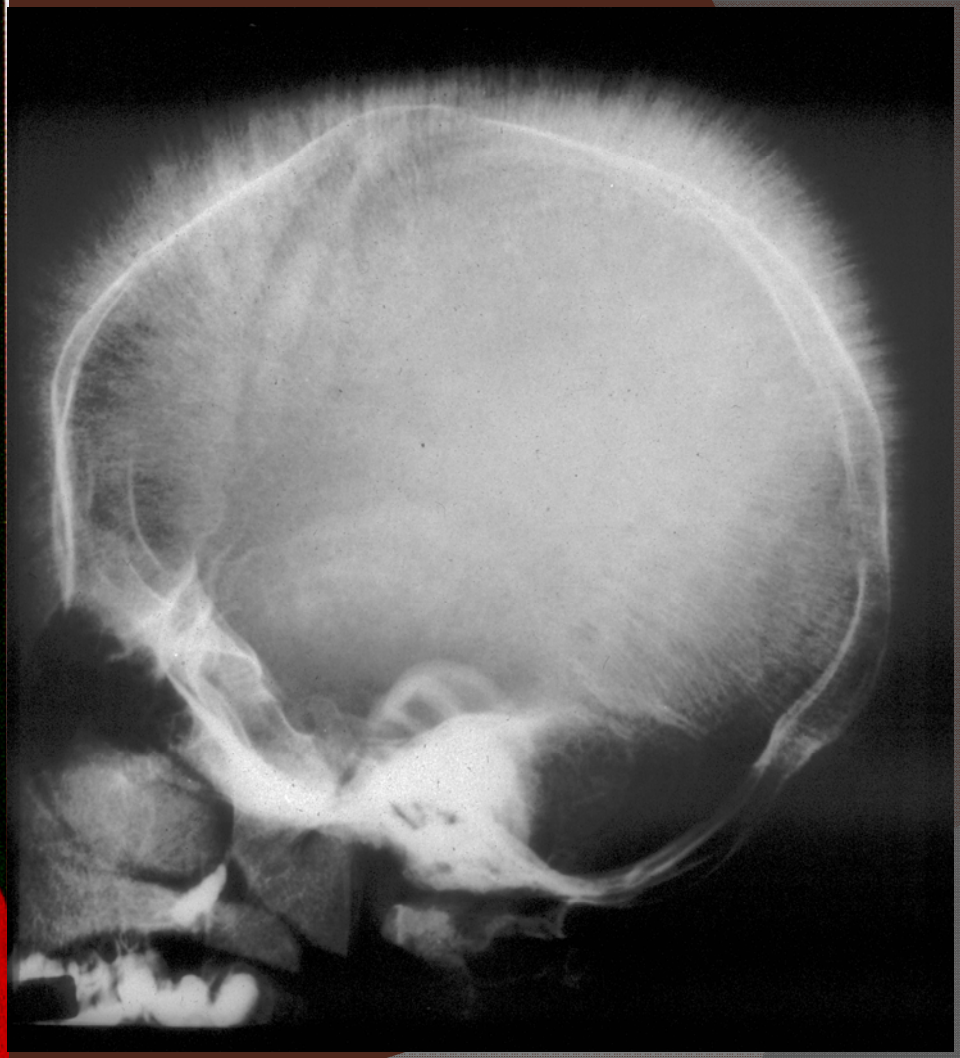
- Beta Thalassaemia Major (Cooley's anemia)
  - Homozygous
  - Failure to thrive
  - Recurrent infections
  - Severe anemia in 3-6 m age
  - Extramedulary hemopoisis
    - Hepatosplenomegaly
    - Bone expansion
  - Hair on end skull x-ray appearance

	Iron deficiency	Thalassemia
MCV	Low	Low
RDW	Increased	Slight increase to normal
RBC count	Normal, slightly decreased	Increased
Others	Target cells +/-	Target cells ++









Primary Laboratory Investigation Thalassemia

- Severe cases present with
  - Microcytosis
  - Hypochromia
  - Poikilocytosis
  - RBC counts higher than expected for the level of anemia

- Serum iron/Ferritin-increased
- β-thal will have an abnormal Hb electrophoresis (↑HbA_2, ↑HbF)
- The more severe α-thal syndromes can have HbH inclusions in RBCs

Course and Treatment Thalassemia

- Untreated β thalassemiaMajor: Death in first or second decade of life

  Intermedia: Usually normal life span

- Minor/Minima: Normal life span

✓Tx:

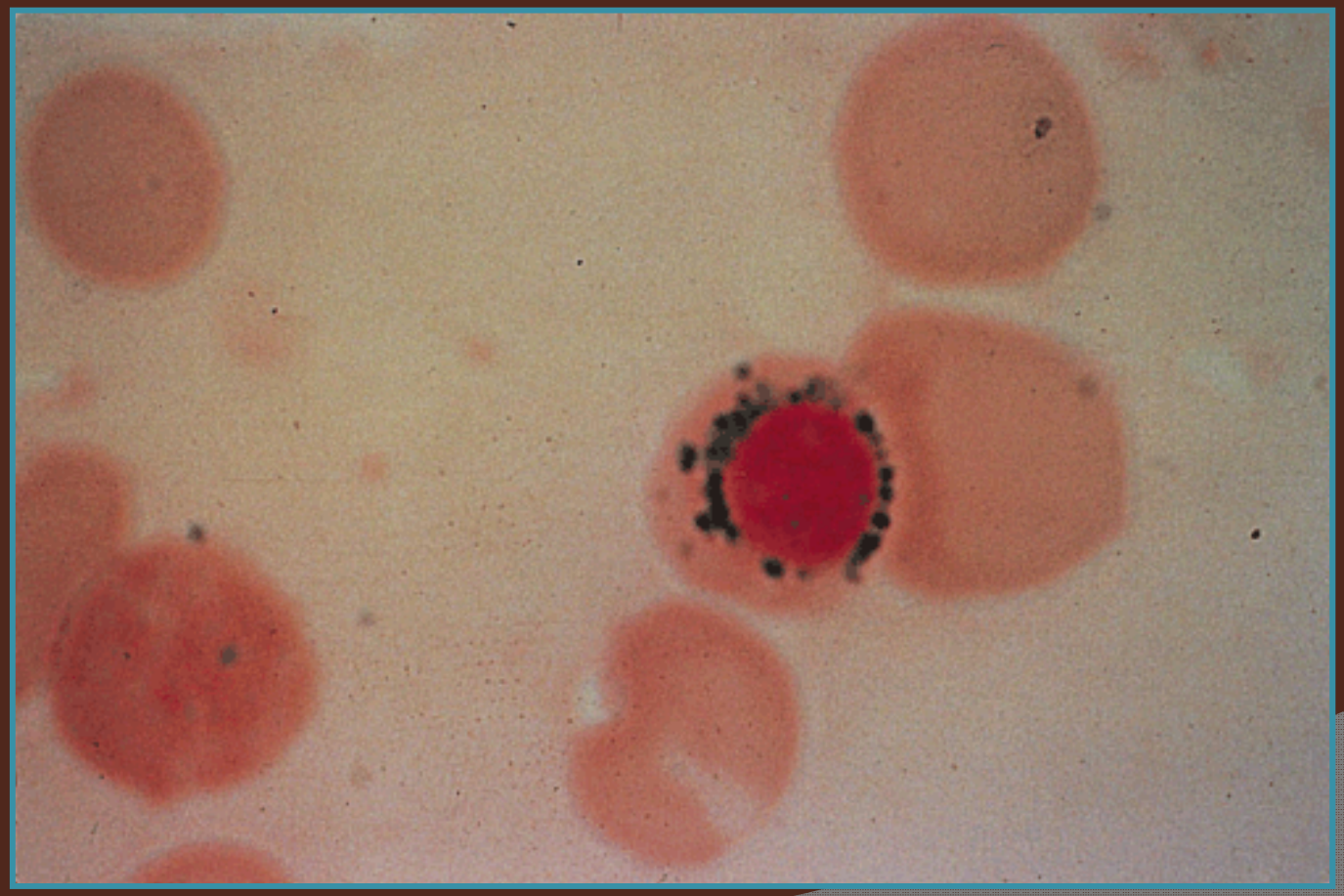
- Mild: None
- Severe: RBC transfusions + Fe chelation, Stem cell transplants

Sideroblastic Anemia

- Inherited or acquired
- Refractory anemia
- Ring sideroblasts – disordered haem synthesis
- Primary acquired is myelodysplastic syndrome
- May be drug induced
  - INH
  - Alcohol
  - Lead toxicity

Ringed Sideroblasts in BM

Prussian Blue Stain



Anemia of Lead Poisoning

- Symptoms
  - NONE!
  - With levels >60 µg/dl
    - lead colic
    - constipation
    - anorexia
    - hyperirritability
    - anemia

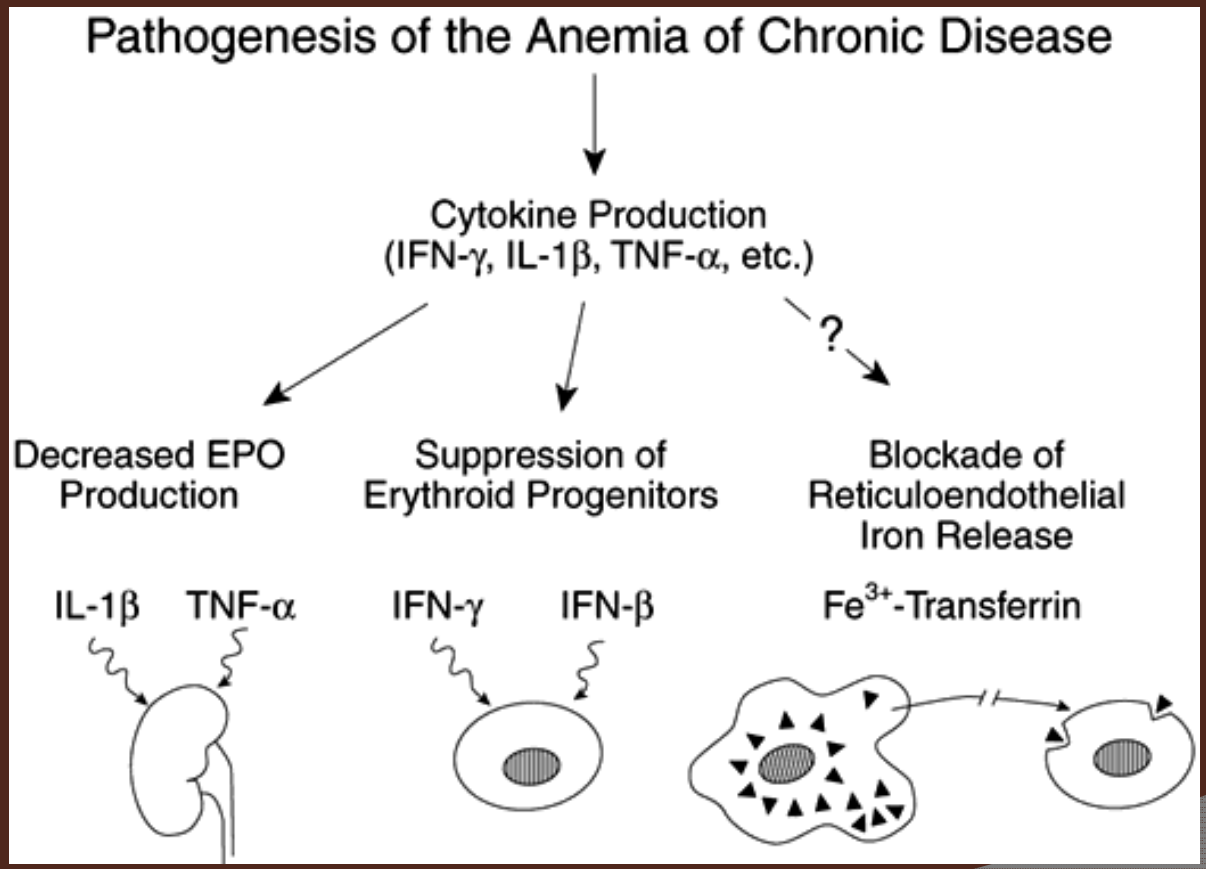
Anemia of Lead Poisoning

- characteristics of smear
  - hypochromic
  - normocytic or microcytic
  - siderocytes
  - basophilic stippling

Anaemia of Chronic Disease

- Malignancy
- Collagen Vascular Disease
  - Rheumatoid Arthritis
  - SLE
  - Polymyositis
  - Polyarteritis Nodosa

- IBD
  - Ulcerative Colitis
  - Crohn's Disease
- Chronic Infections
  - HIV, Osteomyelitis
  - Tuberculosis


Pathogenesis of the Anemia of Chronic Disease
Cytokine Production
(IFN-γ, IL-1β, TNF-α, etc.)
?
Decreased EPO Production
Suppression of Erythroid Progenitors
Blockade of Reticuloendothelial Iron Release
IL-1β
TNF-α
IFN-γ
IFN-β
Fe3+-Transferrin

Anemia of Chronic Disease (con't)

- PBS:
  - If mild: normocytic, normochromic
  - Moderate: microcytic, normochromic
  - Severe: microcytic, hypochromic
- Bone Marrow:
  - Normal or increased Fe stores

Test	IDA	THAL . MINOR	AOCD	SIDEROBLASTIC ANAEMIA
serum iron	low	normal /increased	low	increased
TIBC	high	normal	normal or low	normal
transf. sat.	low	normal	low	normal
serum ferritin	low	normal/increased	normal or increased	normal or increased
marrow iron	absent	normal/increased	normal or increased	normal or increased

MACROCYTIC ANAEMIAS

Macrocytic Anemia

High	MCV
High	MCH
Normal	MCHC

Macrocytic Anemia

High	MCV
High	MCH
Normal	MCHC

Macrocytic Anemia

Megaloblastic : defective DNA synthesis

Non-megaloblastic : numerous mechanisms

Nutritional Requirements for Hematopoiesis

Metals : iron copper cobalt

B_{12} and Folate

Other vitamins: B_6, A, E, C
Riboflavin, Niacin

Causes of Megaloblastic Anaemia

- *Causes OF* COBALAMIN (B12DEFICIENCY)
- Gastric Failure
  - Pernicious Anemia
  - Total gastrectomy
- Ileal Failure
  - Regional enteritis (Crohn's disease)
  - Ileal resection
  - Tropical sprue
- Competing organisms
  - Bacterial overgrowth (Blind loop)
  - Diphyllobothrium latum

- *Cause OF* FOLATE DEFICIENCY
- Folate-poor diet
  - Alcoholism
  - Severe poverty
- Increased folate requirement
  - Pregnancy
  - Severe hemolytic anemia
  - Severe Psoriasis
- Drug therapy
- Malabsorption
- Tropical sprue
- inhibitors of DNA SYN./Folate metabolism
  - MTX

Others

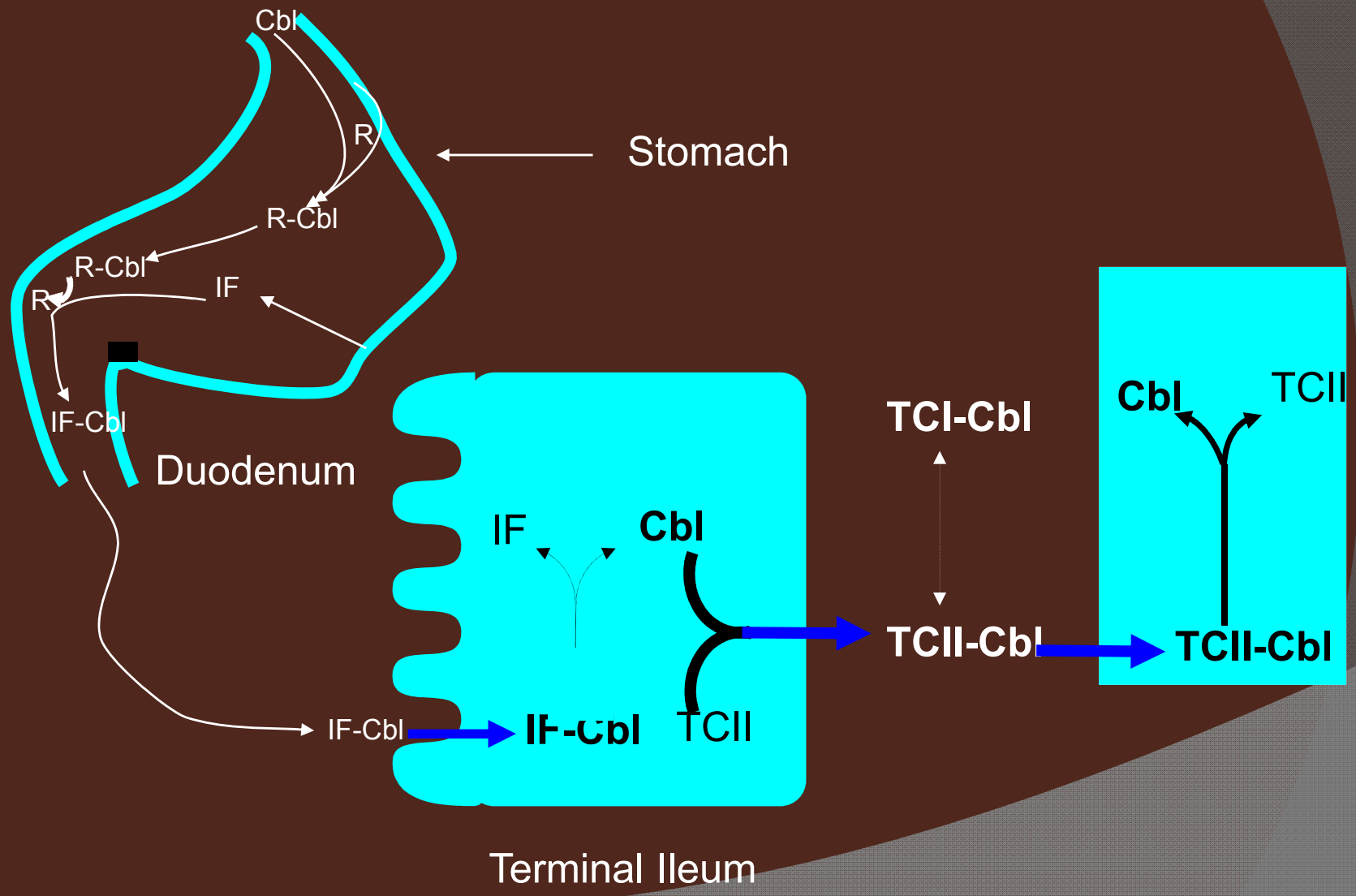
- ERRORS OF METABOLISM

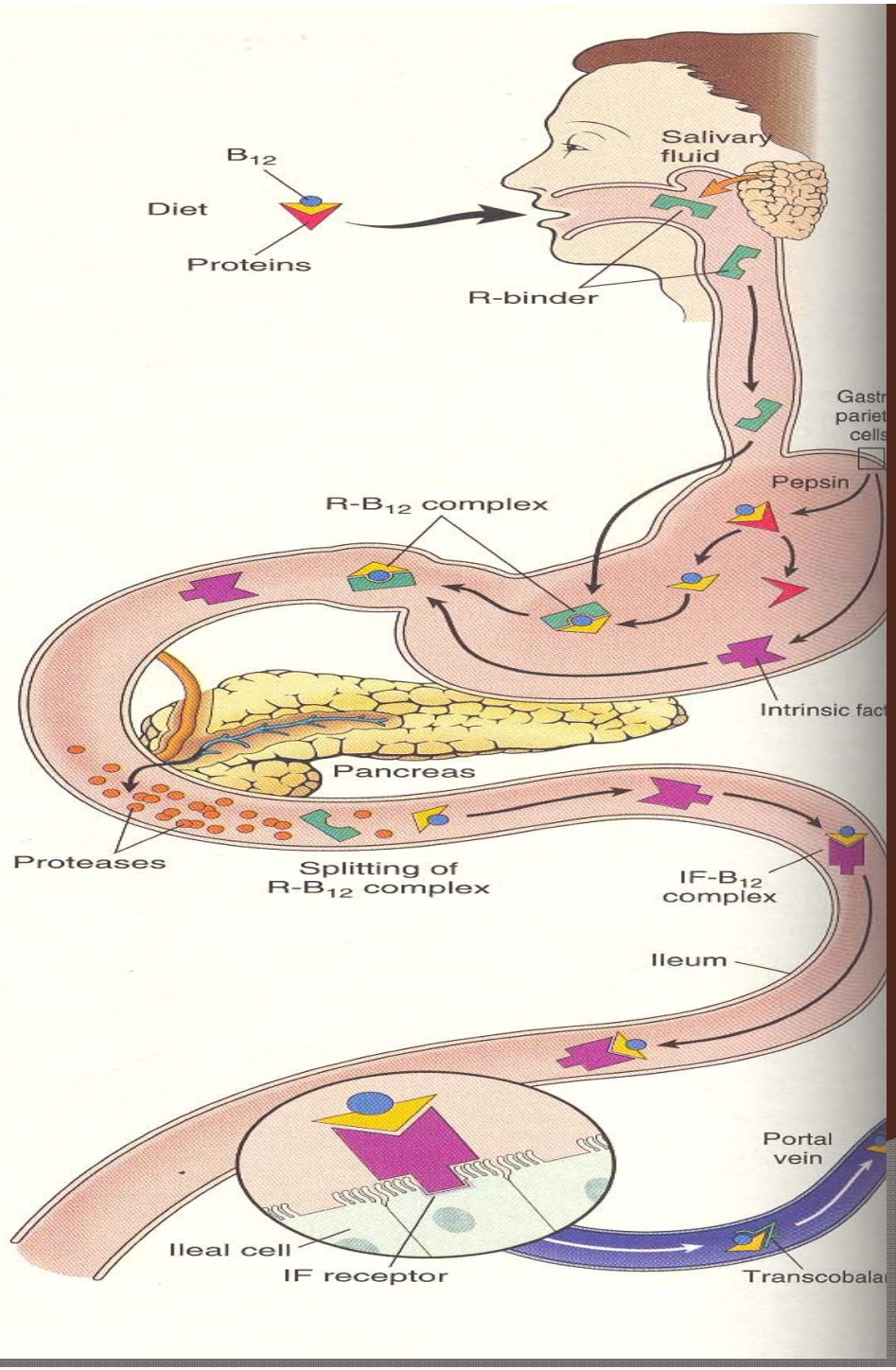
Non-Megaloblastic Anaemia

- AICOHOL
- Liver ds
- Aplastic anaemia
- Hypothyroidsm
- MDS
- MPD

	B 12	Folic Acid
Source	Vegetables-poor	rich
Daily req. (ADULTS)	2-4µg	200 µg
ADULTS Daily intake	5-30µg	100-500µg
Site of absorption	ileum	Dud. &jejunum
Body stores **Serum levels**	2-5mg 160-1000 ng/l	5-20mg 2-15µg/l RBC folate-160-640µg/l

GI ABSORPTION OF COBALAMIN




B_{12}
Diet
Proteins
Salivary fluid
R-binder
Gastr
pariet
cells
Pepsin
R-B_{12} complex
Intrinsic fact
Pancreas
Proteases
Splitting of
R-B_{12} complex
IF-B_{12}
complex
Ileum
Portal
vein
Ileal cell
IF receptor
Transcobala

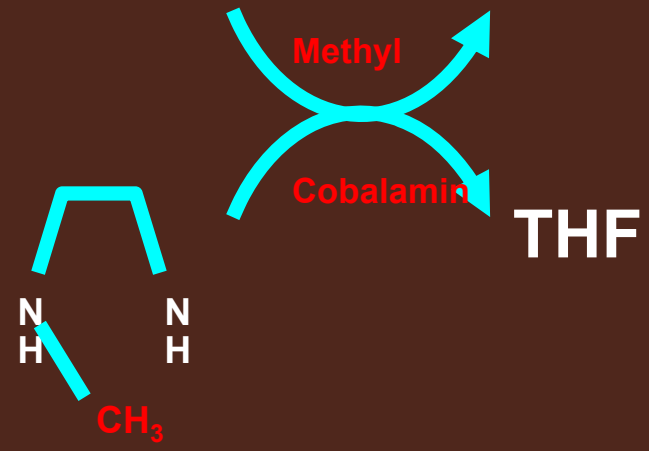
COBALAMIN (Vitamin B_{12})

Functions

- Folate metabolism - Required for demethylation of methyl-THF
- Degradation of certain fatty acids
- Conversion of methylmalonyl CoA to succinyl CoA

COBALAMIN REACTIONS

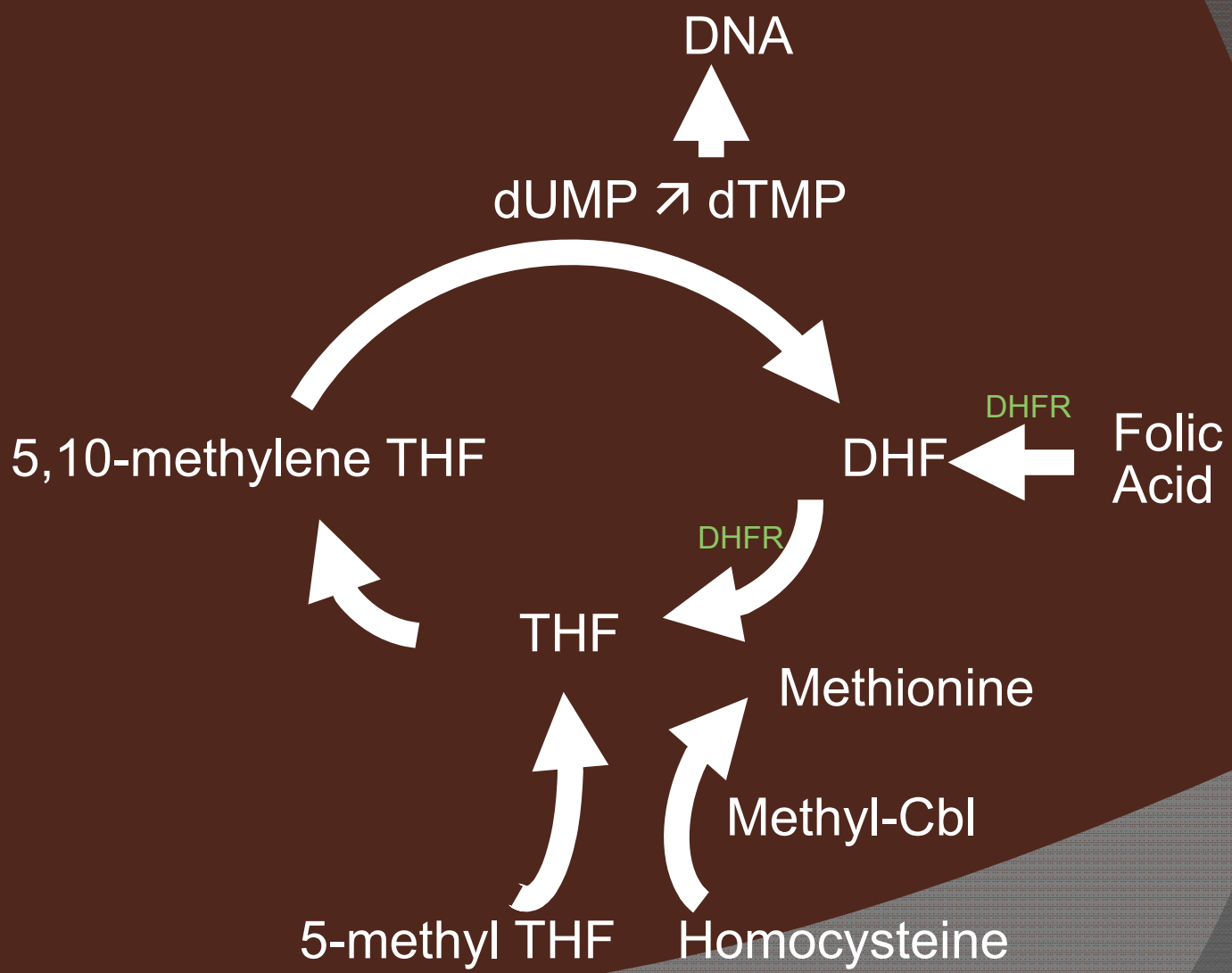
Homocysteine Methionine



Methylmalonyl CoA

Succinyl CoA

THYMIDILATE SYNTHESIS



Impaired DNA synthesis

- B12 /folic acid, coenzymes in DNA synth
- defective nuclear maturation
- asynchrony between nuclear and cytoplasmic maturation
- ineffective granulopoiesis, and thrombopoiesis >>pancytopenia

COBALAMIN DEFICIENCY

Peripheral Manifestations

- Megaloblastic anemia - Indistinguishable from folate deficiency & due to intracellular folate deficiency
- Stomatitis/glossitis
- GI Mucosa alterations
- Can correct all of the above with high dose folate;

DON'T DO THIS!!!!!

COBALAMIN DEFICIENCY

Manifestations-Central

- Both brain and spinal cord
- Brain:
  - Dementia
  - Psychological disturbances
- Spinal cord:
  - Demyelinating disease
  - Loss of posterior & lateral columns- hence name "Combined system disease"
- Neurologic disease stabilized with treatment, but usually not reversed
- Treatment with folate does nothing for neurologic disease

COBALAMIN DEFICIENCY

Usual Sequence of Events

- Serum homocysteine & methylmalonic acid rise
- Serum cobalamin falls
- MCV rises; neutrophil hypersegmentation
- MCV rises above normal
- Anemia
- Symptoms

Folate Deficiency

Hematologic features : same as P.A.

Clinical Picture : no neurologic findings

Folate Deficiency Diagnosis

Dietary history

Clinical conditions

- pregnancy
- malabsorption (sprue)
- hemolytic anemia
- drugs

Laboratory

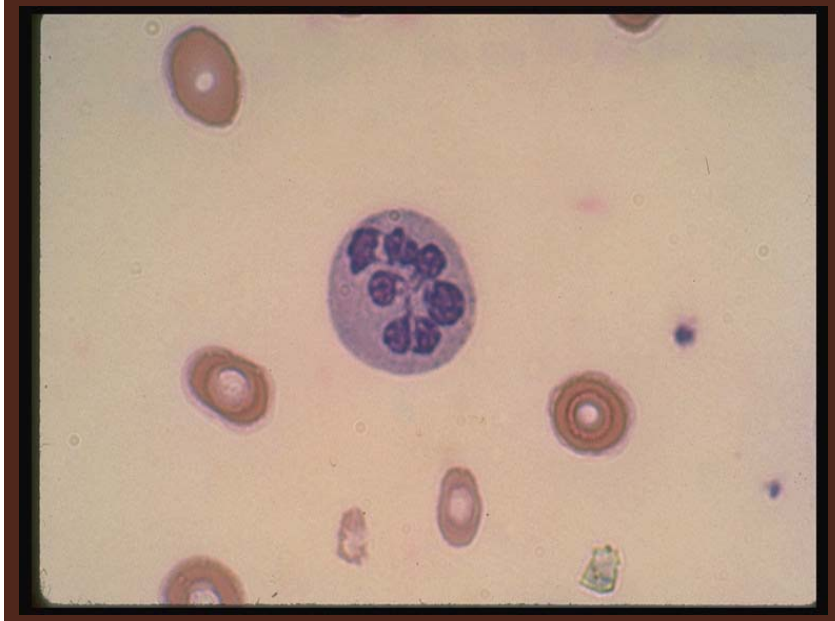
- serum or red cell folate levels

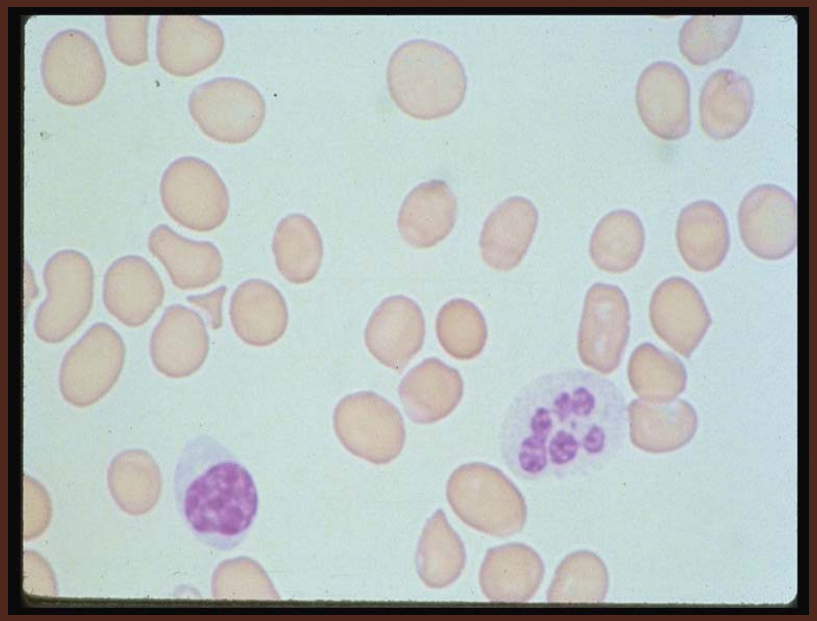
Red cell folate

- more informative than serum folate.
- Red cell folate reflects the body's stores of folate when the red cells were produced whereas serum folate reflects only recent folate intake and absorption

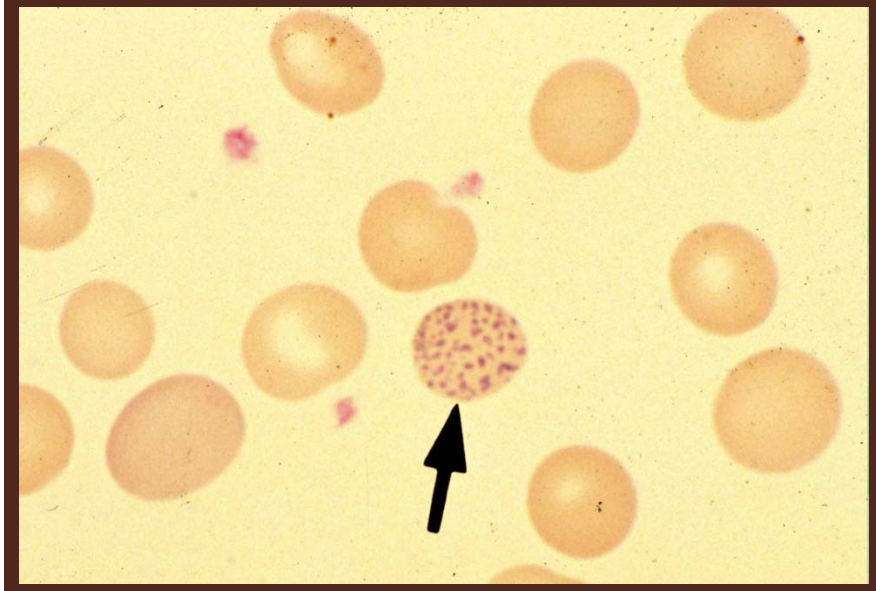
MEGALOBLASTIC ANEMIA

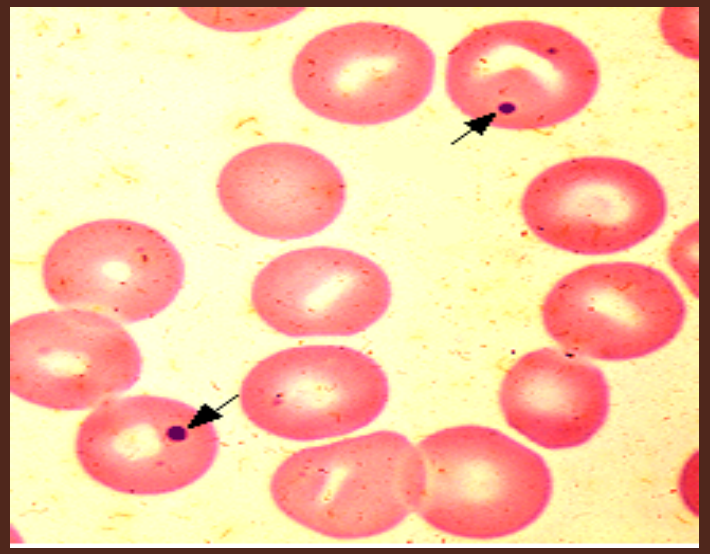
- Trademark cell: Oval macrocyte, (MCV > 100 fl)
- Hypersegmented neutrophils - 98%
- Pancytopenia, esp if anemia severe
- Reticulocytopenia
- LDH elevated (90%)
- Serum Fe normal or elevated
- Serum B_{12} or folate low
- Marrow ➧ classic megaloblastic changes

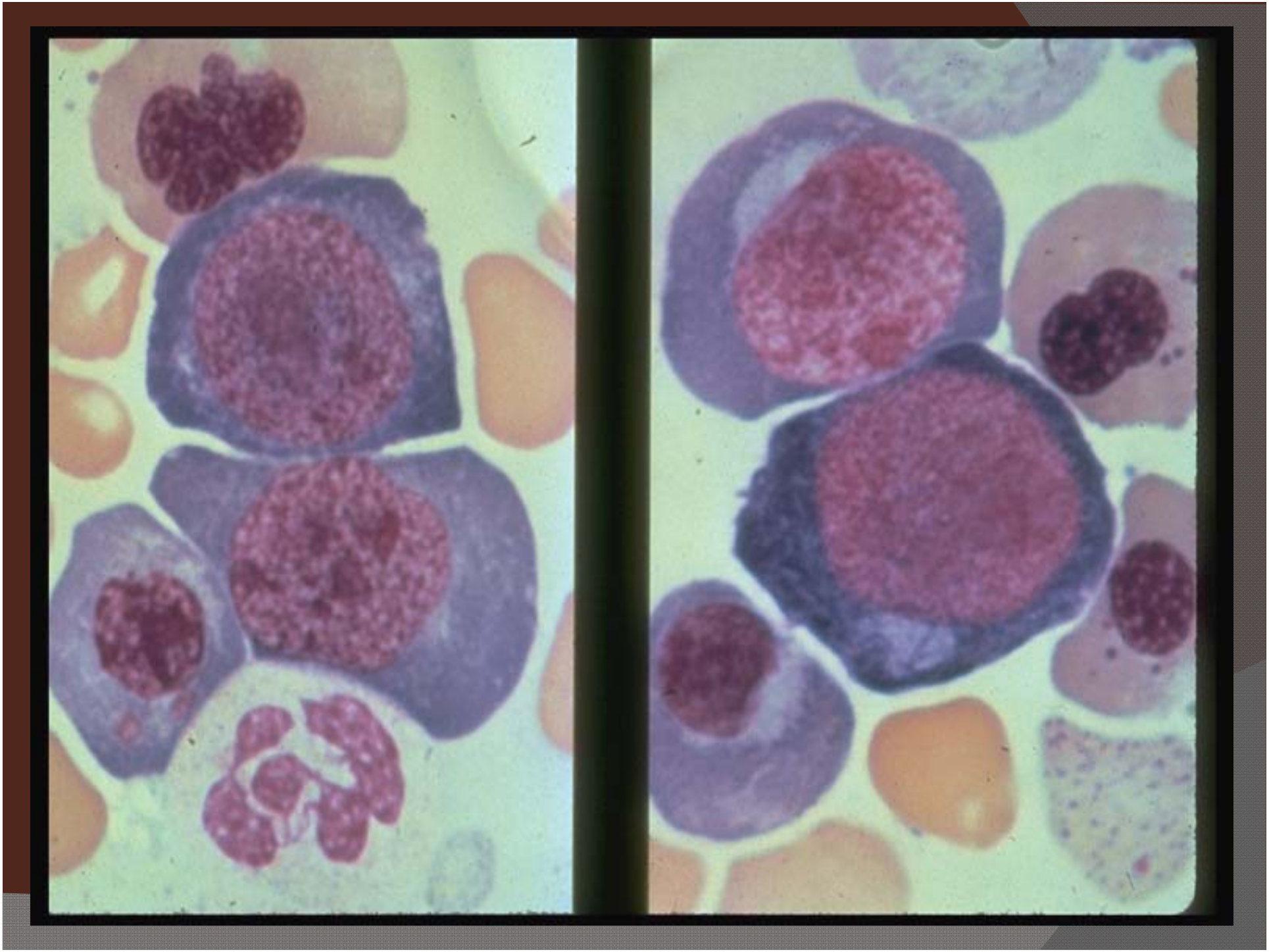




Basophilic stippling







MEGALOBLASTIC ANEMIA

Diagnosis /Therapy

- Draw levels at first suspicion of problem,BEFORE ANY THERAPY
- Once levels drawn, begin treatment with both B_{12} and folate
- Reticuiocyte response-3-4 days.peak 7days
- Once levels are back, can stop the normal vitamin
- Transfusions to be avoided unless hemodynamic compromise is present, or patient having angina

PERNICIOUS ANEMIA

- Autoimmune destruction of parietal cells
- Antibodies vs. parietal cells, intrinsic factor
- Achlorhydria is universal
- Increased incidence of gastric cancer
- Increased incidence American blacks, northern Europeans
- Often associated with other immune diseases
  (eg Hashimoto's thyroiditis)

Pernicious Anemia - Diagnosis

- History and Physical
  - glossitis
  - pallor
  - neurologic exam
- Laboratory
  - blood smear
  - antibody assays
  - B_{12} level
- Other
  - Schilling test

Schilling Test

- Pernicious Anaemia- ST shows reduced absorption of oral vitamin B12 that is corrected if the test is repeated with the addition of oral intrinsic factor.
- small bowel B12 malabsorption there is no correction.

Schilling Test

First stage :

1. Inject B_{12} IM (1,000 ug) to saturate transcobalamin II

2. Administer oral B_{12} - radiolabeled

3. Collect 24 h urine

4. Measure radioactivity in urine

Schilling Test

First stage :

1. Inject B_{12} IM (1,000 ug) to saturate transcobalamin II

2. Administer oral B_{12} - radiolabeled

3. Collect 24 h urine

4. Measure radioactivity in urine

Antibodies

- Testing for antibodies to gastric parietal cells is a sensitive (90%) test for pernicious anaemia but is lacking in specificity.
- Intrinsic factor antibodies has much better specificity although sensitivity (50%) is considerably less.

Haemolytic Anaemias

Hemolytic Anaemia

Anemia of increased RBC destruction

- Normochromic, normocytic anemia
- Shortened RBC survival
- Reticulocytosis – due to RBC destruction
- Will not be symptomatic until the RBC life span is reduced to 20 days – BM compensates 6 times

Haemolysis

- Causes
  - Intracorpuscular
  - Extracorpuscular
- Sites
  - Intravascular
  - Extravascular

Sites of Destruction

- Intravascular
  - severe RBC destruction
  - immediate lysis in intravascular space
- Extravascular
  - less severe RBC damage
  - cells destroyed in monocyte /macrophage RE system, spleen,liver and lymph nodes

HEMOLYTIC ANEMIA

- INHERETED HEMOLYTIC ANEMIA
- ACQUIRED HEMOLYTIC ANEMIA

HEMOLYTIC ANEMIA

Causes

- INTRACORPUSCULAR HEMOLYSIS
  - Membrane Abnormalities
  - Metabolic Abnormalities
  - Hemoglobinopathies
- EXTRACORPUSCULAR HEMOLYSIS
  - Nonimmune
  - Immune

ACQUIRED HEMOLYTIC ANEMIA

- Autoimmune hemolytic anemia
  - Warm AHA
  - Cold AHA
- Alloimmune hemolytic anemia
  - Hereditary disease of newborn
- Drug induced hemolytic anemia
- Non-immune hemolytic anemia
  - Paroxysmal nocturnal hemoglobinuria

INHERETED HEMOLYTIC ANEMIA

- Membrane defect
  - Hereditary spherocytoses
  - Hereditary elliptocytoses
- Hemoglobin abnormalities
  - Thalassaemia
  - Sickle cell anemia
- Metabolic abnormalities
  - G6PD deficiency
  - Pyruvate deficiency

Diagnosis of Haemolysis

Two major tests

- Serum Lactate Dehydrogenase - high
  - released from haemolyzed RBC's
- Haptoglobin - low
  - protein capable of binding Hb
  - binds free Hb in intravascular haemolysis
  - incomplete phagocytosis during extravascular haemolysis

Reticulocyte count

- Normal is 0.5 to 1.5 %
- anaemia causes increased erythropoietin, stimulates erythropoiesis
- increased retic count and percent

  (>4 to 5 %)

- If symptoms related to cold
  - cold agglutinins
  - Donath-Landsteiner Ab

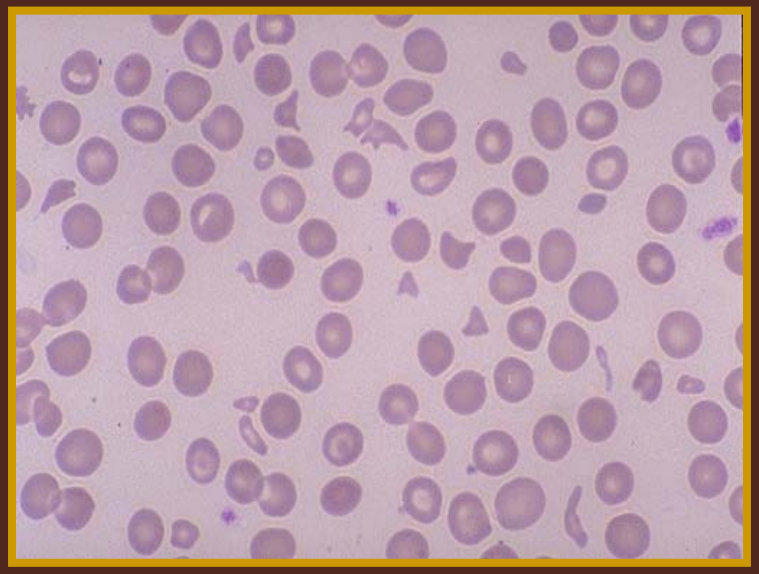
Additional tests for Intravascular Haemolysis

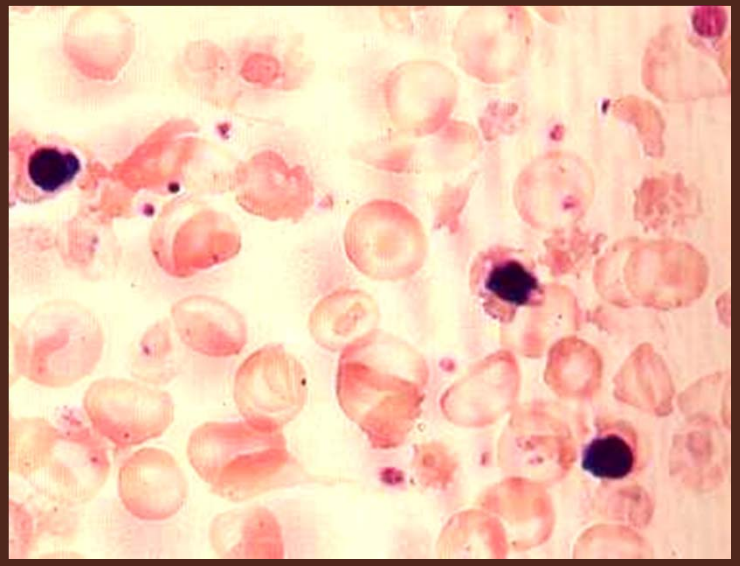
- Haemoglobinaemia- plasma Hb conc (haptoglobin saturated by released free Hb)
- Haemoglobinuria (free Hb saturates renal tubular resorptive capacity)
- Testing for Haemosiderin in shed tubular cells, 7 days later

Blood film in Haemolysis

- Damaged red cells
  - spherocytes, microsperocytes, elliptocytes
- Reticulocytes
  - large polychromatic cells
- Fragmented RBC's- microangiopathic HA
  - schistocytes, helmet cells
- Blister /bite cells of oxidative haemolysis

Blood Film HA



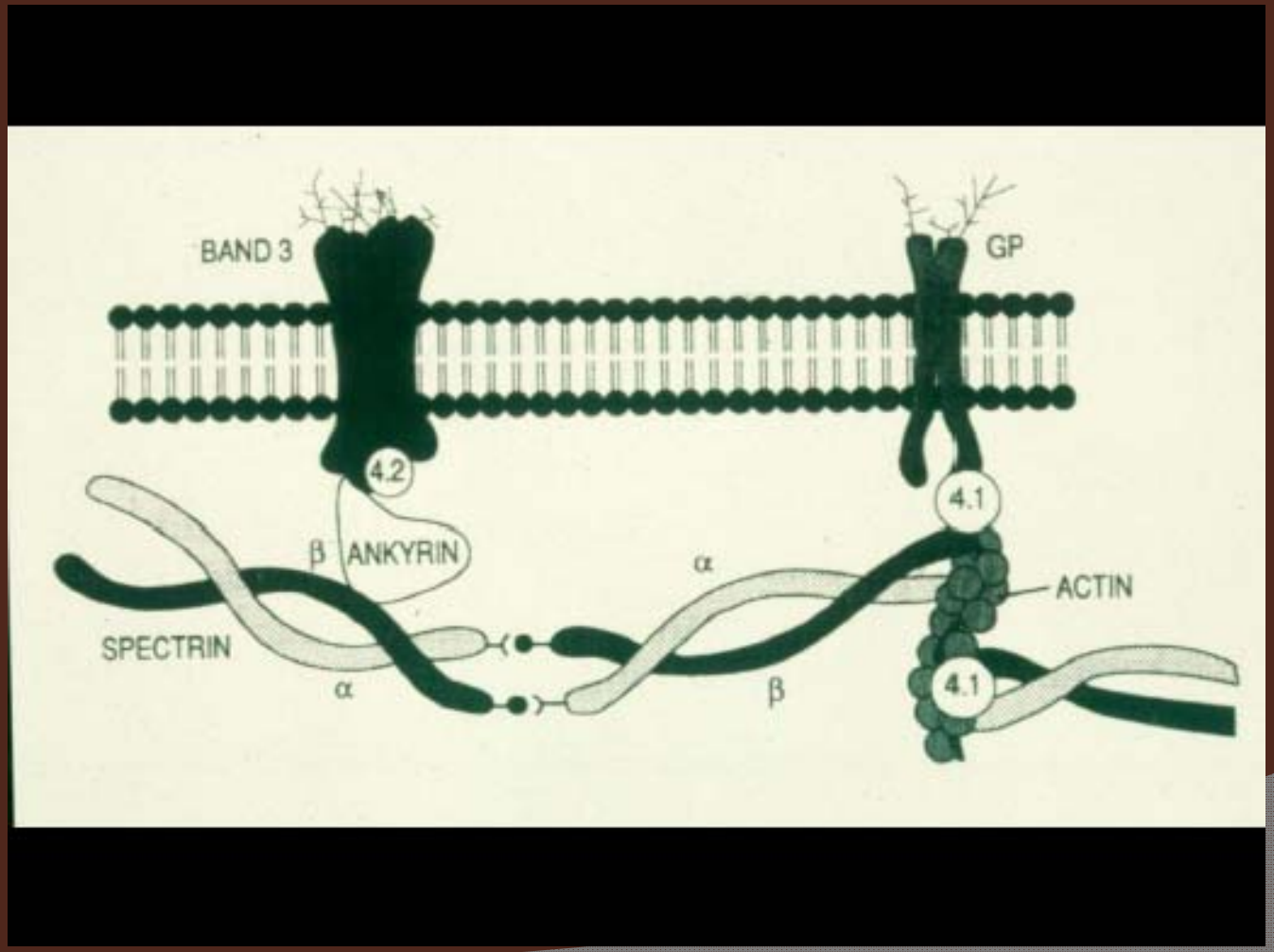


HEREDITARY SPHEROCYTOSIS

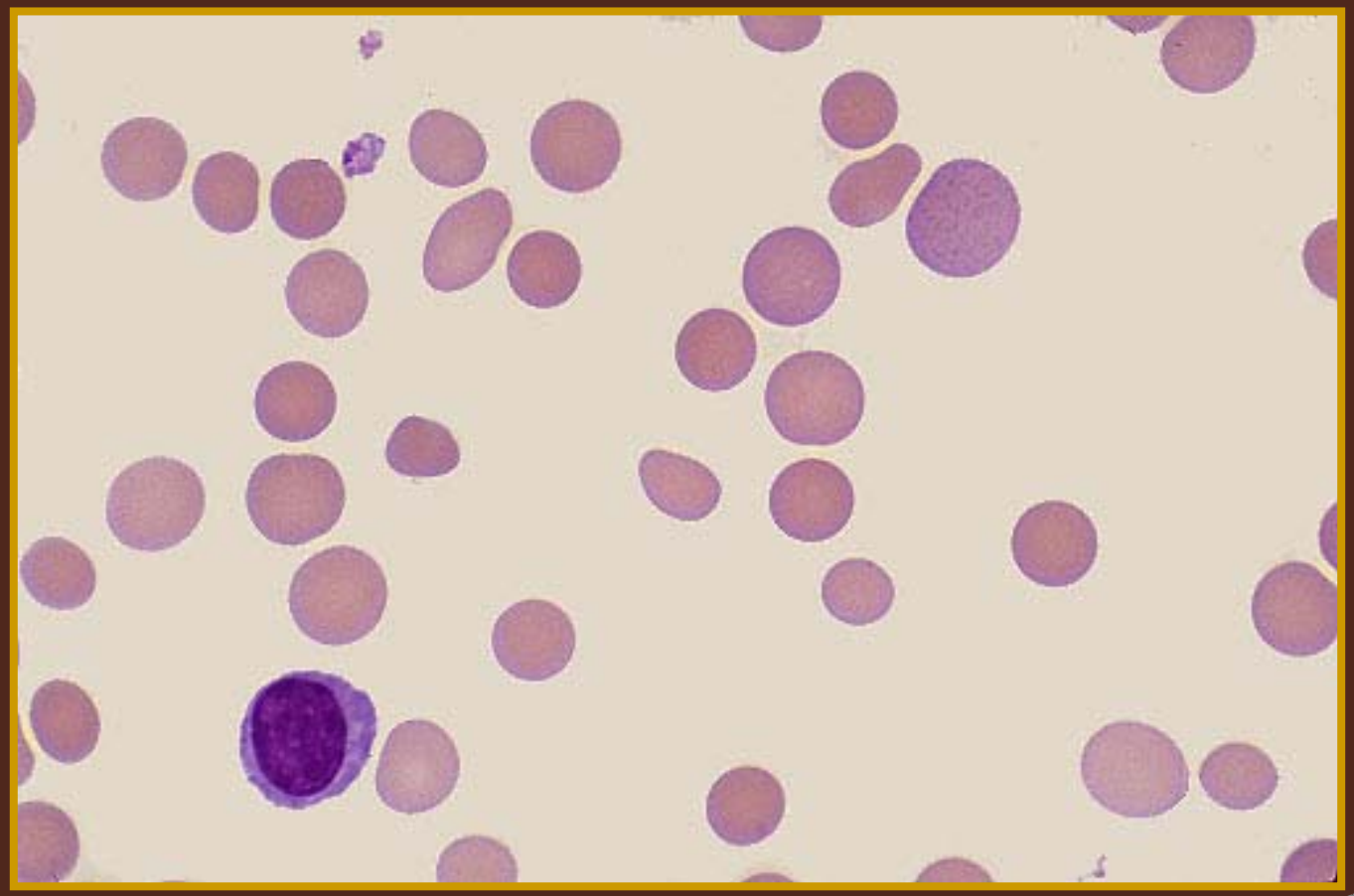
- This disorder is characterized by an inherited (intrinsic) defect in the red cell membrane that renders the cells
  - spheroidal
  - less deformable
  - vulnerable to splenic sequestration and destruction.
- Hereditary spherocytosis (HS) is transmitted most commonly as an autosomal dominant trait
- 25% of patients have a more severe autosomal recessive form of the disease

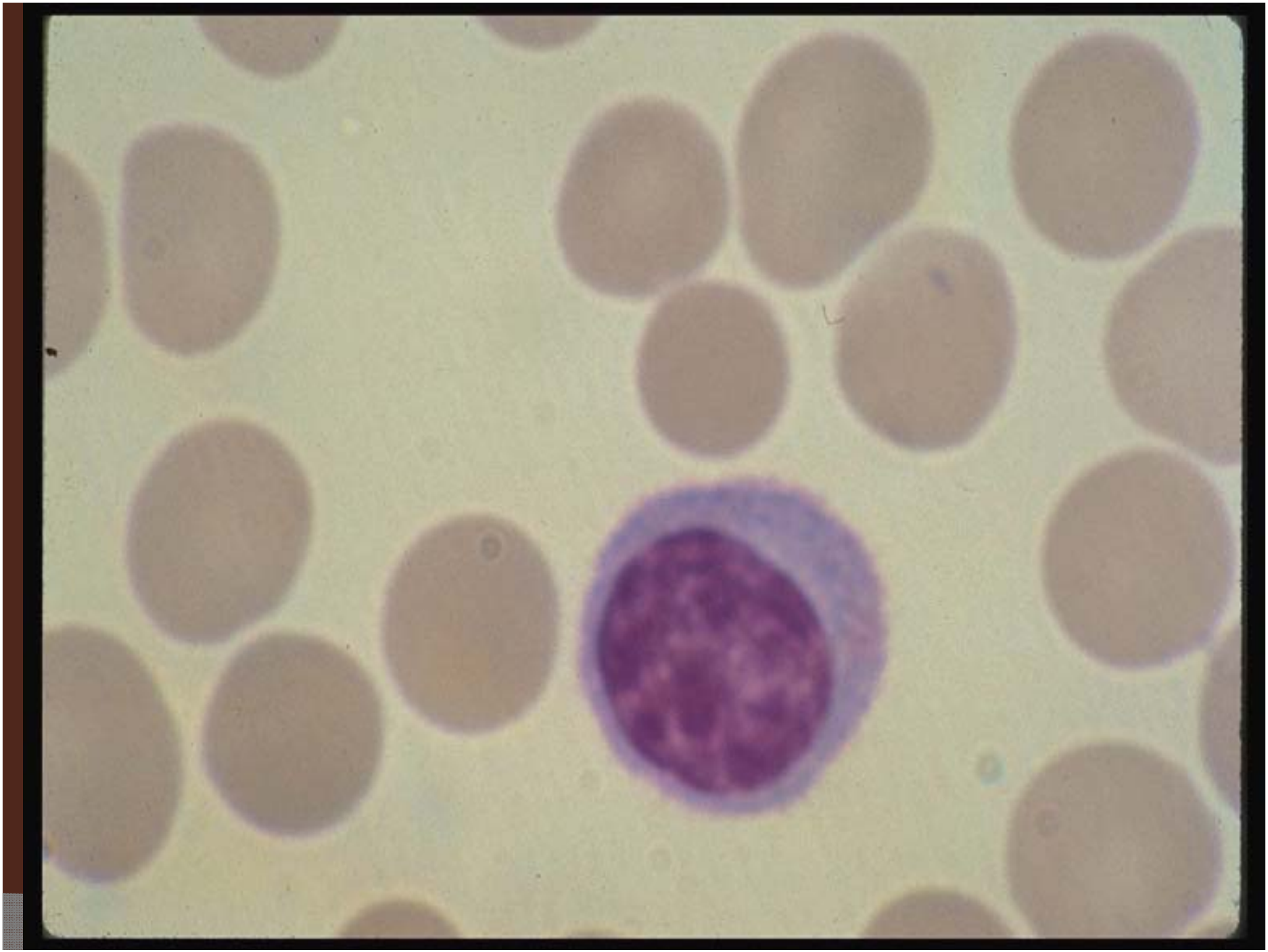
HEREDITARY SPHEROCYTOSIS

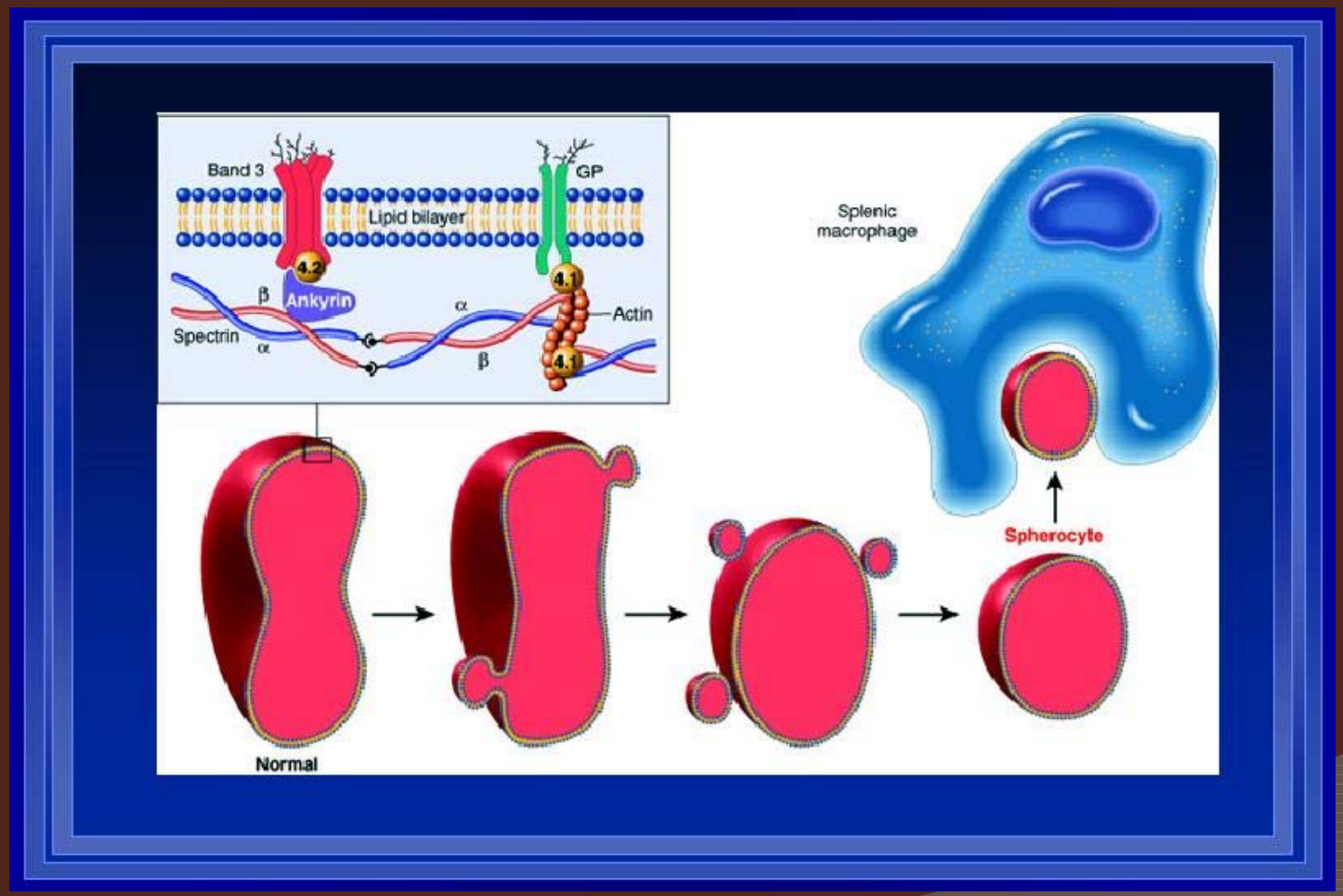
- MC –mutation in ankyrin band 3,spectrin,band 4.2
- Leads to loss of RBC membrane, leading to spherocytosis
- Decreased deformability of cell
- Increased osmotic fragility
- Extravascular hemolysis in spleen

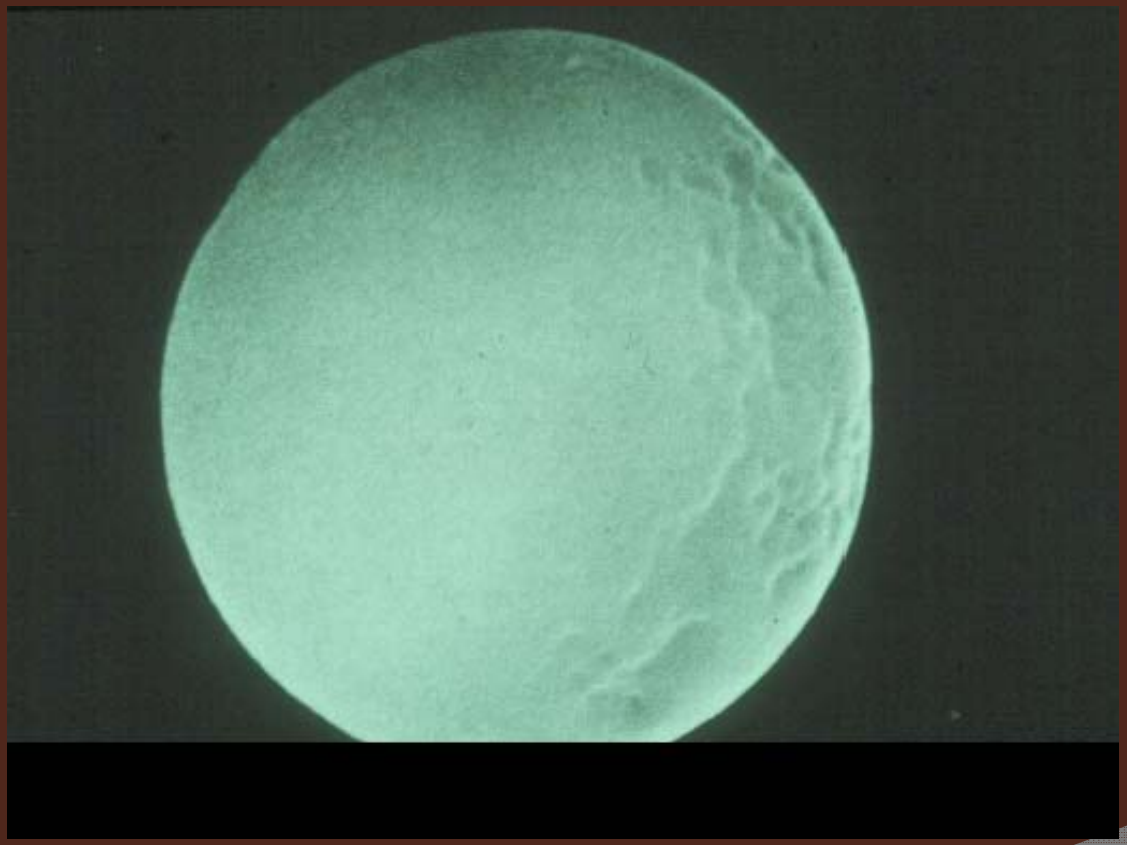

BAND 3
GP
4.2
4.1
β ANKYRIN
α
ACTIN
SPECTRIN
α
β
4.1

Spherocytosis






Band 3
GP
Lipid bilayer
4.2
4.1
β
Ankyrin
α
Actin
Spectrin
α
β
4.1
Splenic
macrophage
Spherocyte
Normal



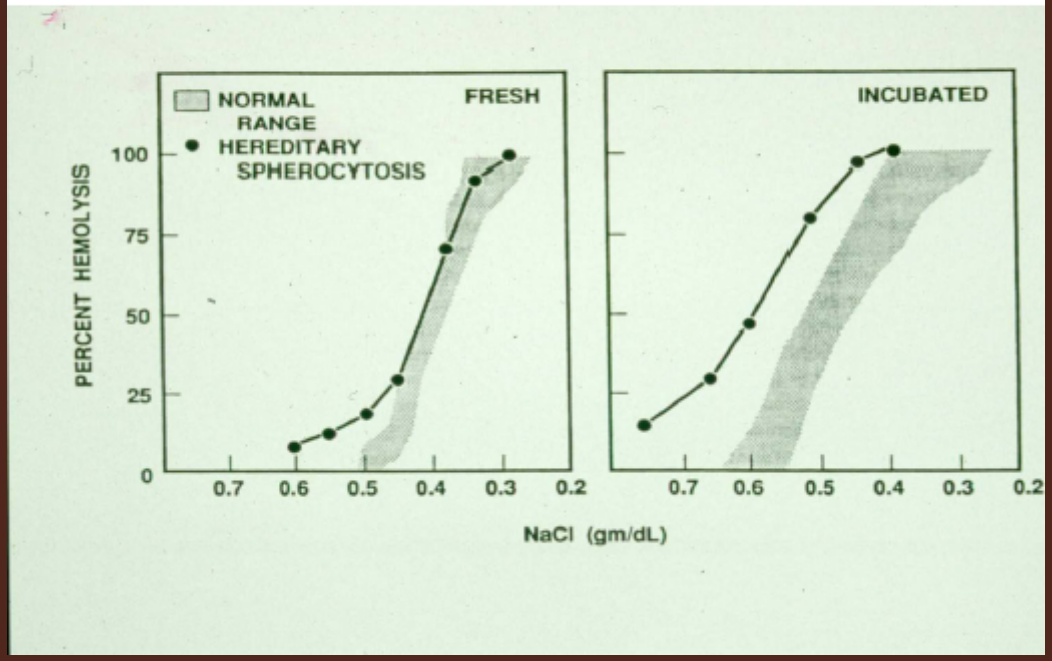
Hereditary Spherocytosis
Clinical Manifestations

- Anemia
- Jaundice
- Splenomegaly
- Aplastic episodes
  - Parvovirus B19
- Cholelithiasis

Diagnosis

- Family history
- ↑ MCHC
- Peripheral blood film
- Osmotic fragility test
- Spectrin and Ankyrin mutations

Osmotic Fragility Test



- Sickle Cell Anemia (HbSS)

Amino Acid Substitution Hemoglobinopathy

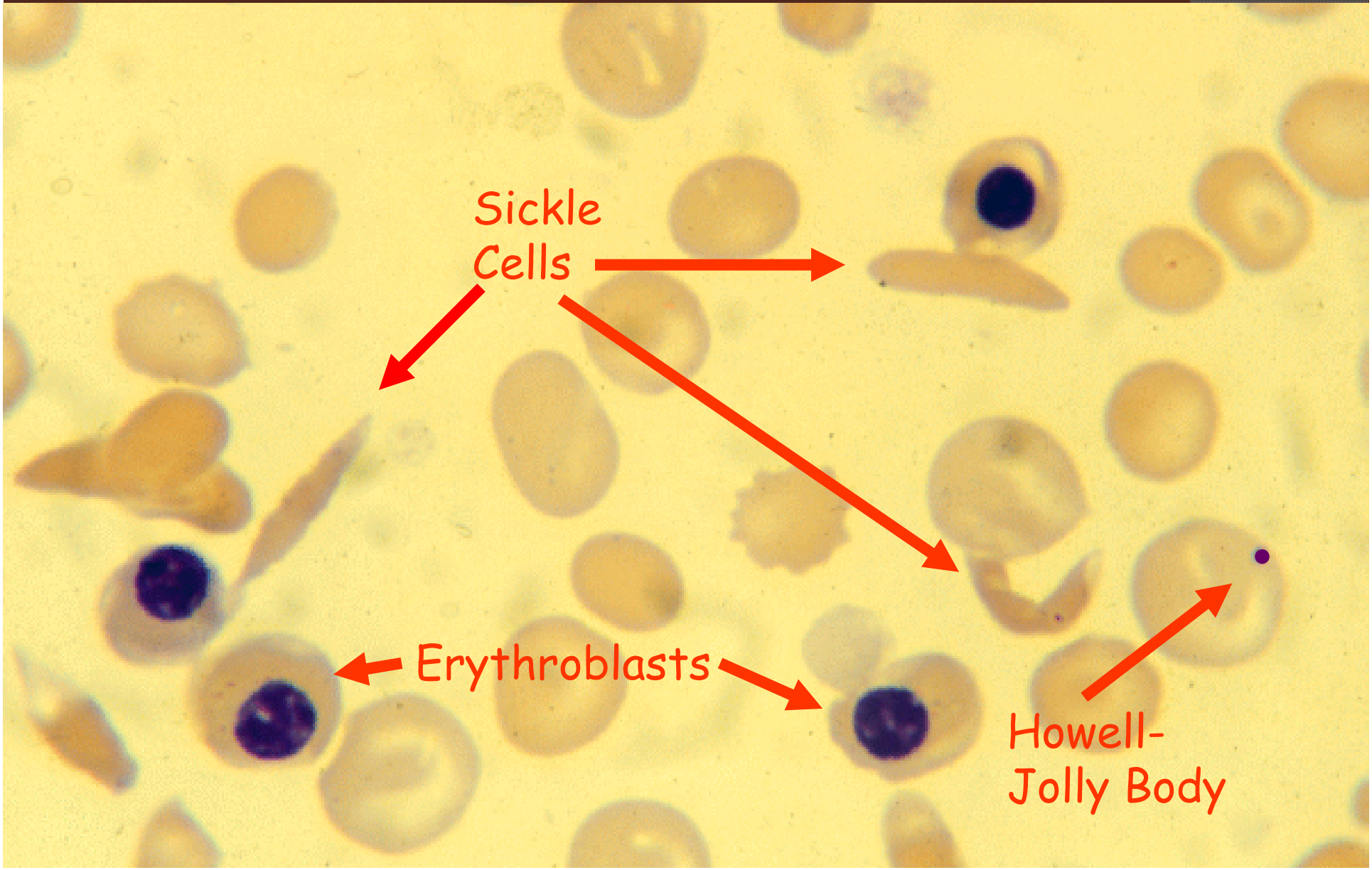
Amino acid substitutions are denoted by the three letter abbreviation for the normally occurring amino acid followed by an arrow followed by the three letter abbreviation for the substituted amino acid:

$$\beta^{6(A3)}\text{Glu} \rightarrow \text{Val}$$

Sickle Cell Anemia (HbSS)

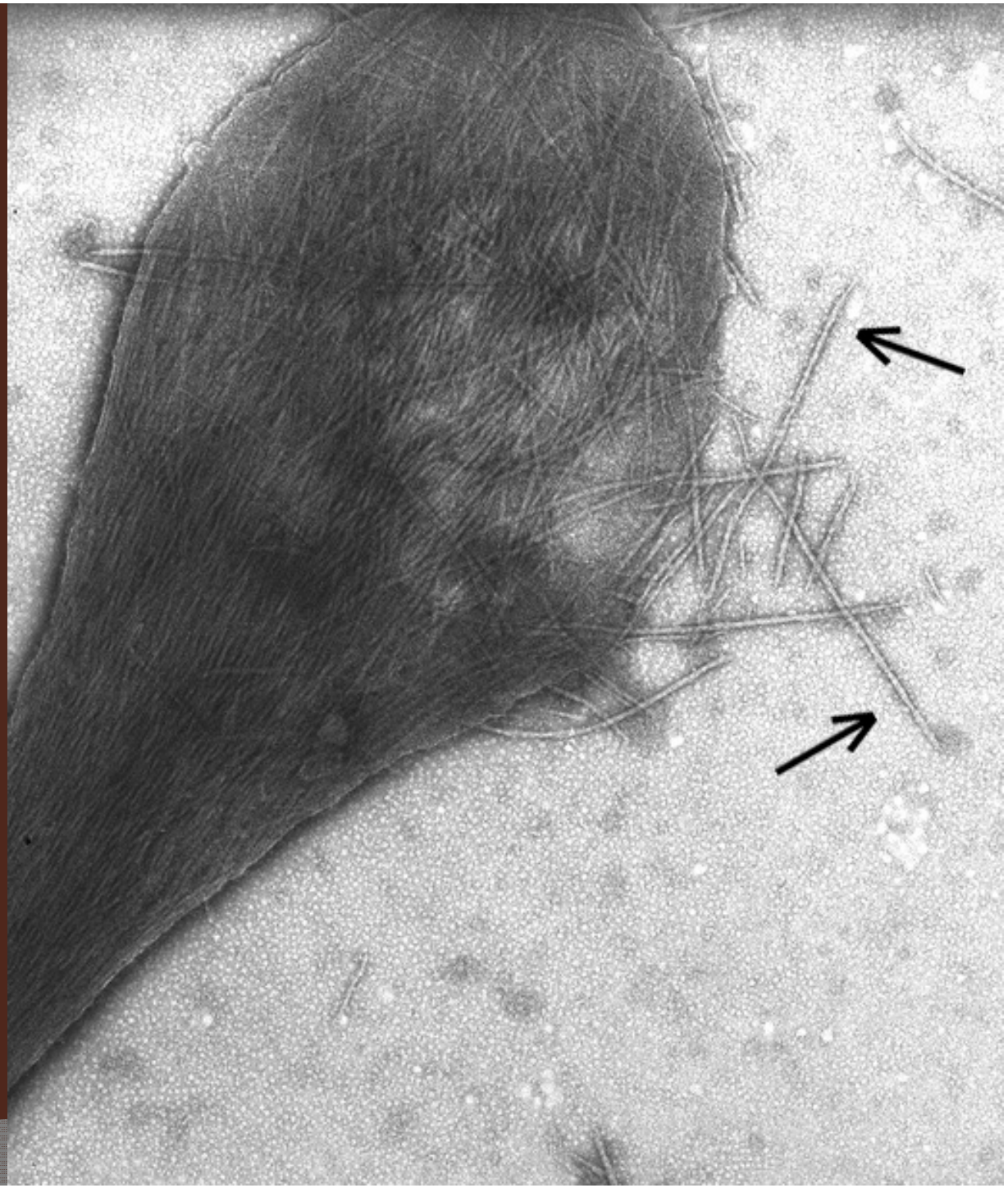
1. β6 glu to val
2. 'tactoids' at low oxygen tension
3. sickled red cells
4. small blood vessel occlusion
5. tissue infarction

Sickle Cell Anemia – blood film



Sickle Cell Anemia –

EM of red cell showing ‘tactoids’



Pathogenesis

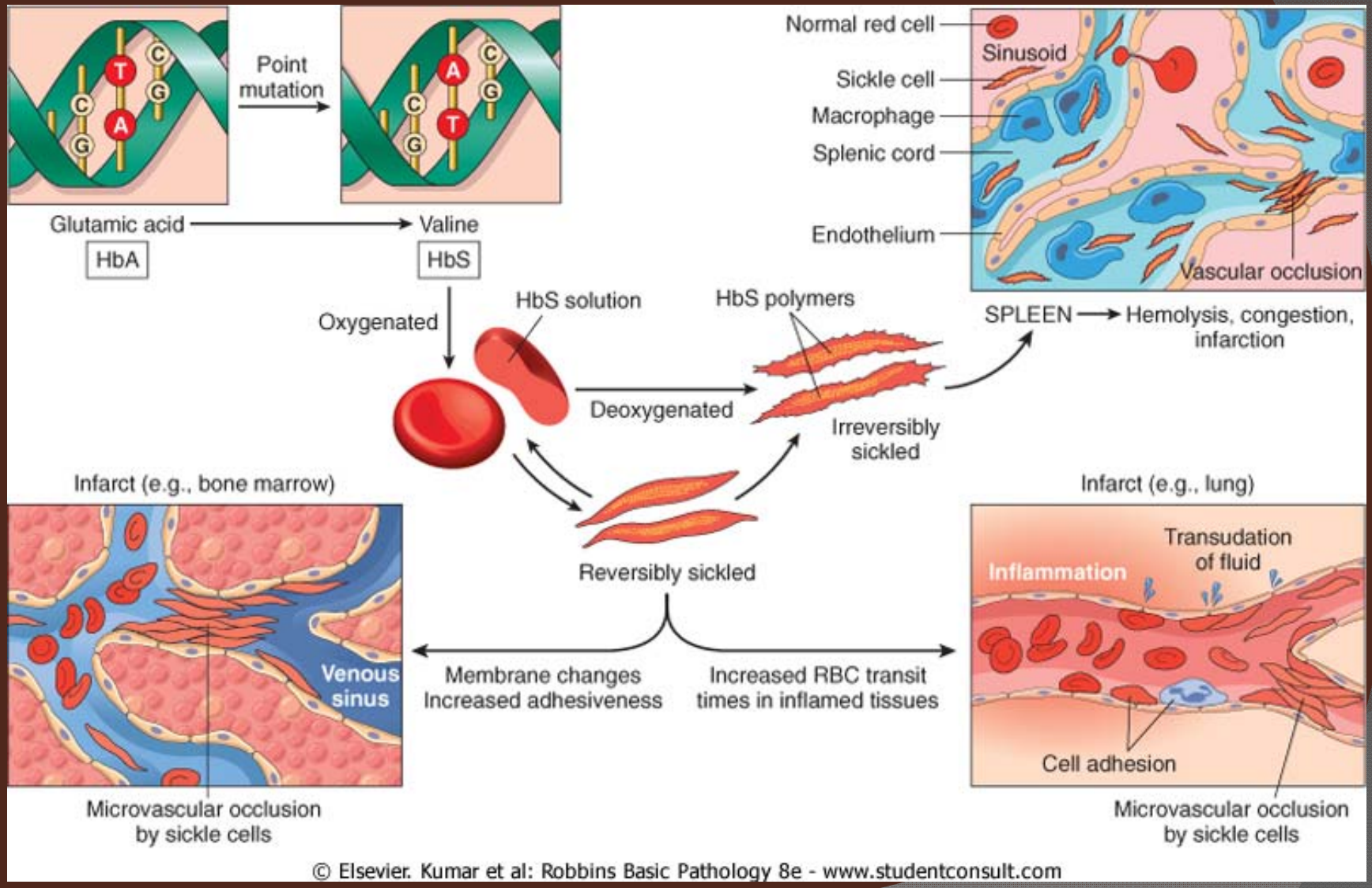
- Factors affecting sickling
  - Amount of HbS and interaction with other Hemoglobins
  - Concentration of hemoglobin
  - ↓ PH
  - Length of time RBCs are exposed to low O_2 tension
  - Susceptibility to infections

- inflammation
- increased red cell adhesion

- **Survival of rbcs is directly prop. To no. of irreversibly sickled cells**

Sickle cell anemia – clinical features

1. Hemolysis
2. Occlusion of blood vessels by sickled red cells



Anemia	Cause	Management
Hemolytic anemia	Removal of irreversibly sickled cells by macrophages	Transfusion for certain indications. Folate supplements. Iron chelation if chronic transfusion
Aplastic crisis	Parvovirus B19	Transfusion

Site of Sickling	Clinical Features	Management
Bone	Painful crisis	Pain relief and hydration. Hydroxyurea
Lung	Acute chest syndrome	Transfusion regime,pain relief and hydration
Brain	Stroke	Transfusion regime.
Heart	Myocardial infarction	Transfusion regimen pain relief and hydration
Spleen	Acute splenic sequestration:	Transfusion, pain relief and hydration
Spleen	Hyposplenism:	Pneumovax
Retina	Proliferative retinopathy	Retinal surveillance. Laser

- Vaso occlusive crises/pain crises
- Sequestration crises
- Aplastic crises

Sickle Cell Anemia - treatment

- Opiates and hydration for painful crises
- Pneumococcal vaccination
- Retinal surveillance
- **Hydroxyurea**
- Transfusion for serious manifestations
- Stem cell transplant
- Support, folate, iron chelation

Sickle Cell Trait

- Heterozygous state for HbS (HbAS)
- No serious clinical consequences
- Sudden death during intensive training
- Hematuria, isosthenuria (renal papillary necrosis)

CLINICAL FEATURES

- Sickle Cell Trait
  - HbA 60%, HbS 40%
  - Asymptomatic
  - Symptomatic in hypoxia
  - Protects against P. falciparum malaria
  - Blood count and film normal
  - Diagnosed by electrophoresis & sickle test

- G-6PD deficiency anaemia

What is G6PD?

- It is an X-linked recessive inheritance. (males usually affected and females are carriers)
- Risk factors: being being male, or having a family history of G6PD deficiency.
- G6PD enzyme functions in the Pentose-Monophosphate shunt and in the process, catalyzes the reduction of NADP+ to NADPH required in triggering a cascade of events that can detoxify the harmful oxidant H_2O_2.

Role of G6PD

- Responsible for maintaining adequate levels of NADPH inside cell.
- The oxidation of NADPH back to NADP+ is coupled with the reduction of oxidized glutathione (GSSG) to glutathione (GSH).
- Thus, NADPH keeps glutathione, a tri-peptide, in its reduced form.

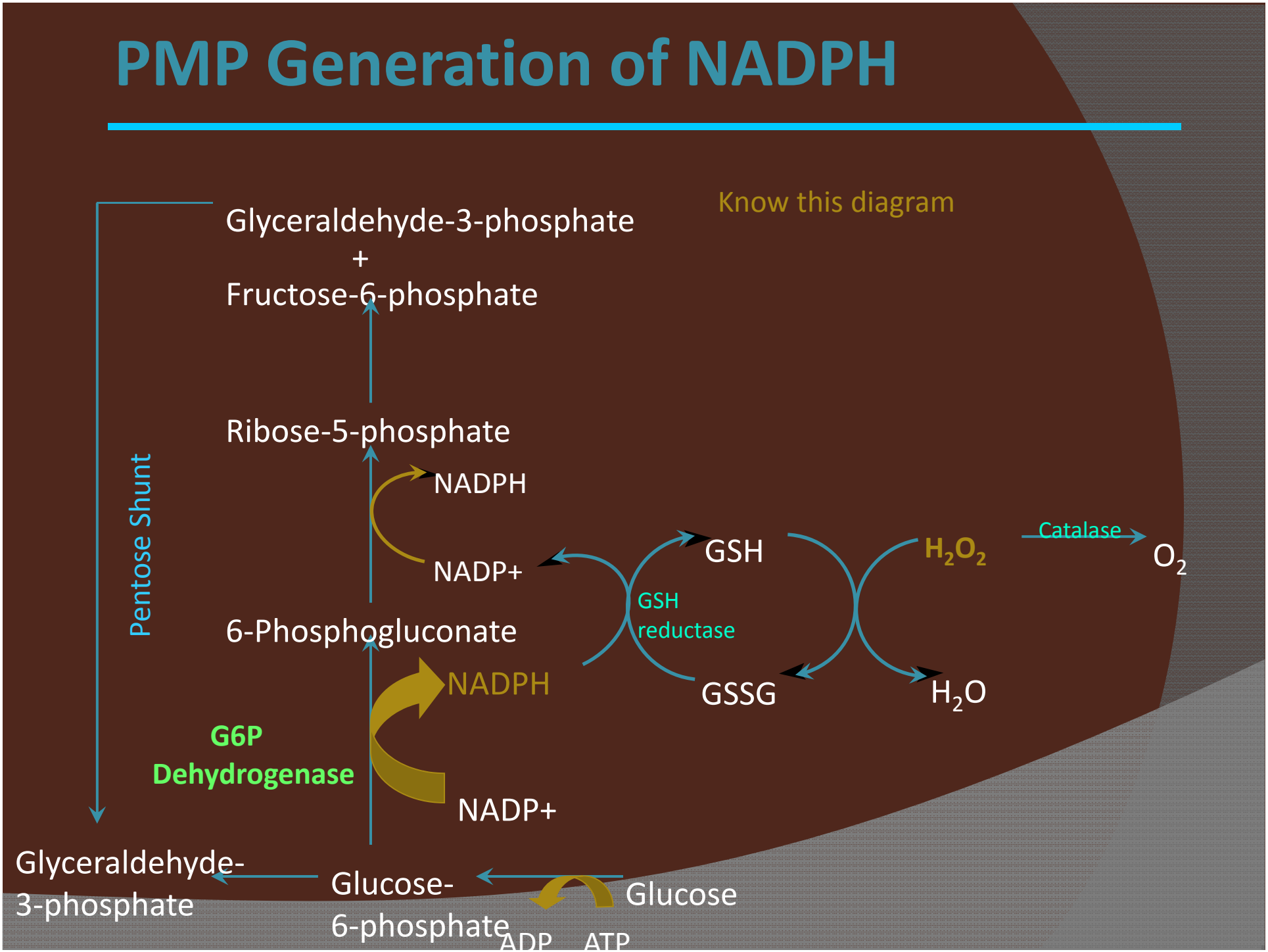
Role of G6PD Cont'd...

- Reduced glutathione (GSH) acts as a scavenger for dangerous oxidative metabolites in the cell.
- GSH converts harmful hydrogen peroxide to water catalyzed by the enzyme, glutathione peroxidase (catalase enzyme also detoxifies H_2O_2).

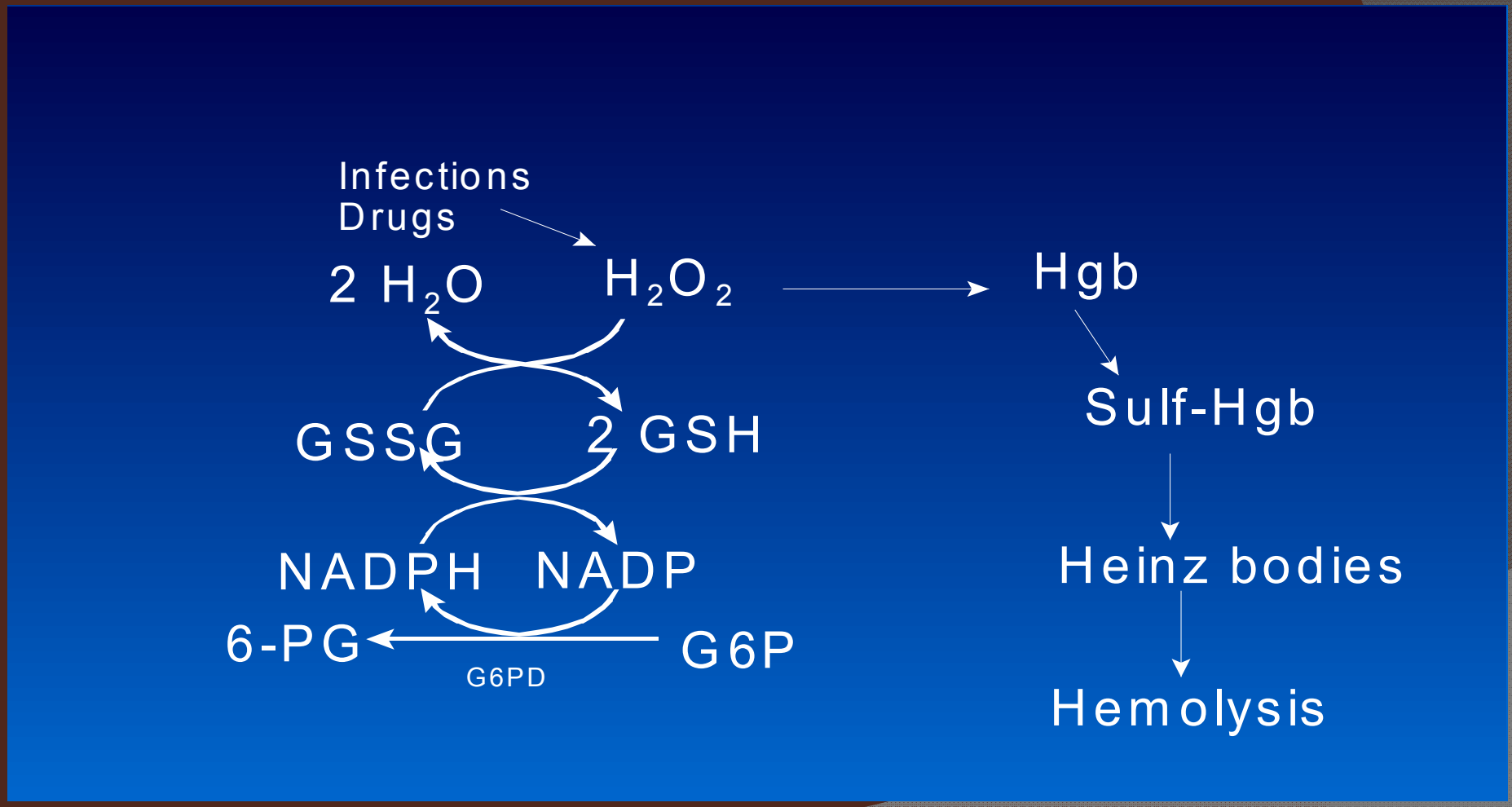
G6PD Deficiency

- Red cells deficient in G6PD are unable to neutralize hydrogen peroxide - H_2O_2 converts to hydroxyl radicals and this can lead to oxidative damage/toxic injury.

PMP Generation of NADPH



G6PD DEFICIENCY



Types

- TypenA-African
- Type-B-Western
- 400 variants
- Type A-
- MEDITERRENEAN

Spectrum of disease

- Acute haemolysis
- Chronic haemolytic anaemia
- Neonatal Jaundice

Drugs that ppt. it

- **Drugs that can precipitate this reaction include:**
  - anti-malarial agents
  - sulfonamides (antibiotic)
  - aspirin
  - non-steroidal anti-inflammatory drugs (NSAIDs)
  - nitrofurantoin
  - quinidine
  - quinine
  - others
- **Also:**

  exposure to certain chemicals such as those in mothballs and flava beans.

Required Tests

- **Blood tests are taken to measure levels of:**
  - red cells, assess size and shape of red cells
  - measure the Hb level
  - determine the number of reticulocytes

- **Other blood tests may include:**

  Heinz body presentation — looks for a deficiency in amount of G6PD enzyme, which results in hemolysis if certain medications or foods are ingested.

- EXTRACORPUSCULAR HEMOLYSIS

EXTRACORPUSCULAR HEMOLYSIS

Nonimmune

- Mechanical

  Macroangiopathic-prosthetic valves

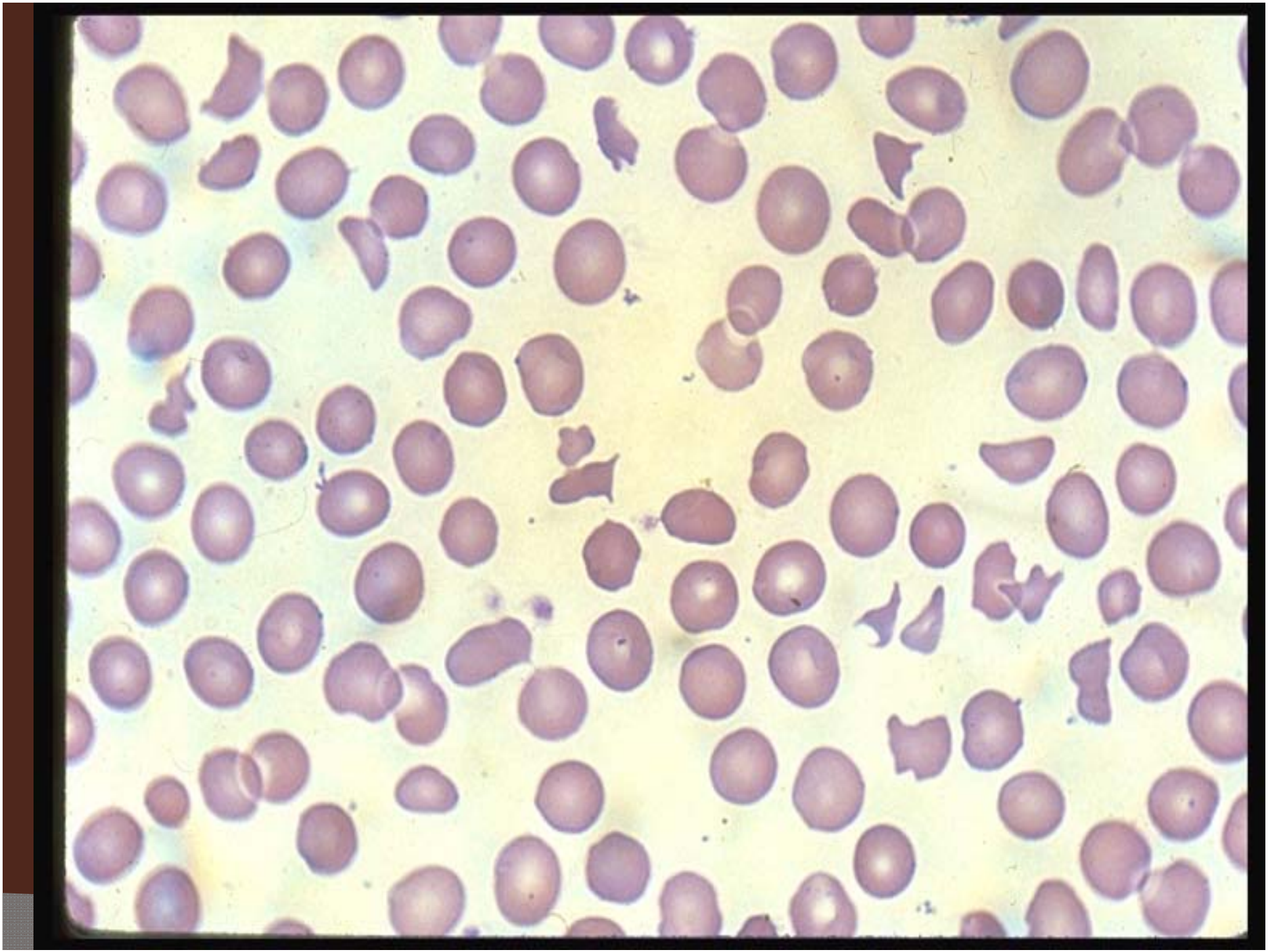
  Microangiopathic
- Infectious
- Chemical
- Thermal

Microangiopathic hemolytic anemia

Traumatic intravascular hemolysis bydeposition of fibrin strands in the lumen of small BV

- DIC
- TTP
- HUS
- Malignant hypertension
- Glomerulonephritis
- Preeclampsia
- Transplant rejection

- Intravascular coagulation predominant
  - Abruptio placentae
  - Disseminated intravascular coagulation
- **PB smear: fragmented RBC, thrombocytopenia**



IMMUNE HEMOLYTIC ANEMIA

General Principles

- All require antigen-antibody reactions
- Types of reactions dependent on:
  - Class of Antibody
  - Number & Spacing of antigenic sites on cell
  - Availability of complement
  - Environmental Temperature
  - Functional status of reticuloendothelial system
- Manifestations
  - Intravascular hemolysis
  - Extravascular hemolysis

IMMUNE HEMOLYTIC ANEMIA

- Antibodies combine with RBC, & either
  1. Activate complement cascade, &/or
  2. Opsonize RBC for immune system
- If 1, if all of complement cascade is fixed to red cell, intravascular cell lysis occurs
- If 2, &/or if complement is only partially fixed, macrophages recognize Fc receptor of Ig &/or C3b of complement & phagocytize RBC, causing extravascular RBC destruction

IMMUNE HEMOLYTIC ANEMIA

Coombs Test - Direct

- Looks for immunoglobulin &/or complement of surface of red blood cell (normally neither found on RBC surface)
- Coombs reagent - combination of anti-human immunoglobulin & anti-human complement
- Mixed with patient's red cells; if immunoglobulin or complement are on surface, Coombs reagent will link cells together and cause agglutination of RBCs

HEMOLYTIC ANEMIA - IMMUNE

- Drug-Related Hemolysis
- Alloimmune Hemolysis
  - Hemolytic Transfusion Reaction
  - Hemolytic Disease of the Newborn
- Autoimmune Hemolysis
  - Warm autoimmune hemolysis
  - Cold autoimmune hemolysis

HEMOLYTIC ANEMIA - IMMUNE

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  - Cold autoimmune hemolysis

IMMUNE HEMOLYSIS

Drug-Related

- Immune Complex Mechanism
  - Quinidine, Quinine, Isoniazid
- “Haptenic” Immune Mechanism
  - Penicillins, Cephalosporins
- True Autoimmune Mechanism
  - Methyldopa, L-DOPA, Procainamide, Ibuprofen

DRUG-INDUCED HEMOLYSIS

Immune Complex Mechanism

- Drug & antibody bind in the plasma
- Immune complexes either
  - Activate complement in the plasma, or
  - Sit on red blood cell
- Antigen-antibody complex recognized by RE system
- Red cells lysed as “innocent bystander” of destruction of immune complex
- REQUIRES DRUG IN SYSTEM

DRUG-INDUCED HEMOLYSIS

Haptenic Mechanism

- Drug binds to & reacts with red cell surface proteins
- Antibodies recognize altered protein, ± drug, as foreign
- Antibodies bind to altered protein & initiate process leading to hemolysis

DRUG-INDUCED HEMOLYSIS

True Autoantibody Formation

- Certain drugs appear to cause antibodies that react with antigens normally found on RBC surface. Methyl Dopa

ALLOIMUNE HEMOLYSIS

Hemolytic Transfusion Reaction

- Caused by recognition of foreign antigens on transfused blood cells
- Several types
  - Immediate Intravascular Hemolysis (Minutes) - Due to preformed antibodies; life-threatening
  - Slow extravascular hemolysis (Days) - Usually due to repeat exposure to a foreign antigen to which there was a previous exposure; usually only mild symptoms
  - Delayed sensitization - (Weeks) - Usually due to 1st exposure to foreign antigen; asymptomatic

ALLOIMMUNE HEMOLYSIS

Hemolytic Disease of the Newborn

- Due to incompatibility between mother negative for an antigen & fetus/father positive for that antigen. Rh incompatibility, ABO incompatibility most common causes
- Usually occurs with 2nd or later pregnancies
- Requires maternal IgG antibodies *vs.* RBC antigens in fetus

ALLOIMMUNE HEMOLYSIS

- Can cause severe anemia in fetus, with erythroblastosis and heart failure
- Hyperbilirubinemia can lead to severe brain damage (kernicterus) if not promptly treated
- HDN due to Rh incompatibility can be almost totally prevented by administration of anti-Rh D to Rh negative mothers after each pregnancy

AUTOIMMUNE HEMOLYSIS

- Due to formation of autoantibodies that attack patient's own RBC's
- Type characterized by ability of autoantibodies to fix complement & site of RBC destruction
- Often associated with either lymphoproliferative disease or collagen vascular disease

AUTOIMMUNE HEMOLYSIS

Warm Type

- Usually IgG antibodies
- Fix complement only to level of C3,if at all
- Immunoglobulin binding occurs at all temps
- Fc receptors/C3b recognized by macrophages; ∴
- Hemolysis primarily extravascular
- ✓ 50% -Pr,othersassociated with other illnesses
- ✓ Lymphomas,leukaemias
- ✓ NEOPLASTIC CONDITIONS
- ✓ SLE
- ✓ Drugs
- Responsive to steroids/splenectomy

Diseases Associated with Warm Autoimmune Antibodies

Autoimmune disorders

Systemic lupus erythematosis	Rheumatoid arthritis
Ulcerative colitis	Scleroderma

Lymphoproliferative disorders

Chronic lymphocytic leukemia,	Hodgkin's disease
Non-Hodgkin's lymphoma	Multiple myeloma
Waldenström's macroglobulinemia	

Others

AIDS	Hypoglammaglobulinemia
Carcinomas	Thymoma
(mucinous adenocarcinomas)	Dysglobulinemia

AUTOIMMUNE HEMOLYSIS

Cold Type

- Most commonly IgM mediated
- Antibodies bind best at 30º or lower
- Fix entire complement cascade
- Leads to formation of membrane attack complex, which leads to RBC lysis in vasculature
- Typically only complement found on cells
- 90% associated with other illnesses
- Mycoplasma,influenza,HIV,IM,LYMPHOMAS
- Poorly responsive to steroids, splenectomy; responsive to plasmapheresis

- Cold Haemolysin haemolytic anaemia
- Intravascular haemolysis
- Donath-Landsteiner Ab

Paroxysmal Nocturnal Hemoglobinuria (PNH)

- **PNH is an acquired chronic hemolytic anemia which arises from a somatic mutation in a hematopoietic stem cell.**
- **Most hematopoitic cell lines may be affected by the intrinsic membrane defect.**
- **This defect renders the red cells highly susceptible to complement mediated lysis resulting in the characteristic hemolysis.**

Epidemiology

- Rare disease -
  - frequency unknown
  - thought to be on the same order as aplastic anemia (2-6 per million)
- Median age at diagnosis
  - ~ 35 yrs
  - PNH reported at extremes of age
- Female:Male ratio = 1.2:1.0
- No increased risk of PNH in patient relatives
- Median Survival after diagnosis ~ 10-15 yrs

Clinical Features

Major symptoms

- Hemolysis
- Cytopenia
- tendency to thrombosis)

Hemolysis

- chronic hemolysis with acute exacerbations (hallmark)
  - most patient at some stage
  - only 1/3 exhibit hemolysis at diagnosis
  - Recurrent attacks of intravascular hemolysis are usually associated with;
    - hemoglobinuria
    - abdominal pain
    - dysphagia

Clinical Features

- cytopenia (varying severity)
  - isolated subclinical thrombocytopenia
  - classical severe aplastic anemia
- tendency to thrombosis
  - venous thrombosis (40%) of patients, main cause of morbidity

- Variable expression of above often causes considerable delay in the diagnosis
- Major cause of death
  - venous thrombosis
  - complications from progressive pancytopenia

Clinical Features - Long term

- 25% of PNH patients survive >25 years - one half of these go on to spontaneous remission
- Remission patients
  - hematological values revert to normal
  - no PNH rbcs or granulocytes detected
  - PNH lymphocytes - still detected but no clinical consequence
- Higher incidence of acute leukemia (6%)
  - “preleukemic condition” most likely bone marrow failure not PNH

Clinical Features - Relationship to aplastic anemia (AA)

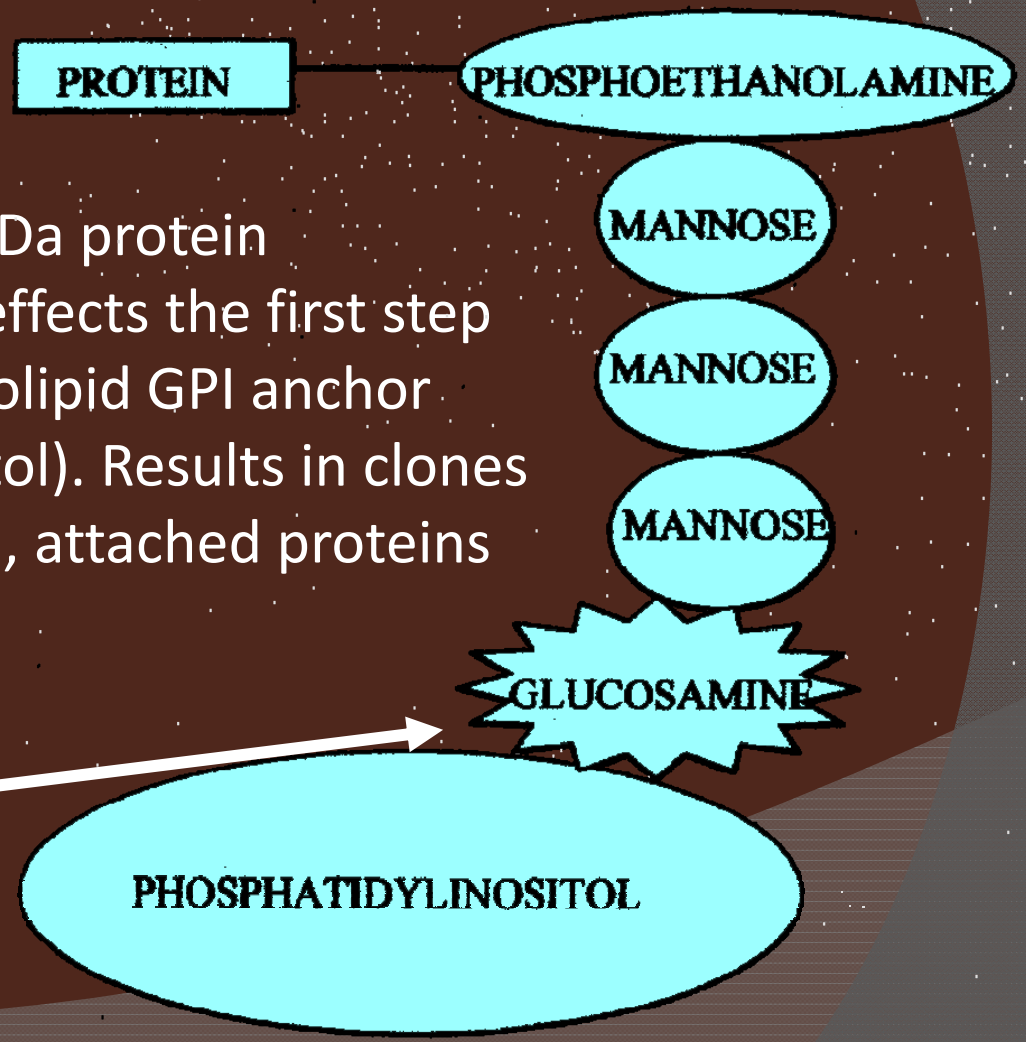
- High percentage of patients with AA develop clinical PNH or have lab evidence of PNH abnormality at some point (52%)
- Supports the theory that bone marrow failure supports the abnormal PNH cells - more later

Pathogenesis - The Defect

- GPI links a series of proteins to outer leaf of cell membrane via phosphatidyl inositol membrane anchor via diacylglycerol bridge
- PIG-A gene, on X-chromosome, codes for synthesis of this anchor; multiple defects known to cause lack of this bridge
- Defect - Somatic mutation of *PIG-A* gene (phosphatidylinositol glycan complementation group A) located on the X chromosome in a clone of a hematopoietic stem cell
  - >100 mutations in *PIG - A* gene known in PNH
  - The mutations (mostly deletions or insertions) generally result in stop codons - yielding truncated proteins which may be non or partially functional - explains heterogeneity seen in PNH

Pathogenesis - The Defect

- *PIG - A* gene codes for 60 kDa protein glycosyltransferase which effects the first step in the synthesis of the glycolipid GPI anchor (glycosylphosphatidylinositol). Results in clones lacking GPI anchor - in turn, attached proteins



Pathogenesis - The Defect
GPI Anchor deficiency

- PNH blood cells deficient in GPI anchor lack membrane proteins linked via the anchor
- Severity & size of deficiency - variable - clinical/diagnostic implications

Proteins anchored by GPI Anchor and Surface Proteins Missing on PNH Blood Cells

Antigen	Expression Pattern
Enzymes	
Acetylcholinesterase (AchE)	Red blood cells
Ecto-5'-nucleotidase (CD73)	Some B- and T-lymphocytes
Neutrophil alkaline phosphatase(NAP)	Neutrophils
ADP-rybosyl transferase	Some T-lymphs, Neutrophils
Adhesion molecules	
Blast-I/CD48	Lymphocytes
Lymphocyte function-associated antigen-3(LFA-3 or CD58)	All blood cells
CD66b	Neutrophils
Complement regulating surface proteins	
Decay accelerating factor (DAF or CD55)	All blood cells
Homologous restriction factor, Membrance inhibitor of reactive lysis (MIRL or CD59)	All blood cells

Pathogenesis - Functional consequences of lack of GPI linked proteins

- *In vivo* function of many of these membrane proteins not fully understood
- However, CD55 and CD59 functions are well known
  - CD55 (decay accelerating factor) inhibits the formation or destabilizes complement C3 convertase (C4bC2a)
  - CD59 (membrane inhibitor of reactive lysis, protectin, homologous restriction factor) Protects the membrane from attack by the C5-C9 complex
  - Inherited absences of both proteins in humans have been described
    - Most inherited deficiencies of CD55 - no distinct clinical hemolytic syndrome
    - Inherited absence of CD59 - produces a clinical disease similar to PNH with hemolysis and recurrent thrombotic events

Laboratory Evaluation of PNH

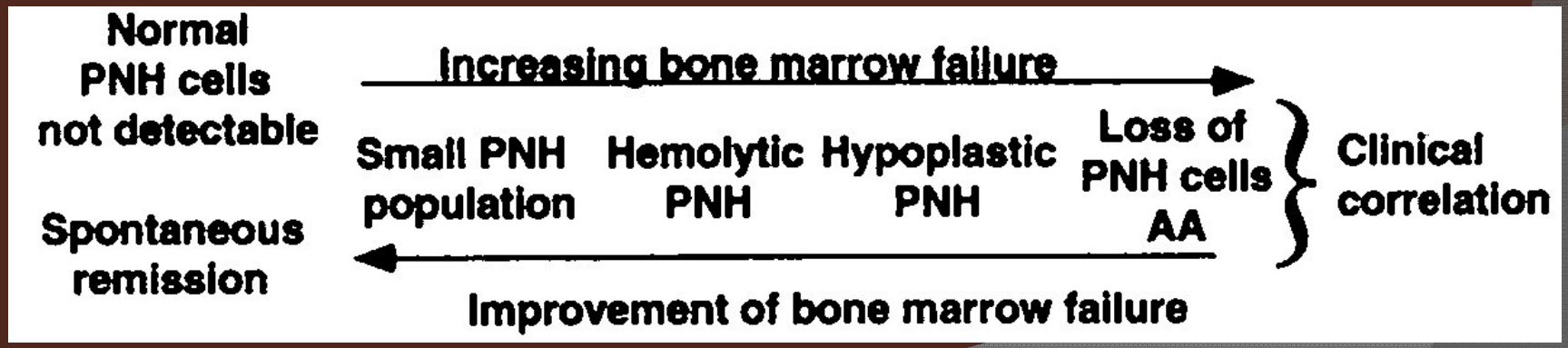
- Acidified Serum Test (Ham Test 1939)
  - Acidified serum activates alternative complement pathway resulting in lysis of patient's rbcs
  - May be positive in congenitial dyserythropoietic anemia
  - Still in use today
- Sucrose Hemolysis Test (1970)
  - 10% sucrose provides low ionic strength which promotes complement binding resulting in lysis of patient's rbcs
  - May be positive in megaloblastic anemia, autoimmune hemolytic anemia, others
  - Less specific than Ham test

Laboratory Evaluation of PNH

- PNH Diagnosis by Flow Cytometry (1986)
  - Considered method of choice for diagnosis of PNH (1996)
  - Detects actual PNH clones lacking GPI anchored proteins
  - More sensitive and specific than Ham and sucrose hemolysis test

PNH Diagnosis by Flow Cytometry

- Flow Cytometry is method of choice but only supportive for/against diagnosis
- More studies are needed to better define whether the type (I, II, or III), cell lineage, and size of the circulating clone can provide additional prognostic information.
- Theoretically - should be very valuable



Therapy

- Bone Marrow Transplantation
  - Only curative treatment
  - chronic condition (possiblity of spontaneous remission) - BMT should be avoided
- Immunosuppressive therapy
  - Antilymphocyte globulin &/or cyclosporine A
    - Does not alter proportion of PNH hemopoiesis
  - Steroids - experimental - controlled studies ??
- Growth Factors
  - Some improvement
  - no evidence that normal clones respond better than PNH clones

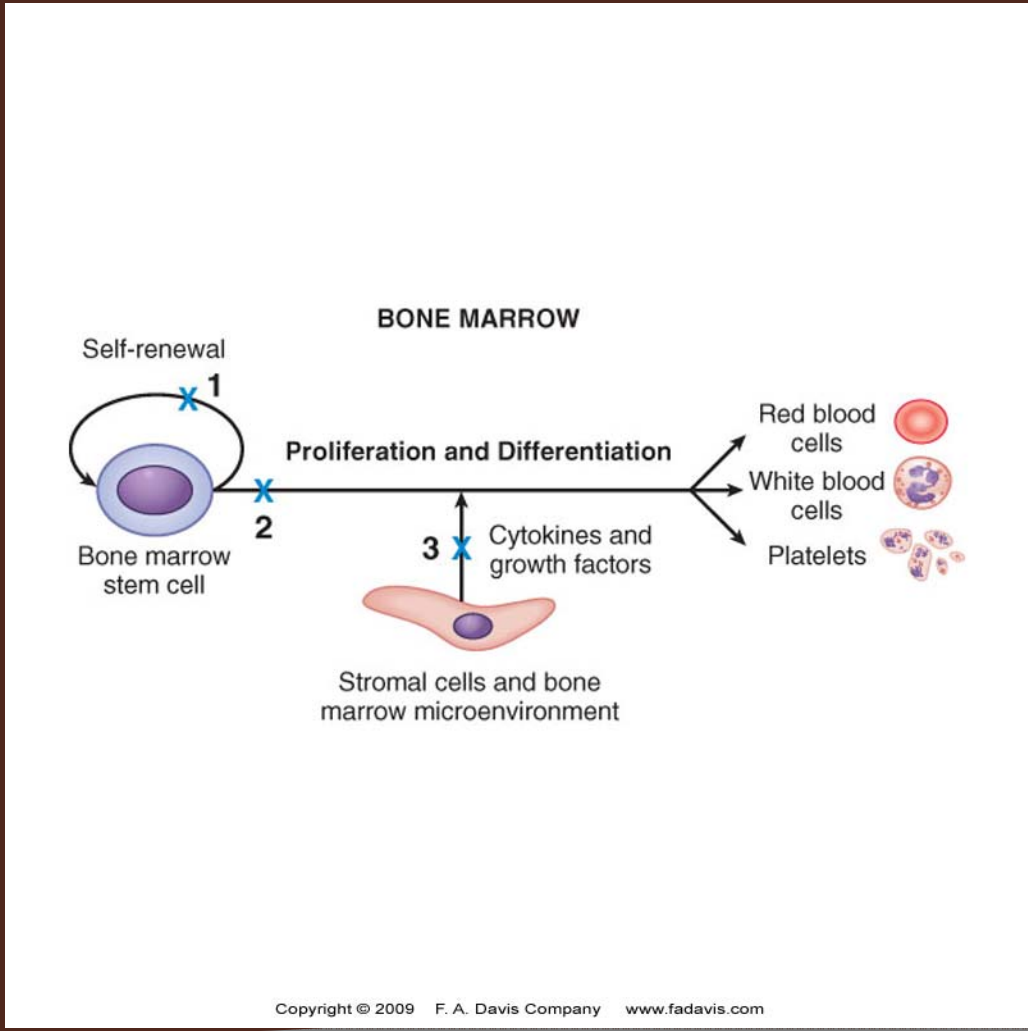
APLASTIC ANEMIA

- Aplastic anemia is a severe, life threatening syndrome in which production of erythrocytes, WBCs, and platlets has failed.
- Aplastic anemia may occur in all age groups and both genders.
- The disease is characterized by peripheral pancytopenia and accompanied by a hypocellular bone marrow.

APLASTIC ANEMIA

- Pathophysiology:
  - The primary defect is a reduction in or depletion of hematopoietic precursor stem cells with decreased production of all cell lines. This is what leads to the peripheral pancytopenia.
    - This may be due to quantitative or qualitative damage to the pluripotential stem cell.
    - or the result of a defective bone marrow microenvironment
    - or from cellular or humoral immunosuppression of hematopoiesis.

Pathophysiology of aplastic anemia



Etiology

✓ Acquired

- Most cases of aplastic anemia are idiopathic and there is no history of exposure to substances known to be causative agents of the disease

❑ Exposure to ionizing radiation – hematopoietic cells are especially susceptible to ionizing radiation. Whole body radiation of 300-500 rads can completely wipe out the bone marrow. With sublethal doses, the bone marrow eventually recovers.

- Chemical agents – include chemical gents with a benzene ring, chemotherapeutic agents, and certain insecticides.
  - Idiosyncratic reactions to some commonly used drugs such as chloramphenicol or phenylbotazone
- Infections – viral and bacterial infections s infectious mononucleosis,
  - ✓ infectious hepatitis
  - ✓ cytomegalovirus infections
  - ✓ and miliary tuberculosis

APLASTIC ANEMIA

- PNH– this is a stem cell disease in which the membranes of RBCs, WBCs and platlets have an abnormality making them susceptible to complement mediated lysis.
- Hereditary
  - Fanconi,
  - Diamond-Shwachman
  - Fanconi's anemia – the disorder usually becomes symptomatic ~ 5 years of age and is associated with progressive bone marrow hypoplasia. Congenital defects such as skin hyperpigmentation and small stature are also seen in affected individuals.

Clinical manifestations

- Fatigue
- Heart palpitations
- Pallor
- Infections
- Petechiae
- Mucosal bleeding

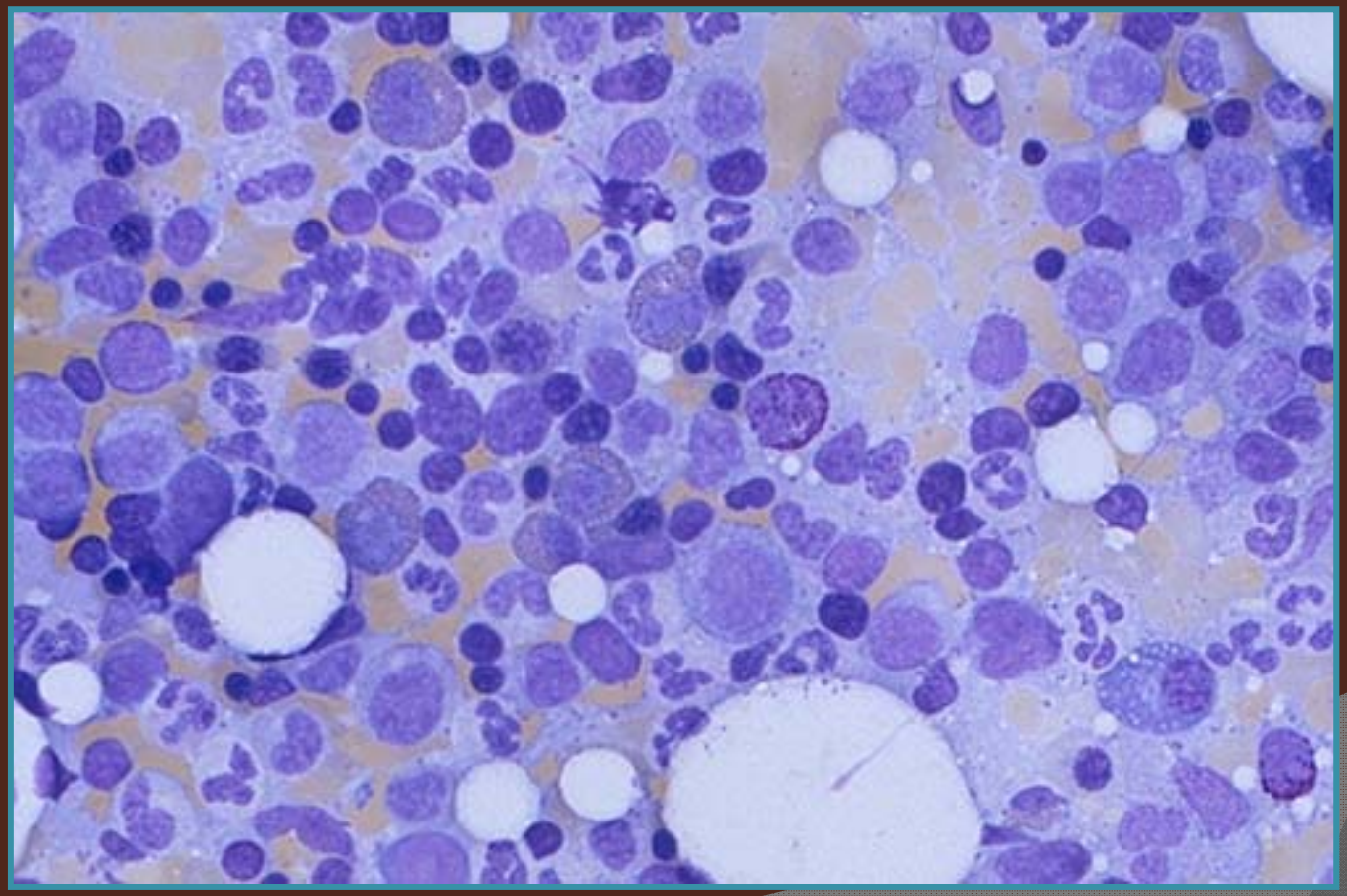
Lab findings

- Severe pancytopenia with relative lymphocytosis (lymphocytes live a long time)
- Normochromic, normocytic RBCs (may be slightly macrocytic)
- Mild to moderate anisocytosis and poikilocytosis
- Decreased reticulocyte count
- Hypocellular bone marrow with > 70% yellow marrow

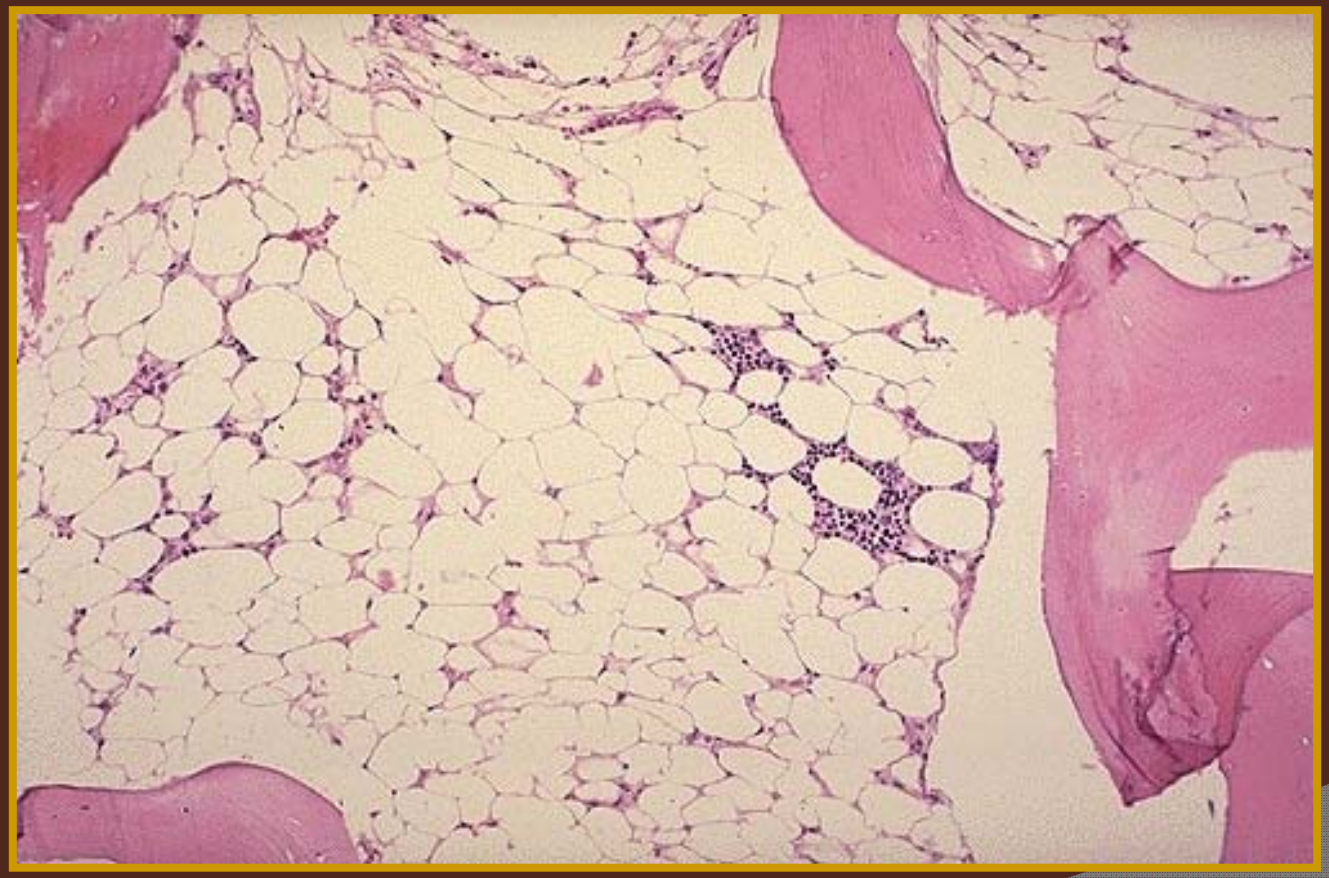
Treatment

- – in untreated cases the prognosis is poor
  - Remove causative agent, if known
  - Multiple transfusions
  - Antithymocyte globulin
  - Methylprednisolone pulse therapy
  - Bone marrow transplant

Normal BM High Power



BM - Aplastic Anaemia



D/D

- Disorders in which there is peripheral pancytopenia, but the bone marrow is normocellular, hypercellular, or infiltrated with abnormal cellular elements
  - Myelopthesic anemia – replacement of bone marrow by fibrotic, granulomatous, or neoplastic cells

D/D

- Myelodysplastic syndromes – are primary, neoplastic stem cell disorders that tend to terminate in acute leukemia. The bone marrow is usually normocellular, or hypercellular with evidence of qualitative abnormalities in one or more cell lines resulting in ineffective erythropoiesis and/or granulopoiesis and/or megakaryopoiesis

D/D

- The peripheral smear shows dysplastic (abnormality in development) cells including nucleated RBCs, oval macrocytes, pseudo-Pelger-Huet PMNs (hyposegmented neutrophils) with hyperchromatin clumping, hypogranulated neutrophils, and giant bizarre platlets.
- Hypersplenism – why can this lead to pancytopenia?

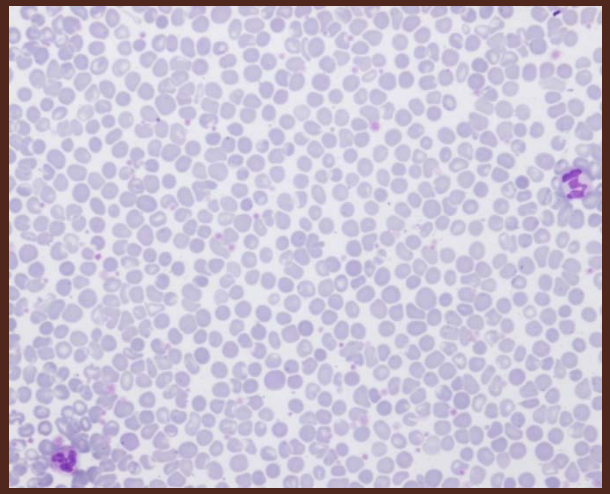
PURE RED CELL APLASIA

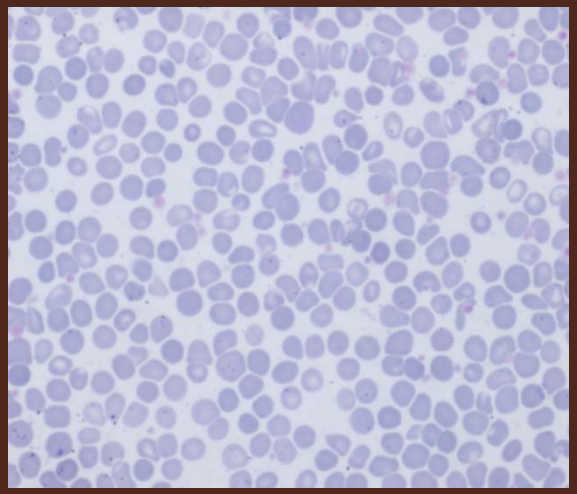
- Pure red cell aplasia is characterized by a selective decrease in erythroid precursor cells in the bone marrow. WBCs and platlets are unaffected.
  - Acquired
    - Transitory with viral or bacterial infections
    - Patients with hemolytic anemias may suddenly halt erythropoiesis
    - Patients with thymoma – T-cell mediated responses against bone marrow erythroblasts or erythropoietin are sometimes produced.

- Erythrocytosis/
- Polycythemia

- **Erythrocytosis**

  Increased Hb/Hct





I. Absolute erythrocytosis (Polycythemia):
II. Relative erythrocytosis (pseudopolycythemia):
    1. Hemoconcentration
    2. Spurious polycythemia (Gaisboek syndrome)

Polycythemia

- Primay polycythemia (polycythemia vera)
- Secondary polycythemia (abnormal increase of serum erythropoietin level)

- Erythrocytosis secondary to decreased tissue oxygenation:

  a) chronic lung diseases

  b) cyanotic congenital heart diseases

  c) high-altitude erythrocytosis

  d) hypoventilation syndromes (Sleep apnoea)

  e) hemoglobin-oxygen dissociation abnormalities

  - hemoglobinopathies associated with high oxygen affinity

  - carboxyhemoglobin in smoker's polycythemia

- . Secondary to aberrant erythropoietin production or response:

  a) **Erythropoietin-producting tumors**: hepatocellular ca, cerebellar hemangioblastoma, pheochromocytoma

  b) **Renal diseases**: renal cell carcinoma

  c) **Androgen abuse**: adrenal cortical hypersecretion, exogenous androgens

Introduction

- Secondary causes of increased red blood cell mass (e.g., heavy smoking, chronic pulmonary disease, renal disease) **are more common than polycythemia vera and must be excluded**

CHRONIC MYELOPROLIFERATIVE DISORDERS (MPD)

1. Polycythemia vera
2. Chronic myeloid leukaemia
3. Essential thrombocythemia
4. Idiopathic myelofibrosis

POLYCYTHEMIA VERA (PV)

Pathogenesis

PV is a clonal disorder involving the hematopoietic stem cells; it leads to an **autonomous proliferation of the erythroid, myeloid, and megakaryocytic cell lines.** Increased erythroid proliferation is usually more prominent than that of the other cell lines and occurs **independently of erythropoietin levels (which are usually very low in PV)**

POLYCYTHEMIA VERA (PV)

Epidemiology

- ✓ The incidence rate of PV is approximately 2 per 100000 population.
- ✓ PV is slightly more prevalent in males with male/female ratio ranging from 1.2 to 2:1.
- ✓ Median age at diagnosis was 60 years in men and 62 years in women.

POLYCYTHEMIA VERA

symptoms

1. Erythrocytosis and hyperviscosity, leading to impaired oxygen delivery:

- ✓ Poor CNS circulation: headaches, dizziness, vertigo, tinnitus and visual disturbances
- ✓ Poor coronary circulation: angina pectoris
- ✓ Peripheral circulation intermittent claudication
- ✓ Patients may present with complaints of pruritus after bathing, burning pains in the distal extremities (erythromelalgia)

POLYCYTHEMIA VERA (PV)

2. Venous thrombosis or thromboembolism
3. Hemorrhage: epistaxis, gingival bleeding, ecchymoses, gastrointestinal bleeding
4. Abdominal pain due to peptic ulcer disease is present because PV is associated with increased histamine levels and gastric acidity or possible Budd-Chiari syndrome (hepatic portal vein thrombosis) or mesenteric vein thromosis
5. Early satiety due to splenomegaly
6. Pruritus is secondary to increased histamine release from the basophils and mast cells

POLYCYTHEMIA VERA
physical examination

1. Splenomegaly – is present in 70% of patients at the time of diagnosis.
2. Hepatomegaly - is present in approximately 40% of patients at the time of diagnosis.
3. Hypertension
4. On examination of the eye , the vessels may be engorged, tortuous, and irregular in diameter; the veins may be dark purple.(fundus policythaemicus)
5. Facial plethora

DIAGNOSTIC CRITERIA FOR POLYCYTHEMIA VERA

(Polycythemia Vera Study Group 75)

CATEGORY A

1. Total red cell mass
   - male $\geq$ 36ml/kg
   - female $\geq$ 32 ml/kg
2. Arterial oxygen saturation $\geq$ 92%
3. Splenomegaly

CATEGORY B

1. Thrombocytosis (platelet count > 400 G/l)
2. Leukocytosis (white cell count > 12 G/l, no fever or infection)
3. Increased leukocyte alkaline phosphatase (score> 100)
4. Serum vitamin B_{12}> 900 pg/ml or vitamin B_{12} binding capacity >2200 pg/ml

PV is diagnosed when A1+A2+A3 or A1+A2 and any two from category B

DIAGNOSTIC CRITERIA FOR POLYCYTHEMIA VERA(WHO)

Major criteria

1. Hb > 18.5 g/dl in men, 16.5 g/dl in women or other evidence of increased red cell volume
2. Presence of *JAK2* mutation

Minor criteria

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis)
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation *in vitro*

Lab findings

- Peripheral blood findings
  - Increased hemoglobin & hematocrit
  - Normal red blood cell morphology, unless iron deficient or spent phase
  - Normoblasts may be present
  - Mild to moderate leukocytosis
  - Mild neutrophilia and/or basophilia
  - Thrombocytosis

Bone marrow findings for Polycythemia vera include

- Moderate to marked hypercellularity
- trilineage hyperplasia
- megakaryocytes increased; hyperlobulated
- dilated sinusoids with intravascular hematopoiesis
- decreased or absent iron stores
- increased reticulin (only in a minority of patients)

- Iron stores are decreased or absent because of the increased red blood cell mass, and macrophages may be masked in the myeloid hyperplasia that is present.

Treatment

- The mainstay of treatment for PV is phlebotomy, which is aimed at reducing hyperviscosity by decreasing the venous hematocrit level to less than 45 percent (0.45) in white men and 42 percent (0.42) in blacks and women.
- The PVSG reported the best median survival, 12.6 years, for this type of treatment.

Treatment

- Patients with hematocrit values of less than 70% may be bled twice a week to reduce the hematocrit to the range of 40%.
- Patients with severe plethora who have altered mentation or associated vascular compromise can be bled more vigorously, with daily removal of 500 mL of whole blood

Treatment

- Elderly patients with some cardiovascular compromise or cerebral vascular complications should have the volume replaced with saline solution after each procedure to avoid postural hypotension

Treatment

- The use of myelosuppressive agents such as radioactive phosphorus (32P), chlorambucil (Leukeran), busulfan (Myleran) and hydroxyurea (Hydrea) in conjunction with phlebotomy has been studied.
- Chlorambucil, busulfan, and pipobroman, all alkylating agents, have fallen out of favor because of concerns about rates of iatrogenic leukemia.
- The agent 32P remains in use with supplemental phlebotomy and has a reported median survival similar to that of phlebotomy alone-10.9 years according to PVSG data.

COURSE OF POLYCYTHEMIA VERA -prognosis

- Untreated patients– the median survival ranges from 1-3 years
- Patients treated with phlebotomy or/and hydroxyurea

  - median survival is 13.9 years
- Patients treated with ^{32}P

- median survival is 11.8 years

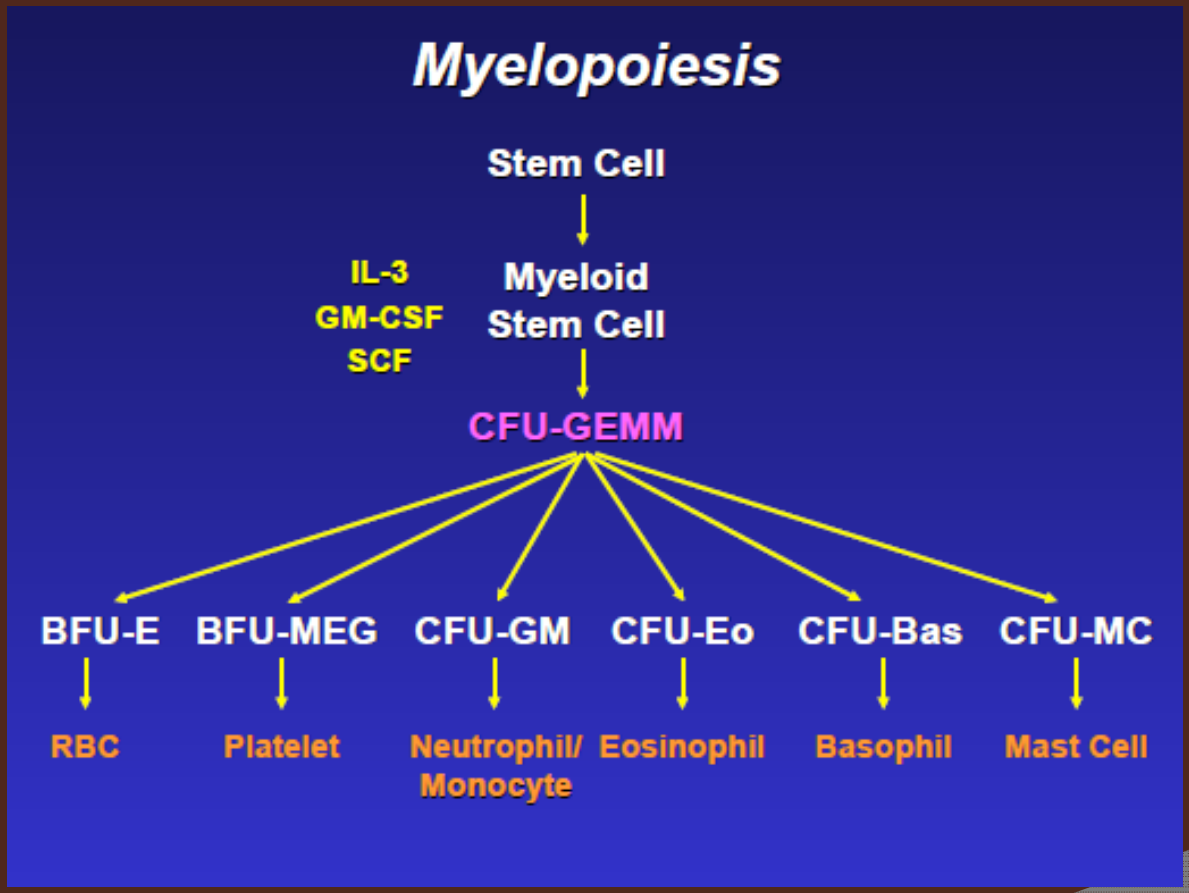
The frequency of acute leukemia (%):

- Patients treated with phlebotomy 1.5 %
- Patients treated with ^{32}P 9.6%

The frequency of myelofibrosis (%):

- Patients treated with phlebotomy 8.6 %
- Patients treated with ^{32}P 7.7%

WBCs


Myelopoiesis
Stem Cell
IL-3
GM-CSF
SCF
Myeloid
Stem Cell
CFU-GEMM
BFU-E
BFU-MEG
CFU-GM
CFU-Eo
CFU-Bas
CFU-MC
RBC
Platelet
Neutrophil/
Monocyte
Eosinophil
Basophil
Mast Cell

Neutrophils

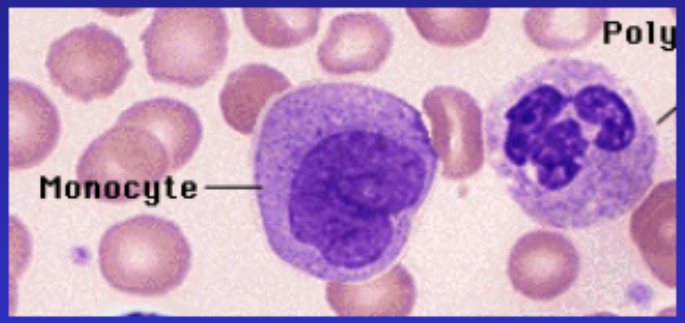
IL-3
GM-CSF
SCF

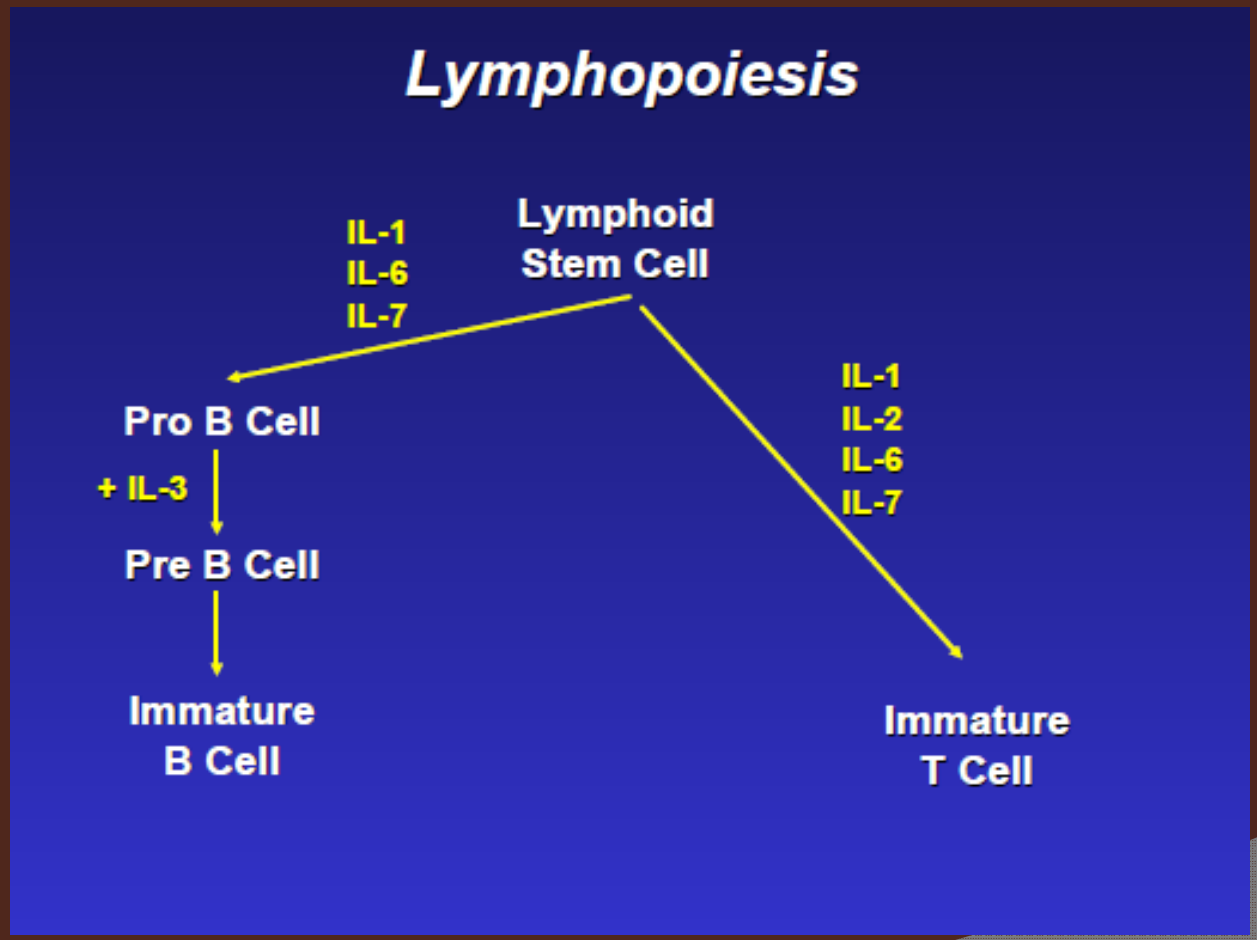
CFU-GEMM → CFU-GM (+ G-CSF) → CFU-G → Myeloblast → Neutrophilic Myelocyte → Neutrophil

Monocytes

IL-3
GM-CSF
SCF

CFU-GEMM → CFU-GM (+ M-CSF) → CFU-M → Monoblast → Promonocyte → Monocyte

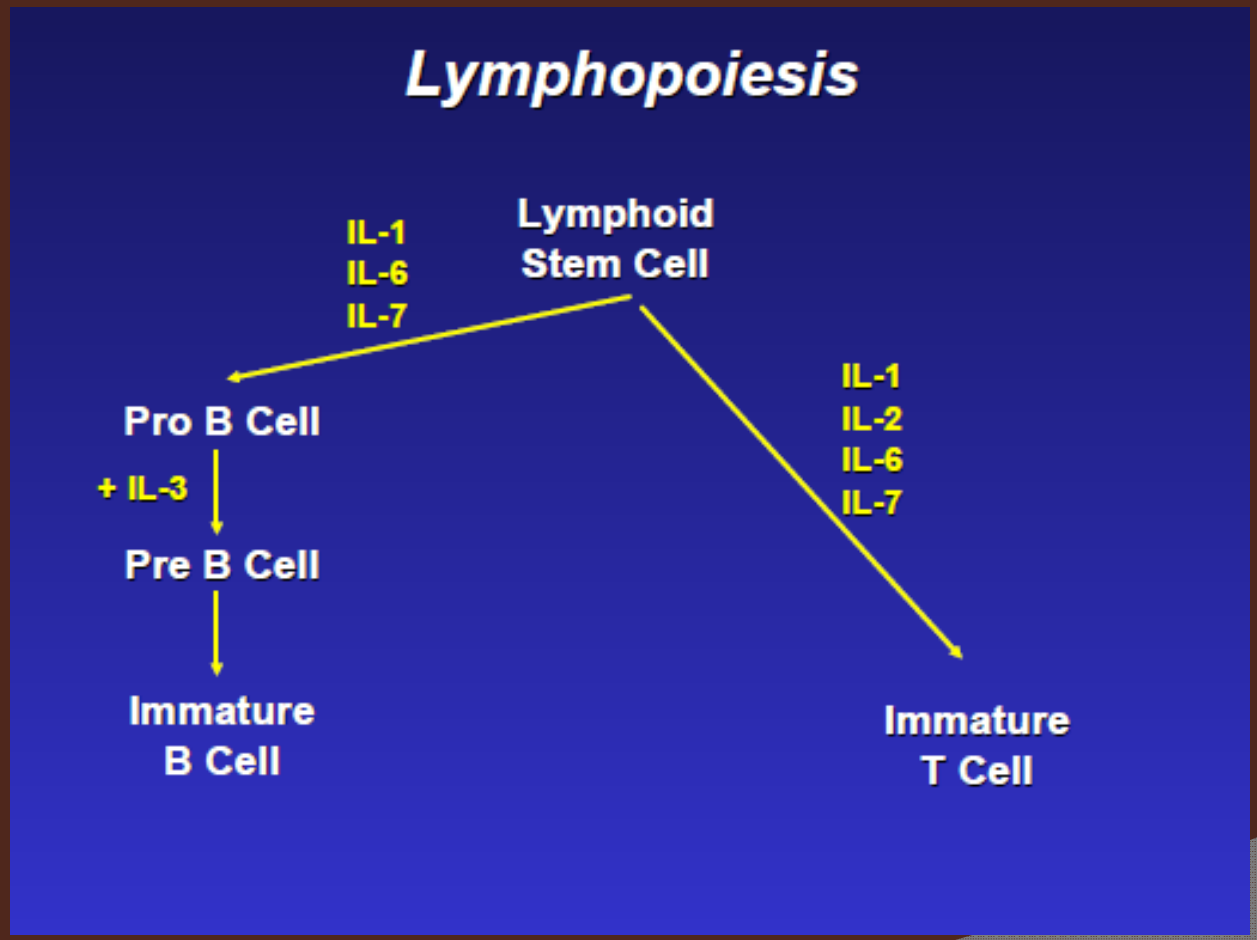



Lymphopoiesis
Lymphoid Stem Cell
IL-1
IL-6
IL-7
Pro B Cell
+ IL-3
Pre B Cell
Immature B Cell
IL-1
IL-2
IL-6
IL-7
Immature T Cell

NONMALIGNANT LEUKOCYTE DISORDERS

Increased TLC

- ✓ **Leukamoid reaction**
- ✓ **Leukemia**
- ✓ **Leukoerythroblastic picture**


Lymphopoiesis
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Pro B Cell
+ IL-3
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Immature B Cell
IL-1
IL-2
IL-6
IL-7
Immature T Cell

NONMALIGNANT LEUKOCYTE DISORDERS

TLC Decreased

- **Isolated**
  - ✓ **Viral Infection**
  - ✓ **Severe Sepsis**
  - ✓ **Drugs**
  - ✓ **Autoimmune diseases**
  - ✓ **Bone Marrow Failure: MDS, AA**
- **Pancytopenia**

Neutrophil disorders

- Changes in leukocyte concentration and morphology often reflect disease processes and toxic challenge.
- The type of cell affected depends upon its primary function:
  - In bacterial infections, neutrophils are most commonly affected
  - In viral infections, lymphocytes are most commonly affected
  - In parasitic infections, eosinophils are most commonly affected.

Neutrophil disorders

- The peripheral neutrophil concentration depends upon
  - Bone marrow production and release
  - The rate of neutrophil movement into the tissues
  - The proportion of circulating to marginating neutrophils
  - Most benign quantitative abnormalities occur in response to physiologic or pathologic processes
- Neutrophilia – an increase in neutrophils
  - Immediate – may occur without tissue damage or other pathologic stimulus.
    - Results from a simple redistribution of cells from the marginal pool to the circulating pool

Neutrophil disorders

- **Neutrophilia – an increase in neutrophils**
  - Immediate – may occur without tissue damage or other pathologic stimulus.
    - Results from a simple redistribution of cells from the marginal pool to the circulating pool
    - May occur after

      violent exercise ,epinephrine administration

      anesthesia

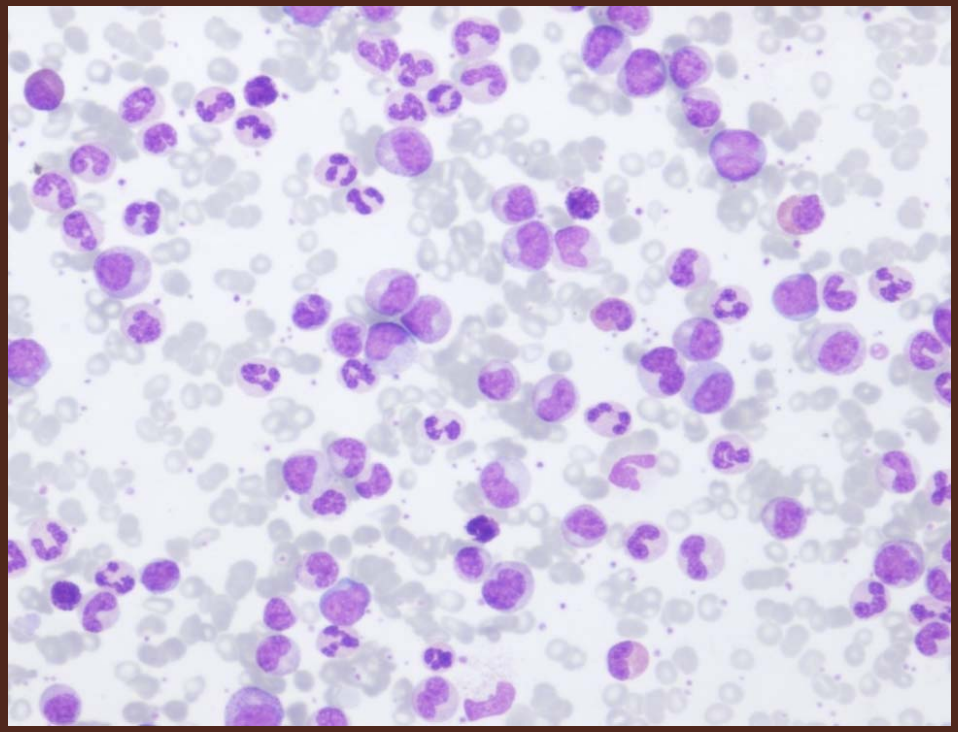
      anxiety
    - also called shift neutrophilia

- Acute neutrophilia – this occurs 4-5 hours after a pathologic stimulus
  - Results from an increased flow of cells from the bone marrow to the peripheral blood
  - Bands and metamyelocytes may be seen
- Chronic neutrophilia – this follows acute neutrophilia
  - The bone marrow starts to throw out younger and younger cells (a shift to the left)

Neutrophil disorders

- Conditions that are associated with neutrophilia are:
  - Bacterial infections (most common cause)
    - This usually causes an absolute neutrophilia
    - In severe infections, the bone marrow stores may be depleted and this can result in neutropenia (typically seen in typhoid fever and brucellosis)
  - Tissue destruction or drug intoxication (tissue infarctions, burns, neoplasms, uremia, gout)
  - SLE

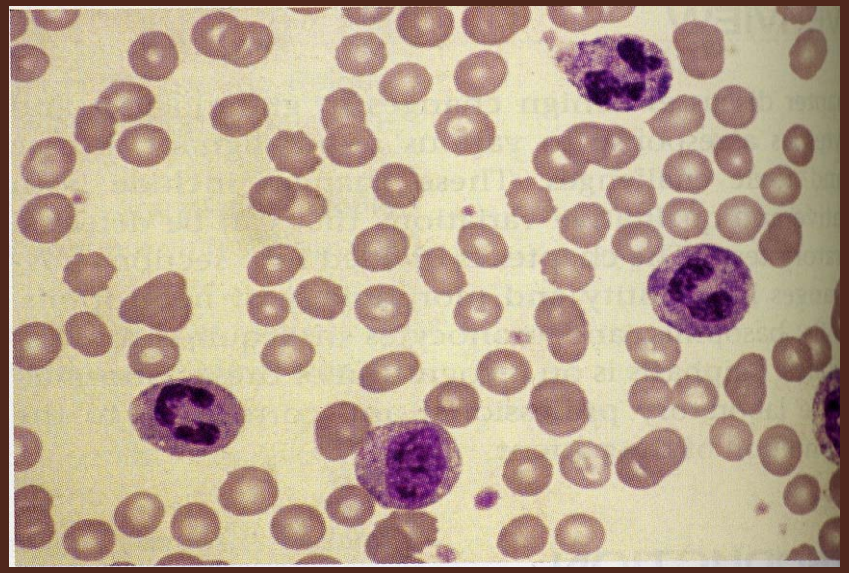
Neutrophilia(40-75%)2000-7000/ µl)

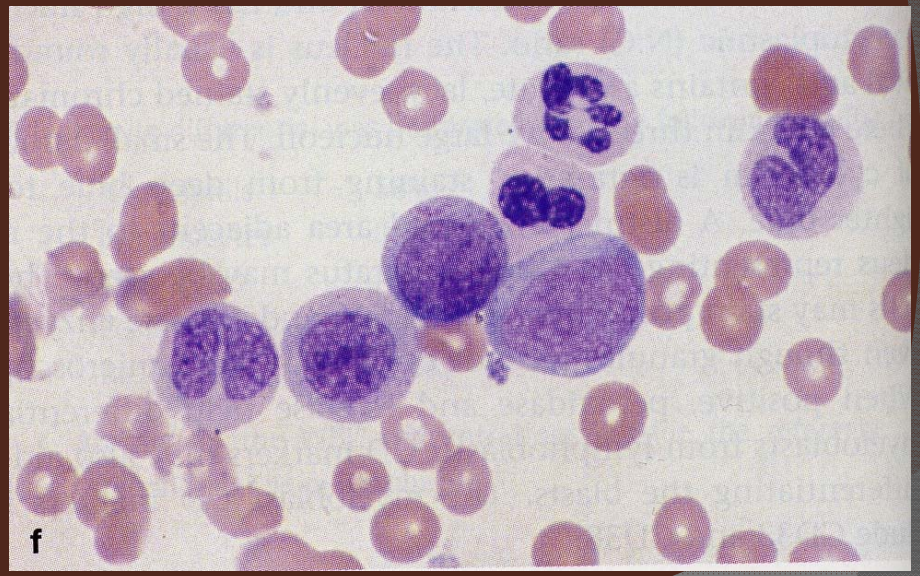


NONMALIGNANT LEUKOCYTE DISORDERS

- Leukemoid reaction – this is an extreme neutrophilia with a WBC count > 30 x 10^9/L
  - Many bands, metamyelocytes, and myelocytes are seen
  - Occasional promyelocytes and myeloblasts may be seen.
  - This condition resembles a chronic myelocytic leukemia (CML), but can be differentiated from CML based on the fact that in leukemoid reactions:
  - There is no Philadelphia chromosome
  - The condition is transient
  - There is an increased leukocyte alkaline phosphatase score (more on this later)
  - No splenomegaly
  - Leukemoid reactions may be seen in tuberculosis, chronic infections, malignant tumors, etc.

LEUKEMOID REACTION





NONMALIGNANT LEUKOCYTE DISORDERS

- Leukoerythroblastic reaction – in this condition nucleated RBCs and neutrophilic precursors are both found in the peripheral blood
  - The WBC count may be increased, normal, or decreased
  - This is associated with myelopthesis (proliferation of abnormal elements in the bone marrow)
- Reactive states
  - With hemorrhage or hemolysis of RBCs, there is increased production of RBCs in the bone marrow and sometimes the granulocyte production also increases.
- Corticosteroid therapy

NONMALIGNANT LEUKOCYTE DISORDERS

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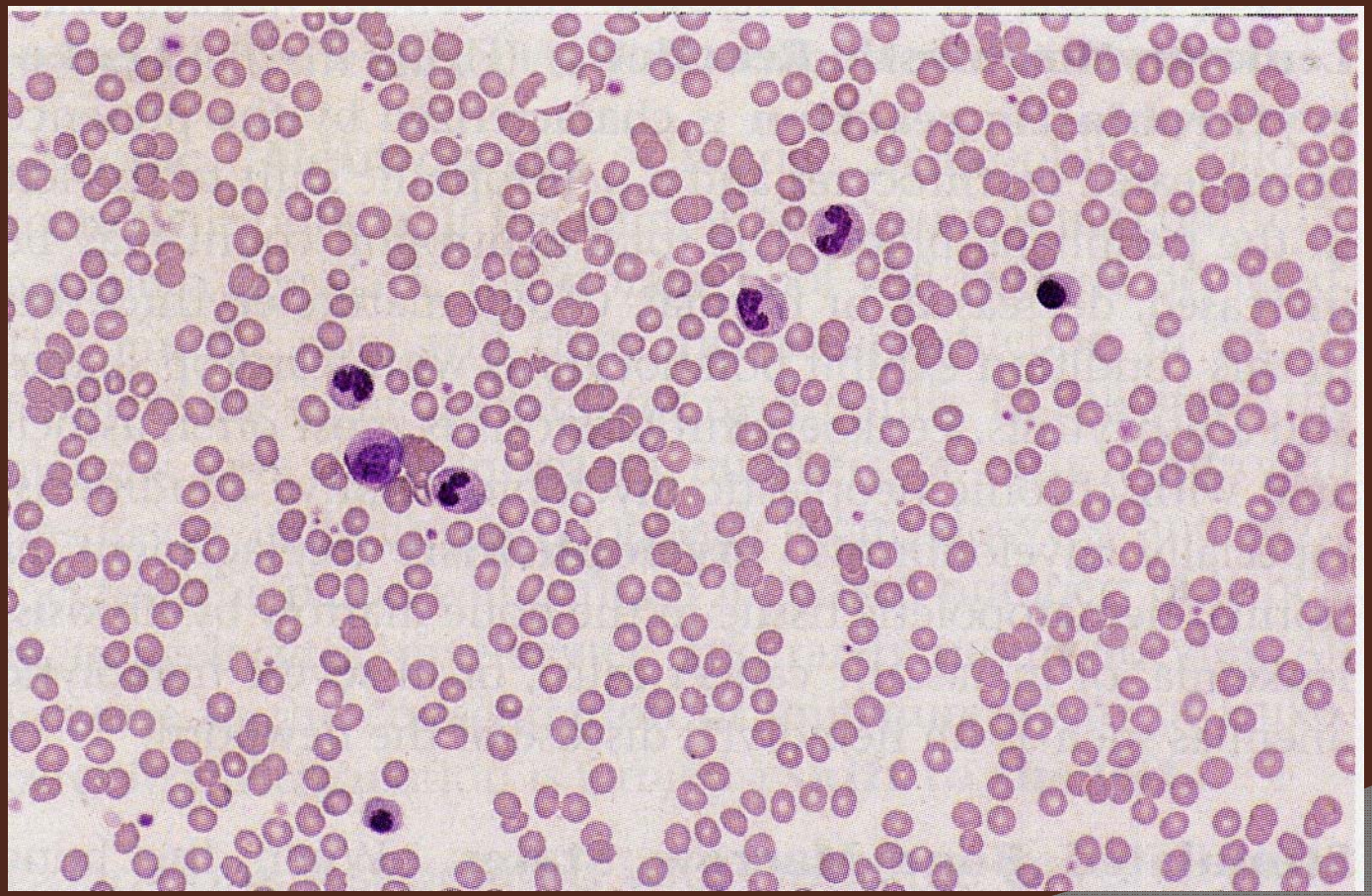
- Reactive states
  - With hemorrhage or hemolysis of RBCs, there is increased production of RBCs in the bone marrow and sometimes the granulocyte production also increases.
  - Storage disorders
- Corticosteroid therapy

LEUKOERYTHROBLASTIC REACTION



Neutrophil disorders

- Neutropenia – this may result from
  - Decreased bone marrow production
    - The bone marrow will show myeloid hypoplasia with a decreased M:E ratio
    - The bone marrow storage pool, and peripheral and marginating pools are all decreased
    - Immature cells may be thrown into the peripheral blood and those younger than bands are ineffective at phagocytosis. This can lead to overwhelming infections.
    - This may be due to stem cell failure, radiotherapy, chemotherapy, or myelopthesis.
  - Ineffective bone marrow production
    - The bone marrow will be hyperplastic
    - Defective production is seen in megaloblastic anemias and myelodysplasic syndromes where the abnormal cells are destroyed before they are released from the bone marrow

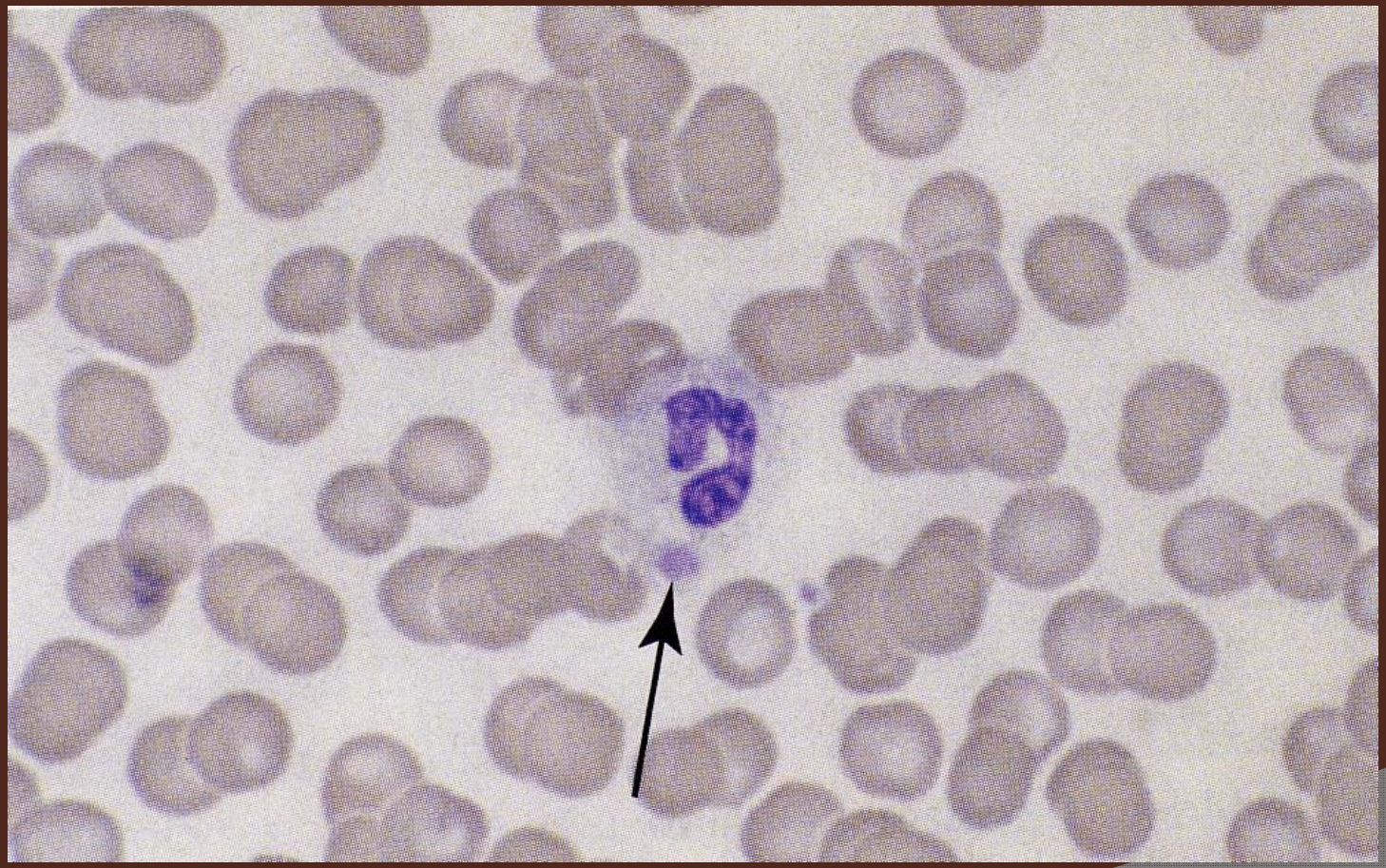
Neutrophil disorders

- Periodic or cyclic – is an inherited autosomal dominant disorder in which every 21-30 days there are several days of neutropenia with accompanying infections. This is followed by asymptomatic periods.
- Familial – this is benign, chronic, and mild with rare clinical symptoms
- Infantile genetic agranulocytosis – this is a rare, congenital, and often fatal disorder in which there is defective bone marrow production of neutrophils.
- False – blood drawn into EDTA in which the cells stick to the side of the tube; disintegration of cells due to age from sitting in a tube for too long; cell clumping

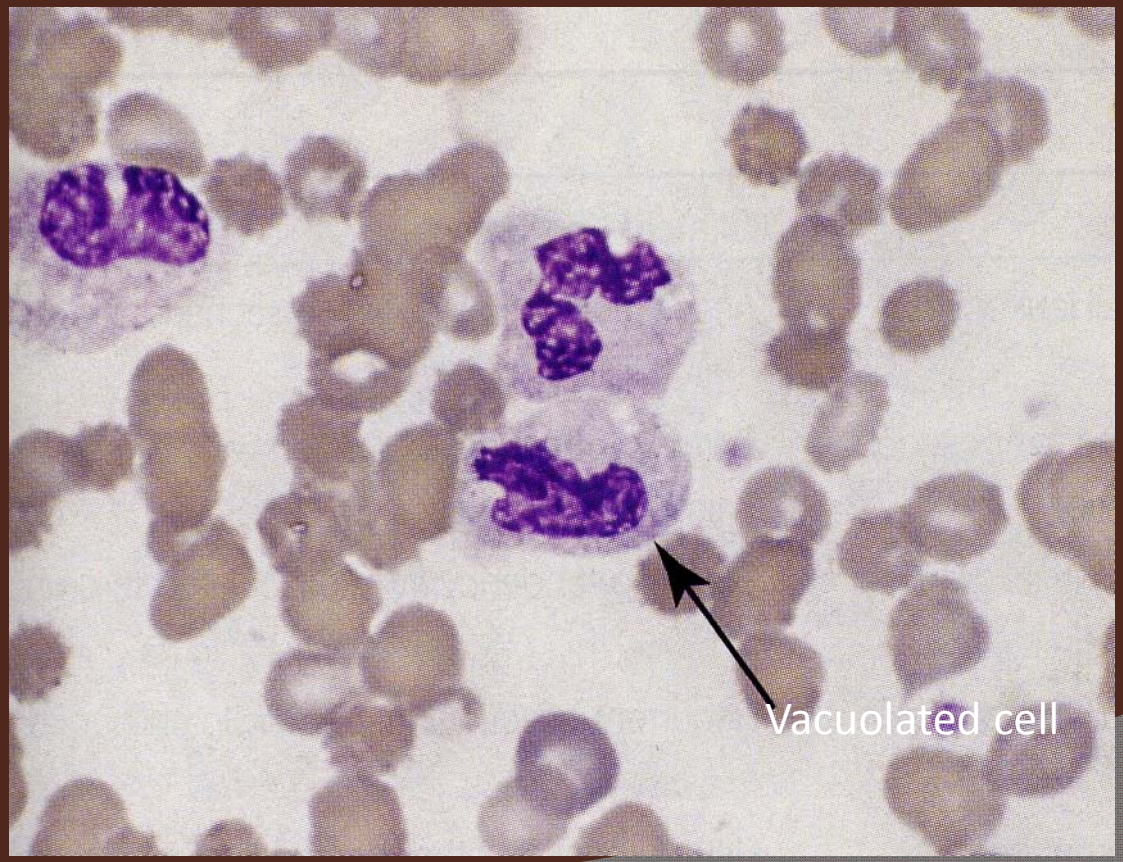
Neutrophil disorders

- **Morphologic and functional abnormalities of neutrophils**
  - Acquired, morphologic – these are reactive, transient changes accompanying infectious states. They include
    - Toxic granulation
    - Dohle bodies
    - Cytoplasmic vacuoles
    - May also see ingested microorganisms

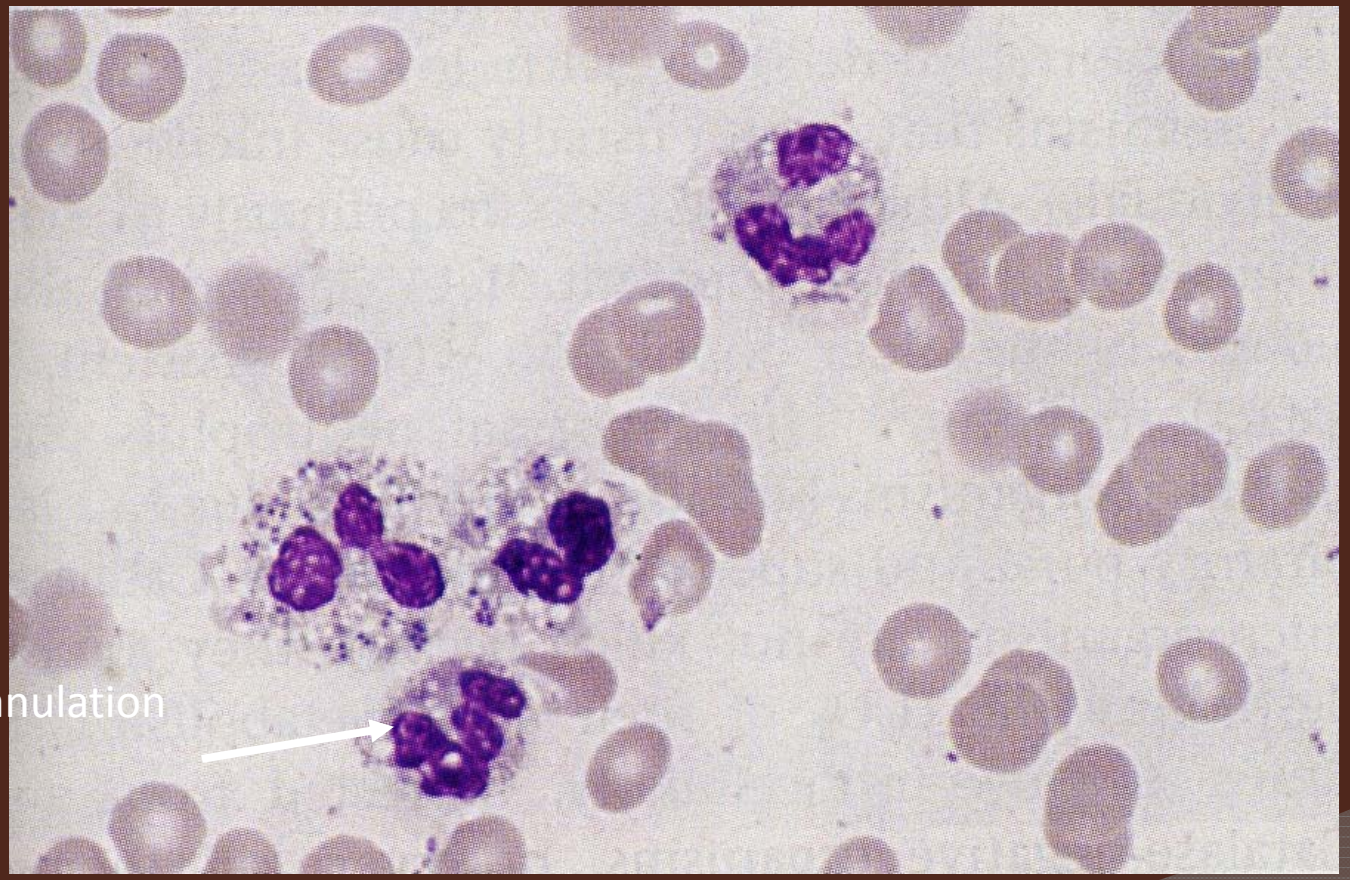
DOHLE BODIES



MORPHOLOGIC NEUTROPHIL CHANGES



MORPHOLOGIC NEUTROPHIL CHANGES

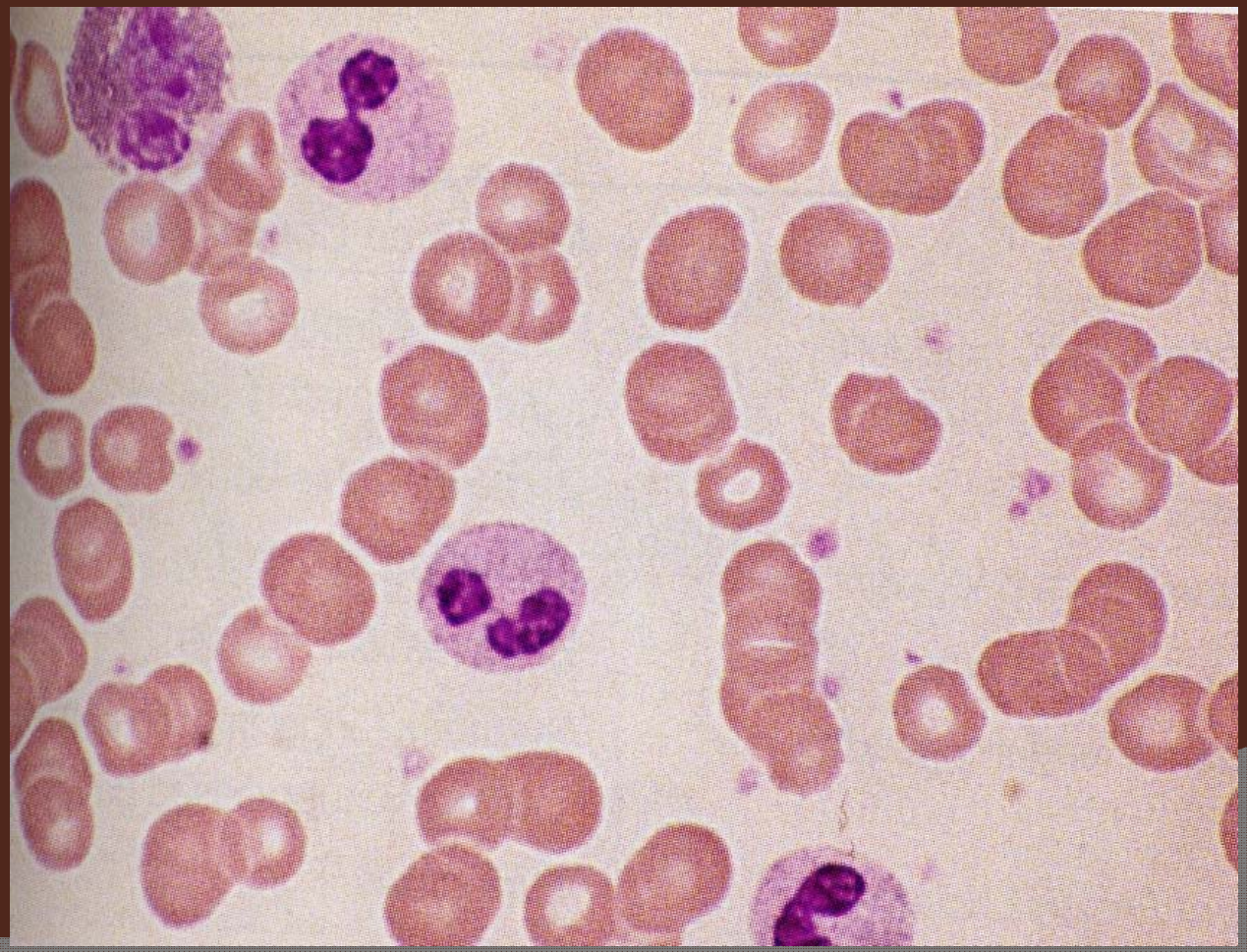


Toxic granulation

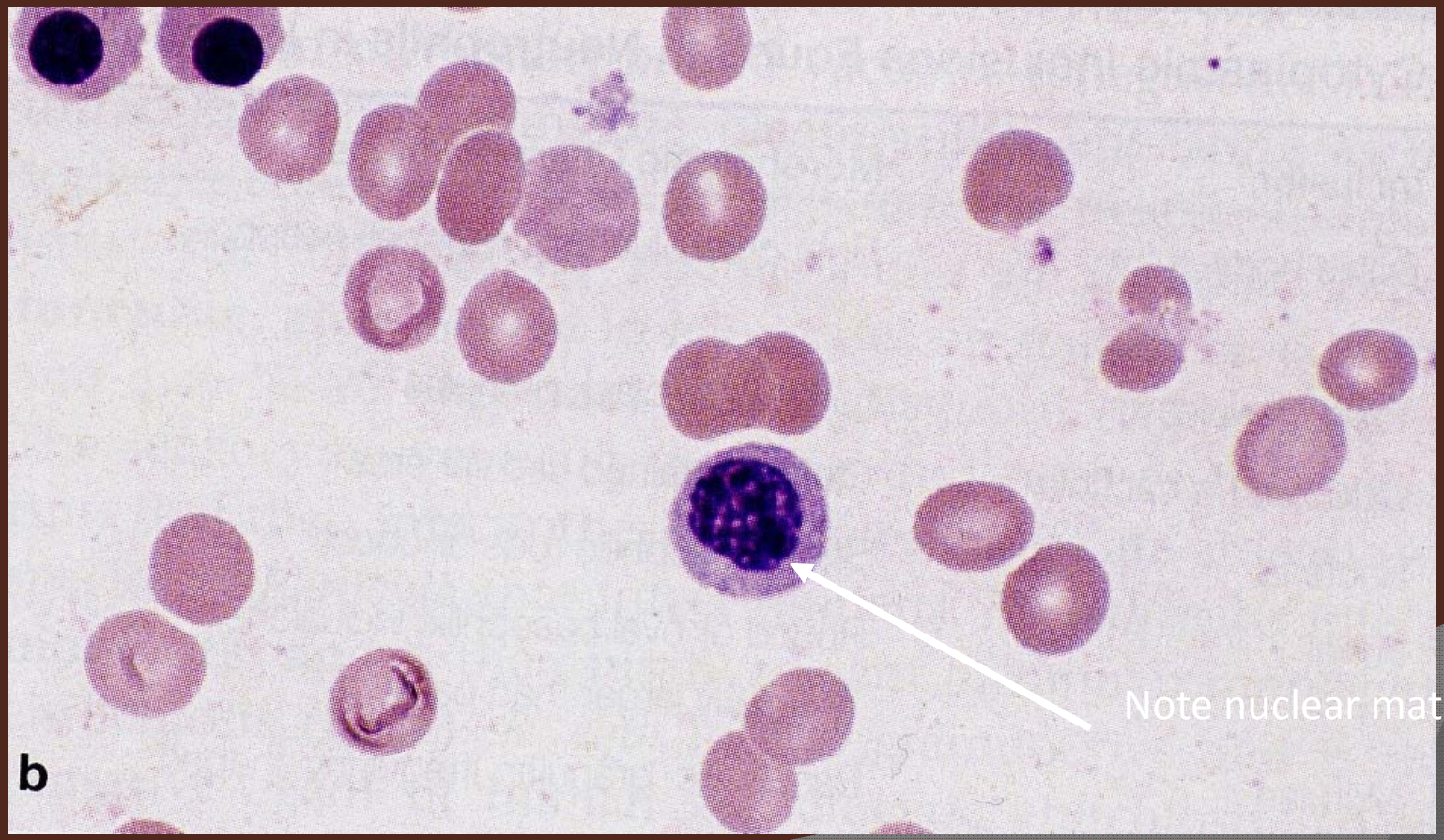
Neutrophil disorders

- Inherited functional and/or morphological abnormalities
  - Pelger- Huet Anomaly – this is a benign, inherited, autosomal dominant abnormality in which the neutrophil nucleus does not segment beyond the bilobular stage ("Prince-nez cells").
    - The cells may sometimes resemble bands, but the chromatin is more condensed (mature).
    - The cells function normally.
    - Acquired or pseudo Pelger-Huet Anomaly is seen in myeloproliferative and myelodysplastic states

PELGER-HUET ANOMALY



PSEUDO PELGER-HUET ANOMALY

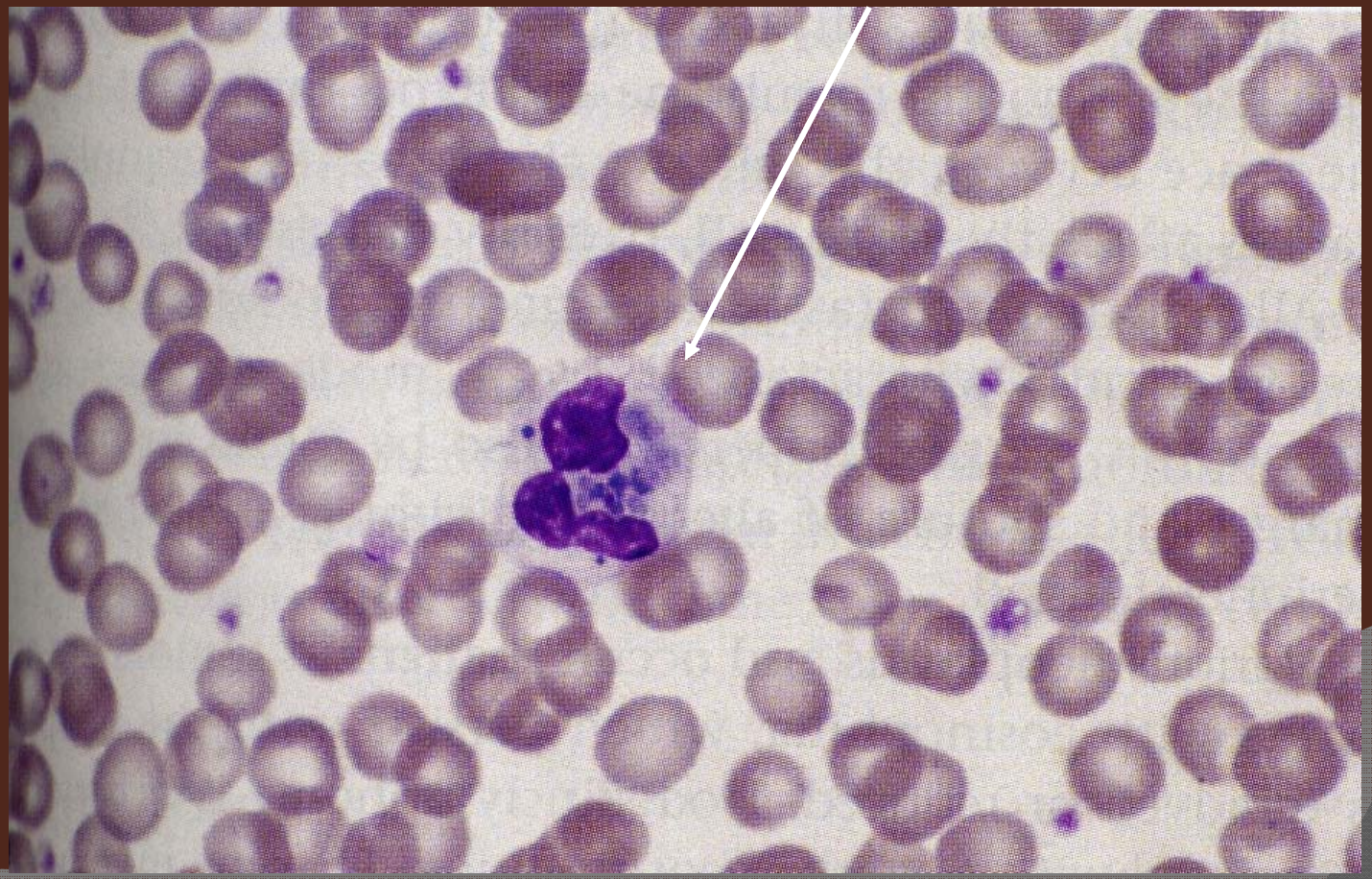


Neutrophil disorders

- Chediak-Higashi Anomaly –
  - This is a rare autosomal recessive disorder in which abnormal lysosomes are formed by the fusion of primary granules. These are seen as grayish-green inclusions
  - The cells are ineffective in killing microorganisms and affected individuals often die early in life from pyogenic infections.

CHEDIAK-HIGASHI ANOMALY

Note abnormal lysosomes



NONMALIGNANT LEUKOCYTE DISORDERS

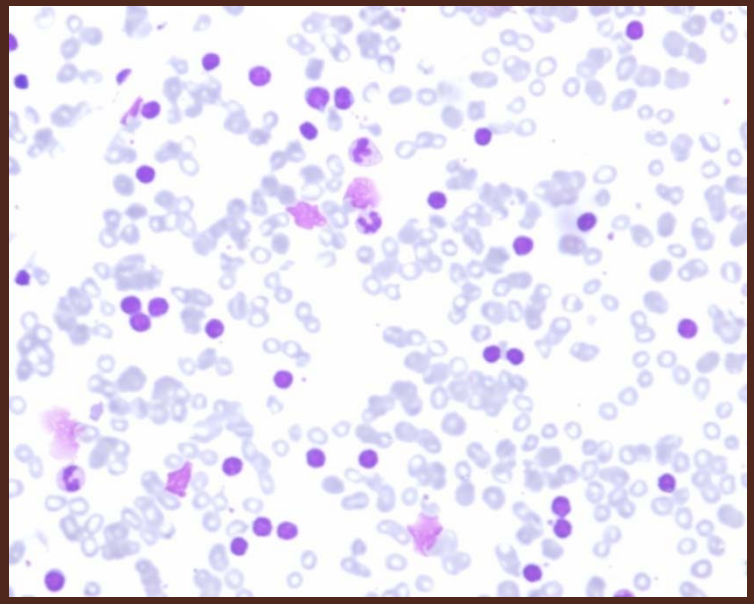
- May-Hegglin Anomaly
  - This is a rare, autosomal dominant disorder in which the leukocytes contain large basophilic inclusions containing RNA that look similar to Dohle bodies.
  - It can be differentiated from an infection because toxic granulation is not seen.
  - The patients also have giant platlets that have a shortened survival time. Because of this, patients may have bleeding problems, but they usually have no other clinical symptoms

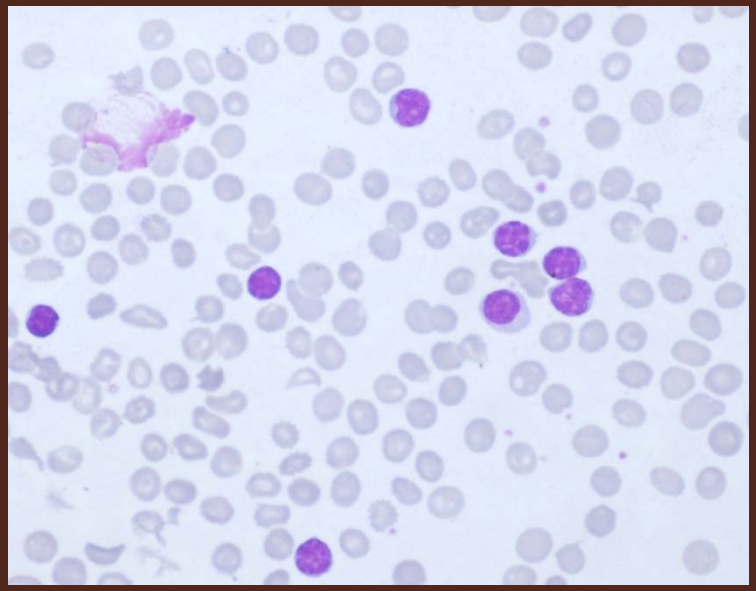
NONMALIGNANT LEUKOCYTE DISORDERS

- Chronic granulomatous disease
  - This is a lethal, sex-linked disorder affecting the function of the neutrophil
  - The neutrophil can function in phagocytosis, but it cannot kill microorganisms because the cells have a defect in the respiratory burst oxidase system.
  - Affected individuals have chronic infections with organisms that do not normally cause infections in normal individuals
- Myeloperoxidase deficiency
  - This is a benign, autosomal recessive disorder characterized by a lack of myeloperoxidase in the neutrophils

Lymphocytes 20–45% of WBCs(1500- 4ooo/ µl)

- **Lymphocytosis**
  CLL
- Viral infection
- Pertussis
- Brucellosis





Lymphopenia

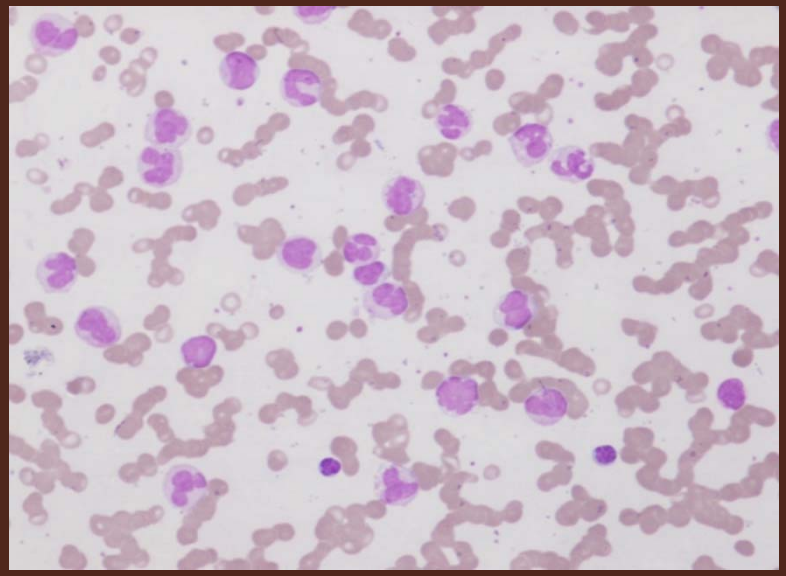
- Steroids
- Uremia
- HIV
- SLE
- Post chemo/RT

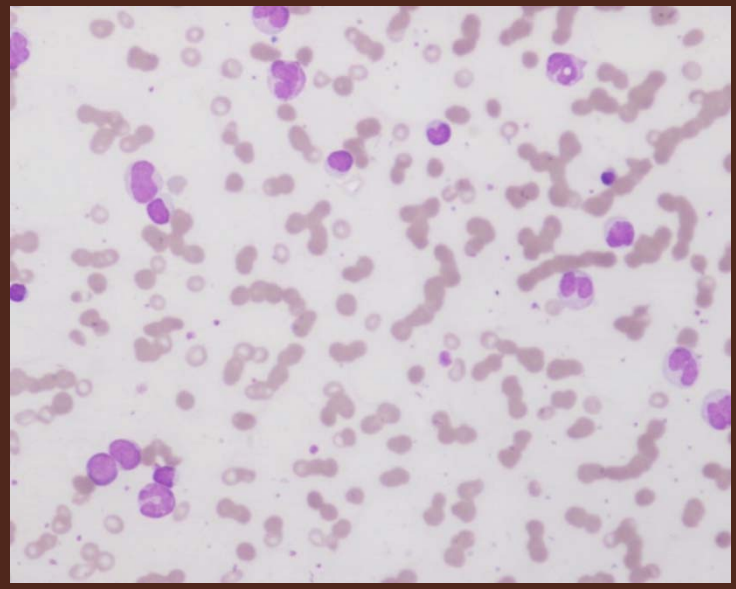
Monocytes

2–10% of WBCs(200-800/ µl l

Monocytosis

- **Infections**
  - ✓ **Tuberculosis,**
  - ✓ **brucellosis**
  - ✓ **protozoan disease)**
- **Malignant disease**
  - ✓ **M4 & M5 AML**
  - ✓ **Hodgkin's disease)**
- **Myelodysplasia**





Eosinophils

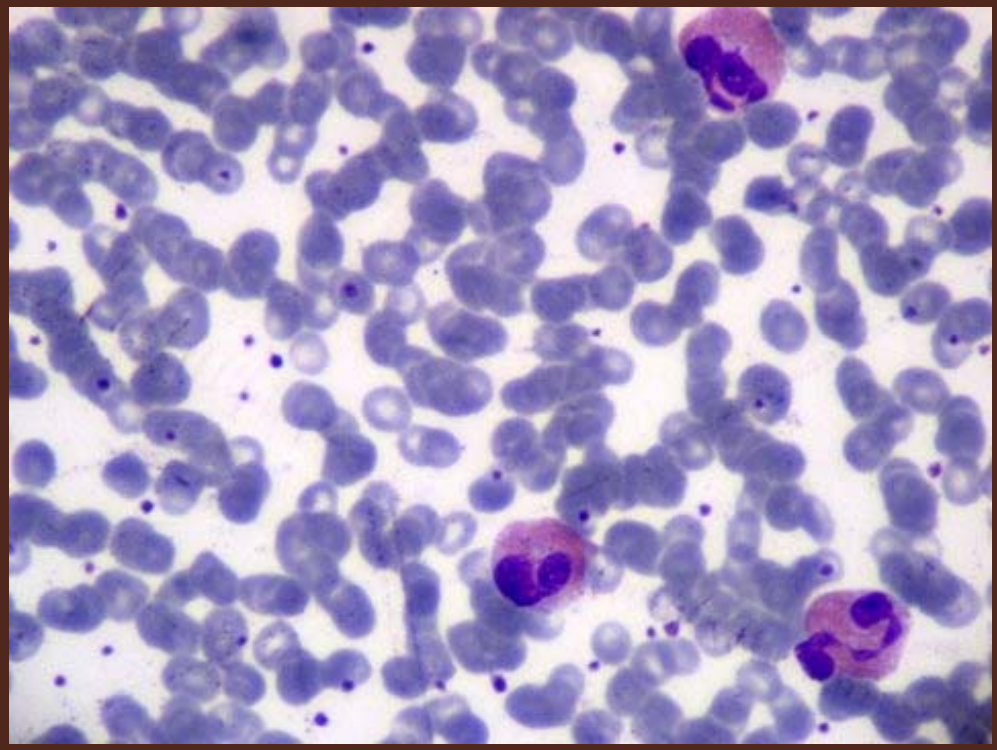
1–6% of WBCs

AEC=40-400/ µl

Eosinophilia

- ✓ **Asthma/ Allergy**
- ✓ **Parasitic Infections**
- ✓ **Skin diseases**
- ✓ **Hypereosinophillic syndrome**
- ✓ **Malignant disease**

Eosinopenia



INFECTIOUS MONONUCLEOSIS

Infectious Mononucleosis

- Epstein Barr Virus (EBV)
  - Herpes Family – (linear DNA virus HHV4)
  - Surrounded by nucleocapsid and glycoprotein envelope
- Also associated with nasopharyngeal carcinoma, Burkitts lymphoma, Hodgkins Disease, B cell lymphoma.

Infectious Mononuclosis Epidemiology

- Worldwide Prevalence of EBV
- Infections peak in early childhood and late adolescence/young adulthood.
- By adulthood , 90% of individuals have been infected and have antibodies to the virus.

Introduction

- The virus is spread by person-to-person contact, via saliva. In rare instances, the virus has been transmitted by blood transfusion or transplacentally.
- In underdeveloped countries, people are exposed in early childhood where they are less likely to develop noticeable symptoms.
- In developed countries such as the United States, the age of first exposure may be delayed to older childhood and young adult age when symptoms are more likely to result.

Introduction

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Infectious Mononucleosis Pathogenesis

- Memory B cells (? epithelial cells) are reservoir for EBV.
- EBV receptor is CD21 (found on B cell surface)
- Cellular immunity (suppressor T cells, NK cells, cytotoxic T cells) more important than humoral immunity in controlling infection

- .
- EBV infected B-lymphocytes express a variety of “new” antigens encoded by the virus. Infection with EBV results in expression of:
  1. Viral Capsid Antigen (VCA)
  2. Early Antigen (EA)
  3. Nuclear Antigen (NA)

  Each antigen expression has corresponding antibody responses.

Epstein-Barr Virus (VCA)

- Viral capsid antigen (VCA) is produced by infected B cells and can be found in the cytoplasm.
- Anti-VCA IgM is usually detectable early in the course of infection, 4 to 7 days after onset of signs and symptoms, but it is low in concentration and disappears within 2 to 4 months.

Epstein-Barr Virus (EA)

- Early antigen (EA) is a complex of two components, early antigen-diffuse (EA-D), which is found in both the nucleus and cytoplasm of the B cells, and early antigen-restricted (EA-R), which is usually found as a mass only in the cytoplasm.
- Anti-EA-D of the IgG type is highly indicative of acute infection, but it is not detectable in 10% to 20% of patients with IM. EA-D disappears in about 3 months; however, a rise in titer is demonstrated during reactivation of a latent EBV infection.
- Anti-EA-R IgG is not usually found in young adults during the acute phase. Anti-EA-R IgG appears transiently in the later convalescent phase. In general, anti-EA-D and anti-EA-R IgG are not consistent indicators of the disease stage.

Epstein-Barr Virus (EBNA)

- Epstein-Barr nuclear antigen (EBNA) is found in the nucleus of all EBV-infected cells. Although the synthesis of NA precedes EA synthesis during the infection of B cells, EBV-NA does not become available for antibody stimulation until after the incubation period of Infectious Mono, when activated T lymphocytes destroy the EBV genome-carrying B cells. As a result, antibodies to NA are absent or barely detectable during acute IM.
- Anti-EBNA IgG does not appear until a patient has entered the convalescent period. EBV-NA antibodies are almost always present in sera containing IgG antibodies to VCA of EBV unless the patient is in the early acute phase of IM.

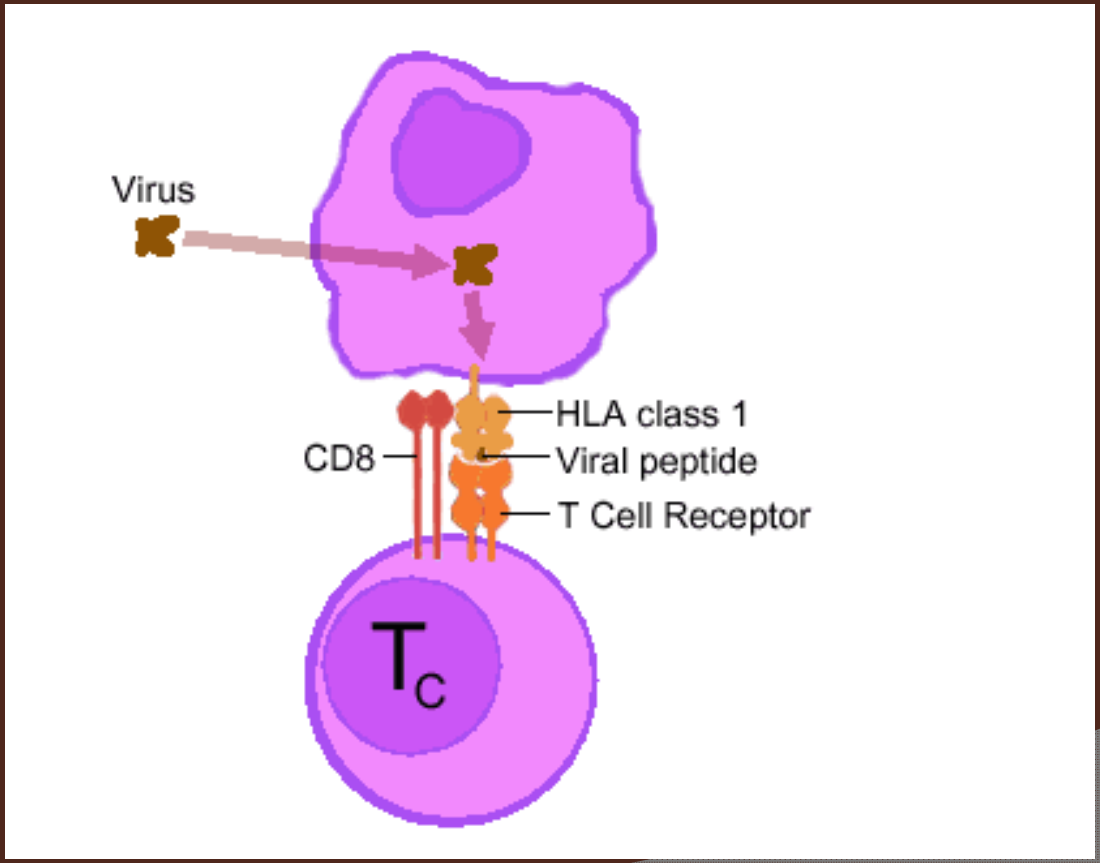
Epstein-Barr Virus (EBNA)

- Under normal conditions, antibody titers to NA gradually increase through convalescence and reach a plateau between 3 and 12 months postinfection. The antibody titer remains at a moderate, measurable level indefinitely because of the persistent viral carrier state established following primary EBV infection.
- Test results of antibodies to EBV-NA should be evaluated in relationship to patient symptoms, clinical history, and antibody response patterns to EBV-VCA and EA to establish a diagnosis.

Immunopathogenesis : IM

- In acute stage, proliferating EBV-infected B cells are controlled principally by NK cellsand CD8 cells.
- After T-cell response, number of EBV-infected B cells falls dramatically.

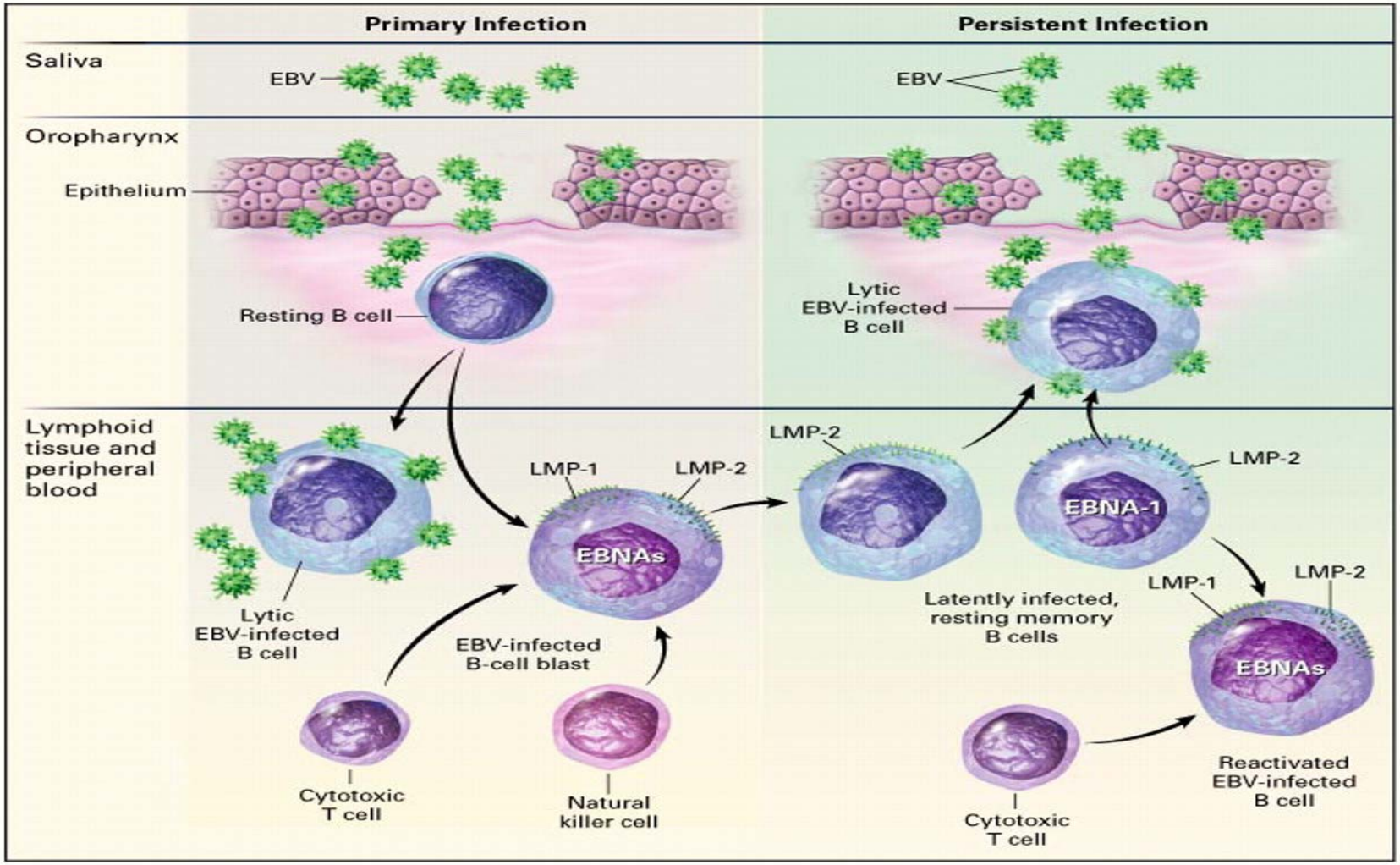
Infectious Mononucleosis Pathogenesis



Infectious Mononucleosis Signs & Symptoms

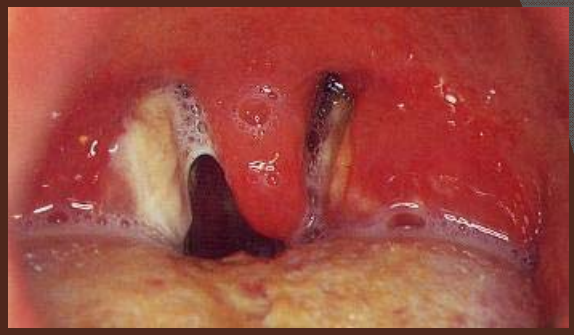
- Incubation in adults 4-6 wks
- Prodrome (1-2 weeks before illness)
  Fatigue, Malaise, Myalgias
- Symptoms
  - Sore throat, Malaise, Headache, Abdominal Pain, Nausea/Vomiting, Chills
- Signs
  - Lymphadenopathy, Fever, Pharyngitis, Splenomegaly, Hepatomegaly, Rash, Periorbital Edema, Palatal Enanthem, Jaundice.

Infectious Mononucleosis



NEJM;343:481-492.

Infectious Mononucleosis



- Pharyngitis is the most consistent physical finding.
  - 1/3 of patients : exudative pharyngitis.
  - 25-60% of patients : petechiae at the junction of the hard and soft palates.
  - Tonsillar enlargement can be massive, and occasionally it causes airway obstruction.

Infectious Mononucleosis

- Lymphadenopathy : 90%
  - symmetrical enlargement.
  - mildly tender to palpation and not fix.
  - posterior cervical lymph nodes.
  - anterior cervical and submandibular nodes.
  - axillary and inguinal nodes.
  - Enlarged epitrochlear nodes are very suggestive of infectious mononucleosis.

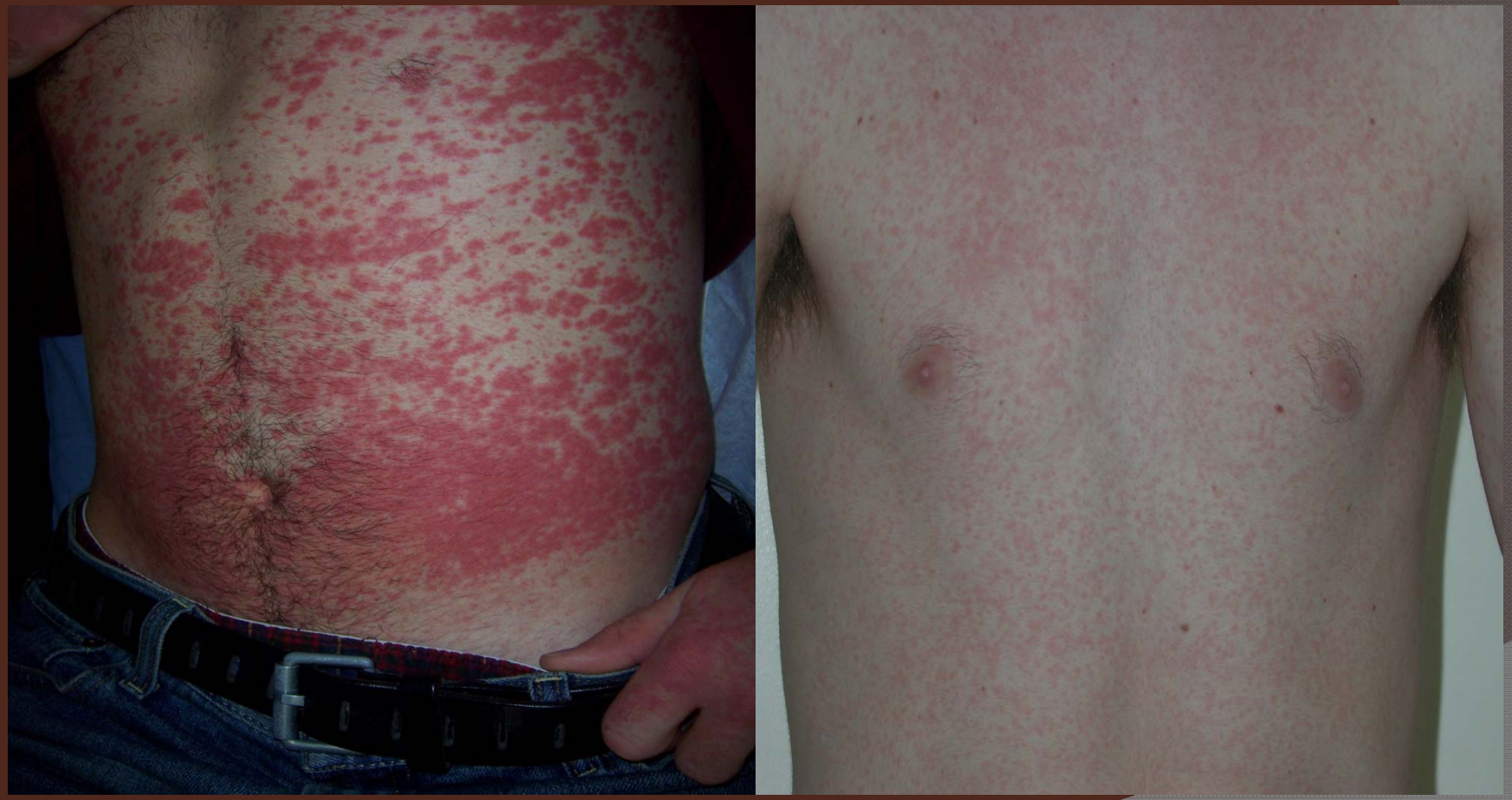
Infectious Mononucleosis

- Hepatomegaly :
  - jaundice is rare.
  - Percussion tenderness over the liver is common.
- Splenomegaly :
  - palpable 2-3 cm below the left costal margin and may be tender.
  - rapidly over the first week of symptoms, usually decreasing in size over the next 7-10 days.
  - spleen can rupture from relatively minor trauma or even spontaneously.

Infectious Mononucleosis

- Maculopapular rash : 15%
  - usually faint, widely scattered, and erythematous
  - occurs in 3-15% of patients and is more common in young children.
  - 80% of patients, treatment with amoxicillin or ampicillin is associated with rash
  - Circulating immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to ampicillin are demonstrable.

Infectious Mononucleosis



IM with rash after treatment with amoxicillin or ampicillin

NEJM;343:481-492.

Infectious Mononucleosis

- Eyelid edema : 15%
  - may be present, especially in the first week

- Children younger than 4 years : more commonly
  - splenomegaly or hepatomegaly
  - rash
  - symptoms of an upper respiratory tract infection

Infectious Mononucleosis : Lab

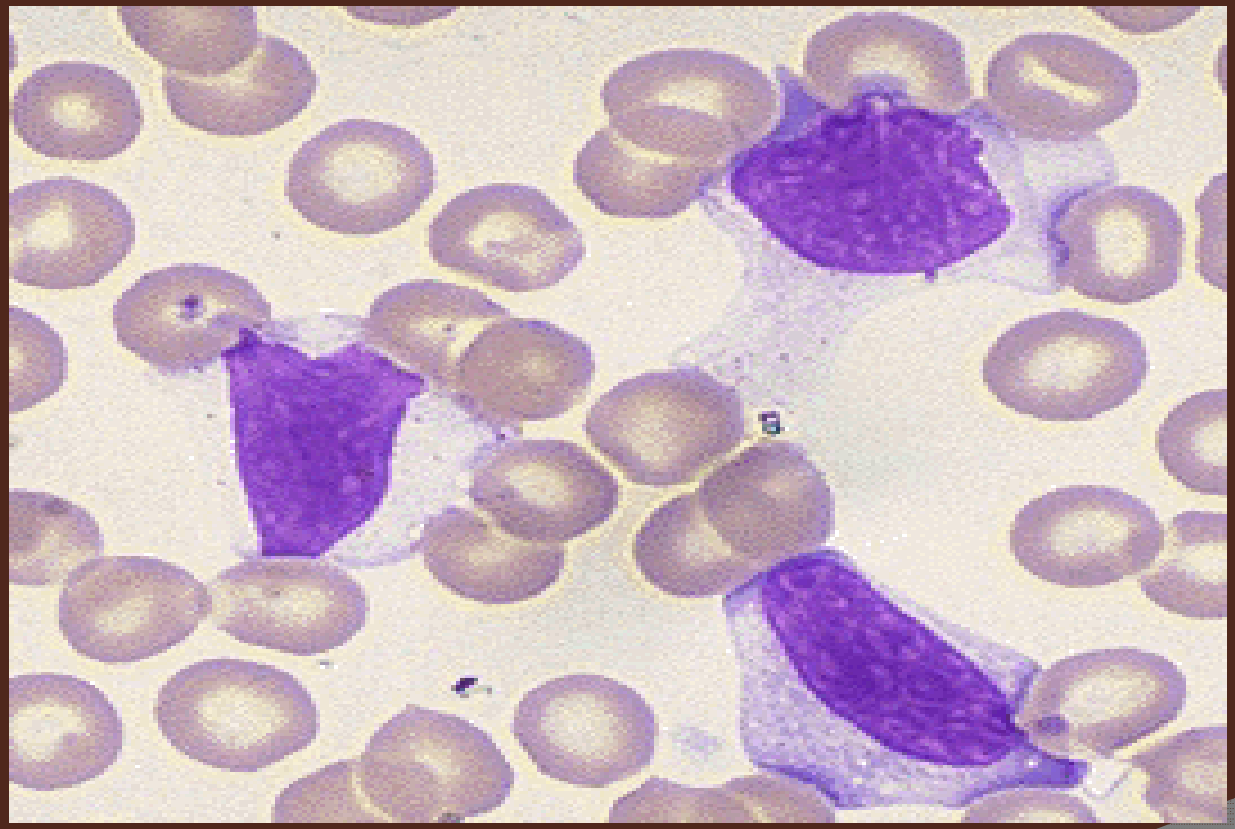
- The 3 classic criteria for laboratory confirmation

  1 leucocytosis lymphocytosis(60%) & the presence of at least 20% atypical on peripheral smear

  2 positive serologic test for Epstein- Barr virus (EBV).

  3.Heterophil Ab 1:256

Infectious Mononucleosis



atypical lymphocytes : Downey types

Lab diagnosis

- Heterophile antibodies
  - 50% in first week of illness
  - 60-90% in the second or third weeks
  - begins to decline during the fourth or fifth week and often is less than 1:40 by 2-3 months after symptom onset
  - 20% of patients have positive titers 1-2 years after acquisition

Infectious Mononucleosis : Lab

- Liver function tests
  - 80-100% of patients : elevated LFT
  - Alkaline phosphatase, AST and bilirubin peak 5-14 days after onset
  - GGT peaks at 1-3 weeks. Occasionally, GGT remains mildly elevated for up to 12 months
  - 95% of patients : elevated LDH
  - most liver function test results are normal by 3 months.

IM Treatment

Medical Care :

- self-limited illness : not require specific therapy.
- Inpatient therapy of medical and surgical complications may be required.
- Acyclovir
  - inhibit viral shedding from the oropharynx
  - clinical course is not significantly
- IVIG
  - immune thrombocytopenia associated with

Andersson J et al. *J Infect Dis*. Feb 1986;153(2):283-90.
Cyran EM et al. *Am J Hematol*. Oct 1991;38(2):124-9.

IM Treatment

Medical Care :

- Short-course corticosteroids
  : prednisolone (1 mg/kg/d, max 60 mg/d for 7 d
  and tapered over another 7 d)
  - Marked tonsillar inflammation with impending airway obstruction
  - Massive splenomegaly
  - Myocarditis
  - Hemolytic anemia
  - Hemophagocytic syndrome
  - Seizure and meningitis

Surgical Care :

- Splenic rupture.

AAP. Red book2006;286-288.
Nelson. Textbook of Pediatrics17th ed;977-981.

IM : Complication

- Hepatitis : > 90% of patients
  - LFT : < 2-3 times of NUL in the second and third weeks of illness
  - 45% of patients : elevated bilirubin, but jaundice occurs in only 5%.

- Platelet count : nadir approximately 1 week after symptom onset (100,000-140,000/mm^3.), then gradually improves over the next 3-4 weeks. Mild thrombocytopenia occurs in approximately 50% of patients with infectious mononucleosis.

IM : Complications

- Hemolytic anemia
  - 0.5-3%, associated with cold-reactive antibodies, anti-I antibodies, and with autoantibodies to triphosphate isomerase
  - mild and is most significant during the second and third weeks of symptoms.
- Upper airway obstruction
  - 0.1-1%, due to hypertrophy of tonsils and other lymph nodes of Waldeyer ring
  - treatment with corticosteroids may be beneficial

IM : Complications

- Splenic rupture : 0.1-0.2%
  - Spontaneous or history of some antecedent trauma.
  - occur during the second and third weeks.
  - mild-to-severe abdominal pain below the left costal margin, sometimes with radiation to the left shoulder and supraclavicular area.
  - Massive bleeding : Shock

IM : Complications

- Hematologic complications
  - hemophagocytic syndrome.
  - Immune thrombocytopenic purpura occurs and may evolve to aplastic anemia.
  - accelerate hemolytic anemia in congenital spherocytosis or hereditary elliptocytosis.
  - Disseminated intravascular coagulation associated with hepatic necrosis has occurred.

IM : Complications

- Neurologic complications : < 1%
  - during the first 2 weeks.
  - negative for the heterophile antibody.
  - Severe (fatal), complete recovery
  - aseptic meningitis, acute viral encephalitis, coma, meningitis, and meningoencephalopathy.
  - Hypoglossal nerve palsy, Bell palsy, hearing loss, brachial plexus neuropathy, multiple cranial nerve palsies, Guillain-Barré syndrome, autonomic neuropathy, gastrointestinal dysfunction secondary to selective cholinergic dysautonomia, acute cerebellar ataxia, transverse myelitis.

IM : Complications

- Cardiac and pulmonary complications
  - rare
  - chronic interstitial pneumonitis.
  - myocarditis and pericarditis.

IM : Prognosis

- Immunocompetent : full recovery in several months.
- The common hematologic and hepatic complications resolve in 2-3 months.
- Neurologic complications
  - Children : resolve quickly
  - Adults : neurological deficits
- All individuals develop latent infection
  - asymptomatic.

- Leukaemias