Peripheral Smear Basics (Part 1)

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11-16-2005

Peripheral Smear

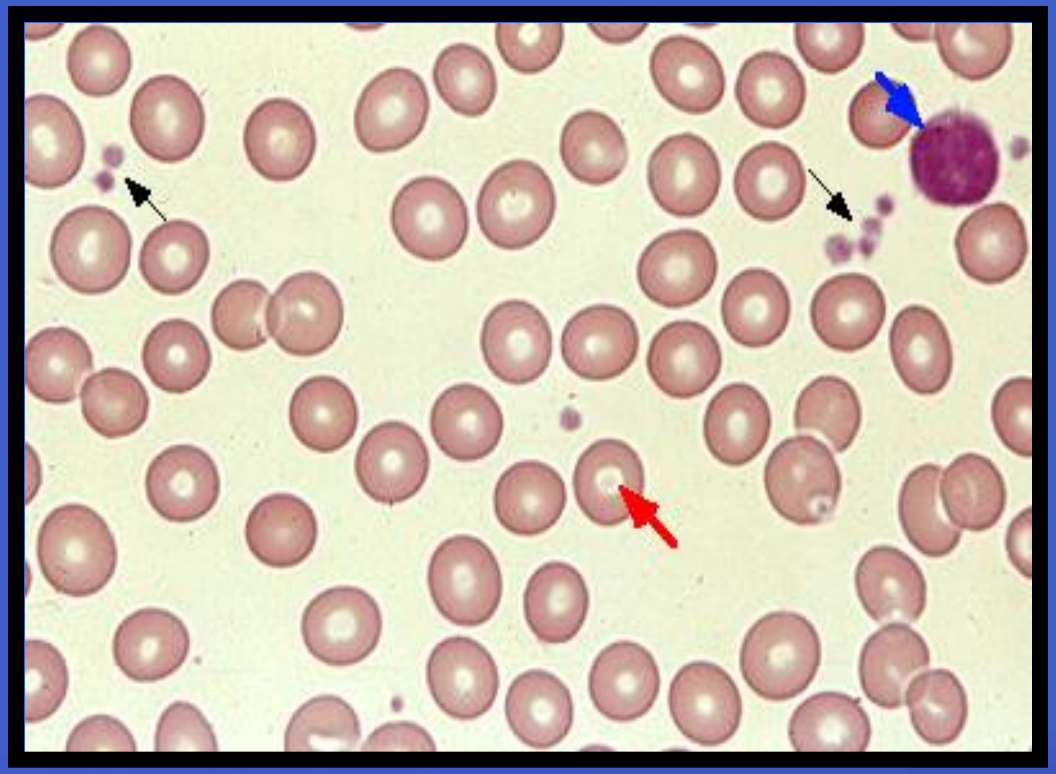
- Inexpensive, rapid, reliable
- Window into the functional status of the bone marrow
  - especially cytopenic states

Vs. automated machines

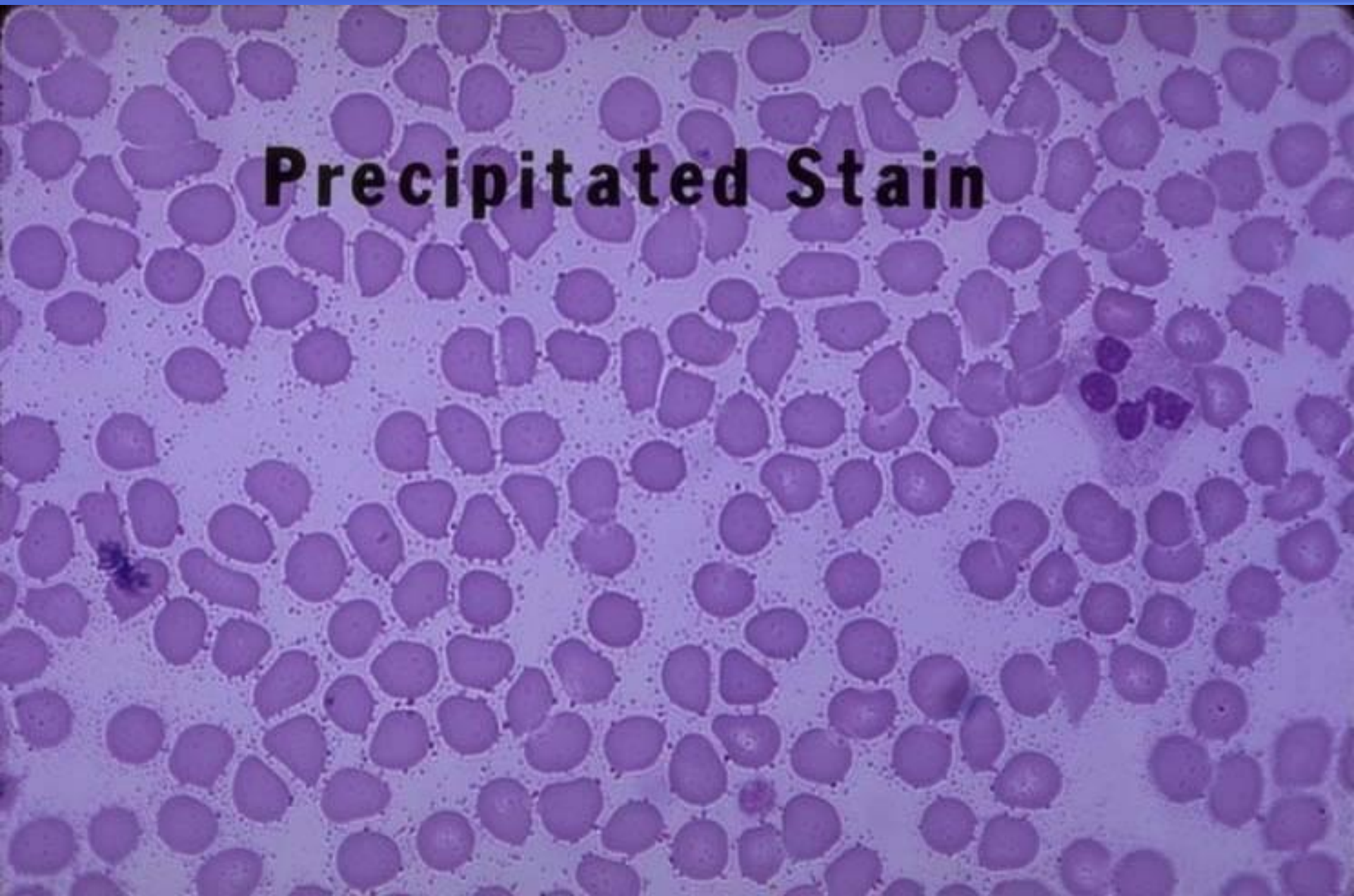
- Able to weigh importance of observed findings and assess their importance within context of clinical data
- Machines tend to generalize a wide array of morphologic abnormalities

Systematic approach

- Slide preparation
- Optimal area of review
- RBCs
  - Size
  - Shape
  - Pallor
  - Premature forms
  - Inclusions
- White cells
  - Lymphocytes
  - Neutrophils
  - Eosinophils
  - Basophils
  - Monocytes
  - Blasts
  - Organisms
- Platelets

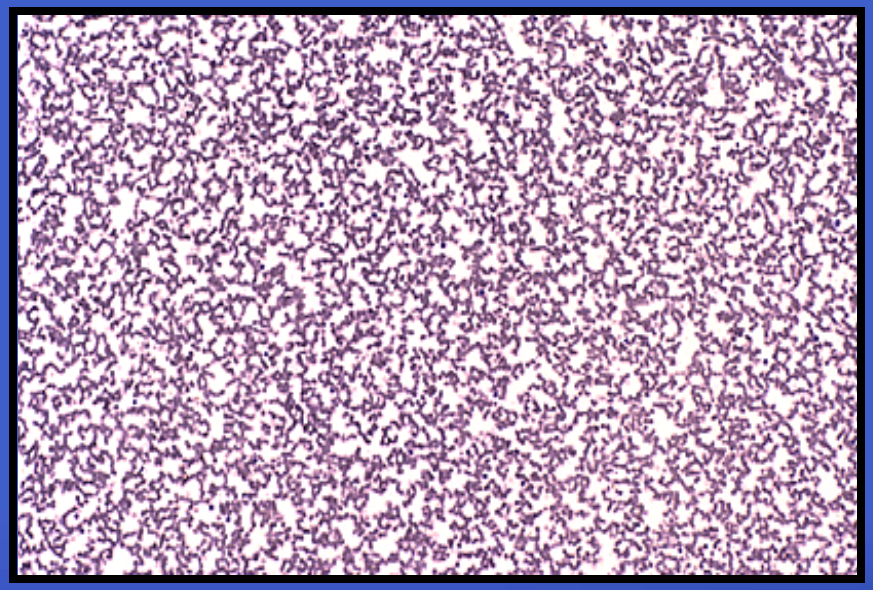


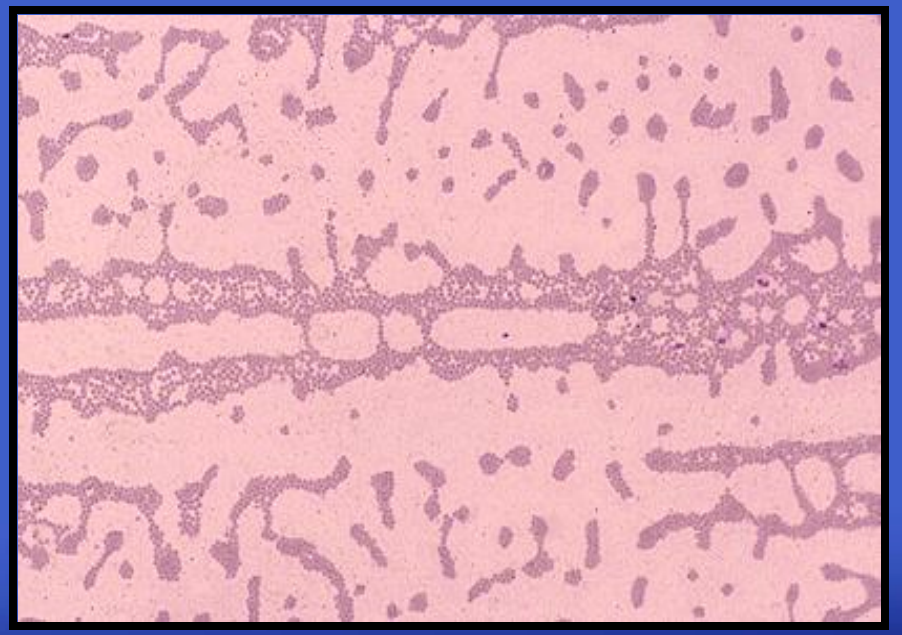
Slide preparation



Optimal area of review

Ideal thickness



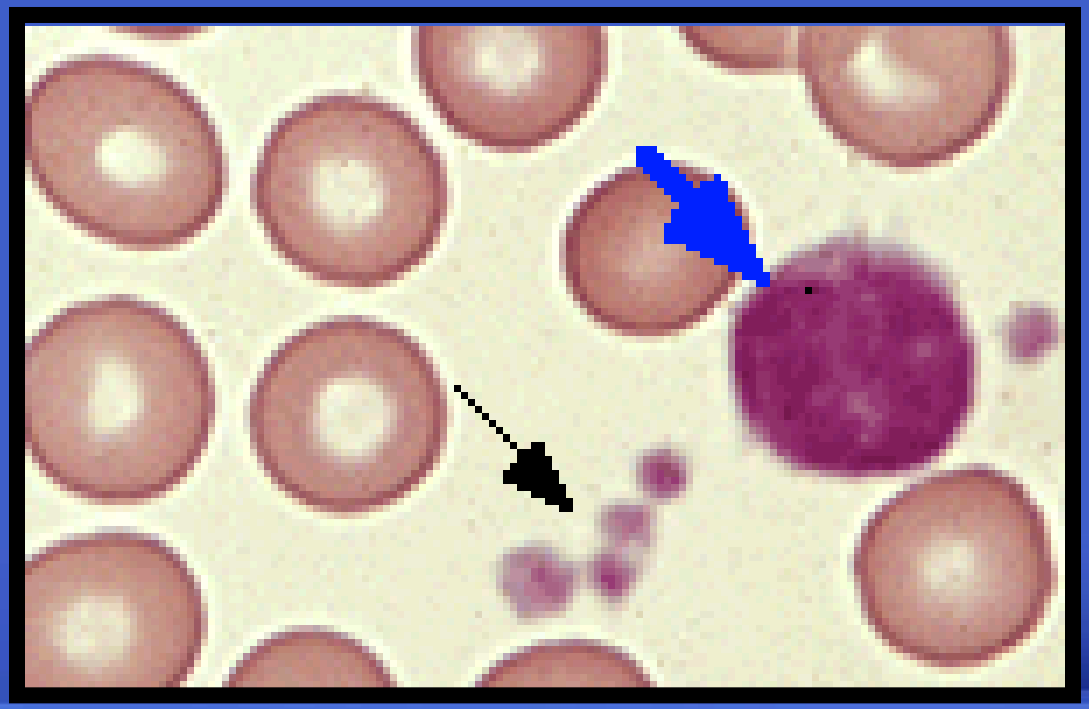


RBCs

SIZE

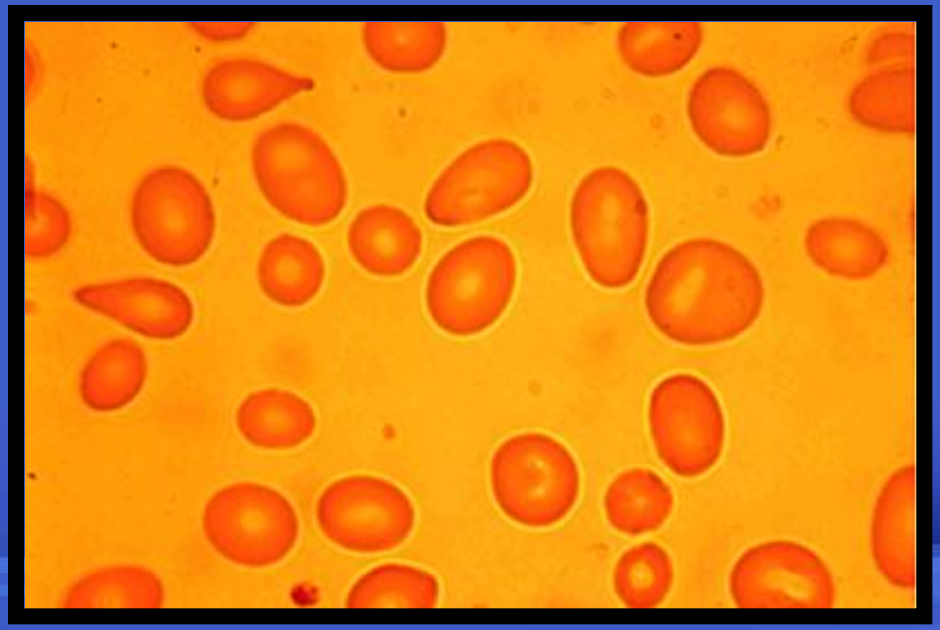
RBC Size

- Normal RBC should be the size of a *mature* lymphocyte nucleus
- Measured by the MCV



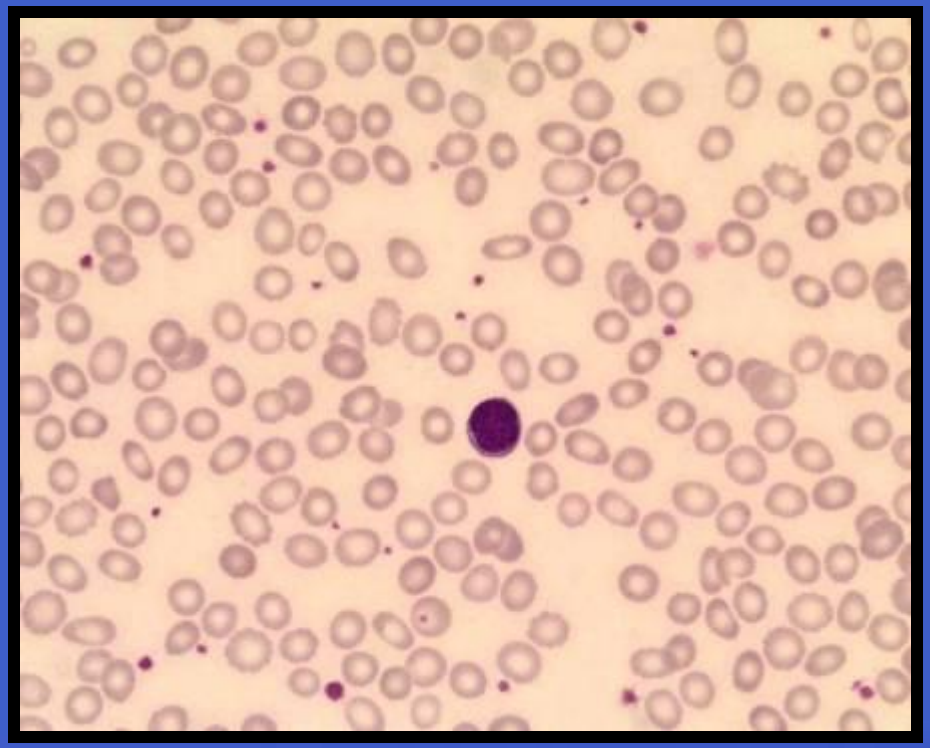
Elevated MCV = Macrocytosis
MCV > 100um^3

- B12/Folate deficiency, aplastic anemia
- Autoimmune hemolytic anemia
- Liver and thyroid disease, alcoholism
- Cold agglutinin disease



Decreased MCV = Microcytosis
MCV < 80um^3

- Iron deficiency
- Thalassemias
- Anemia of chronic disease
- Hemoglobinopathies
  - C, E, S

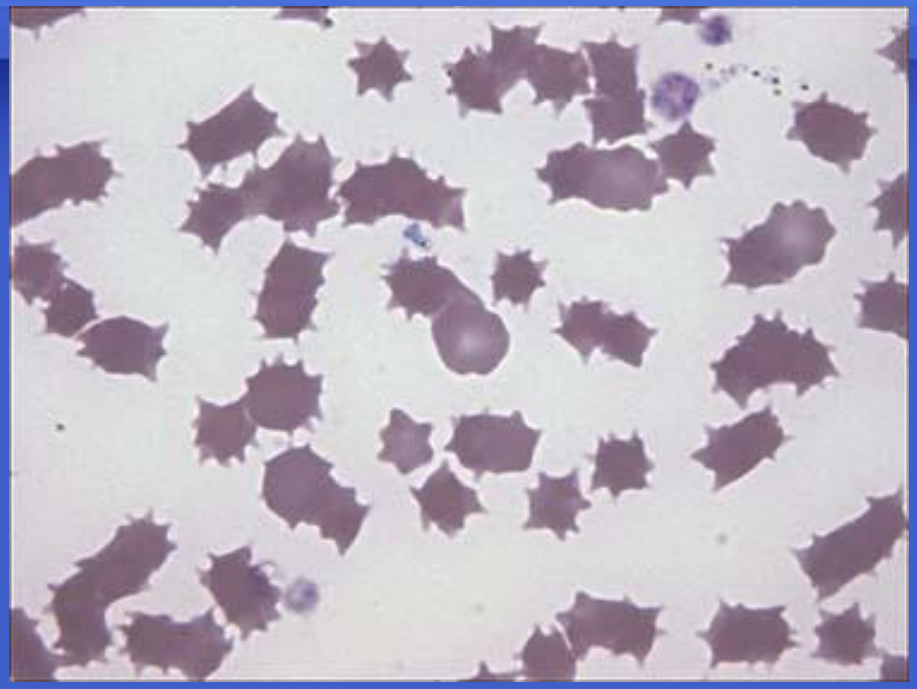


SHAPE

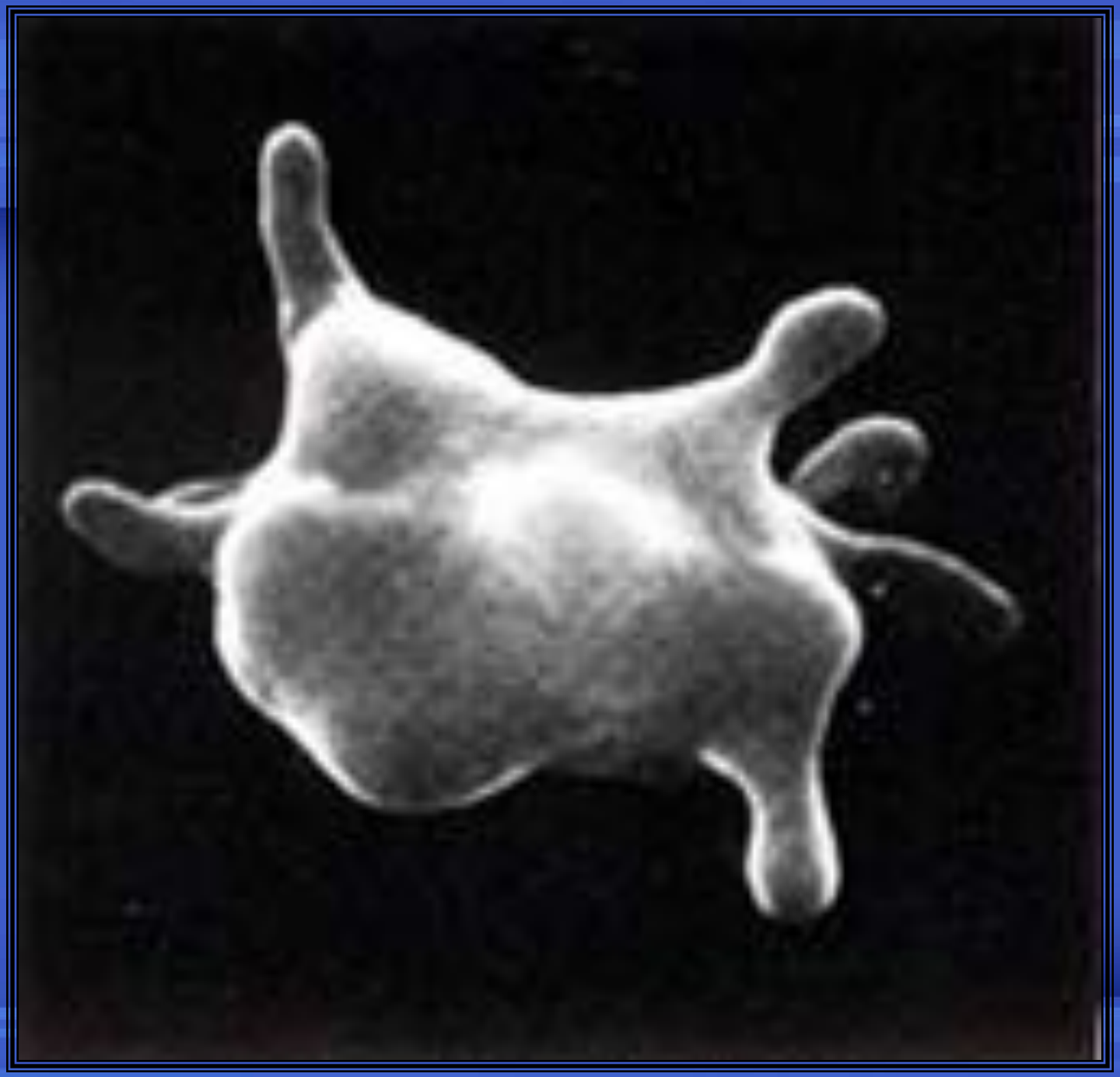
Pathologic Red Blood Cells in Peripheral Blood Smears

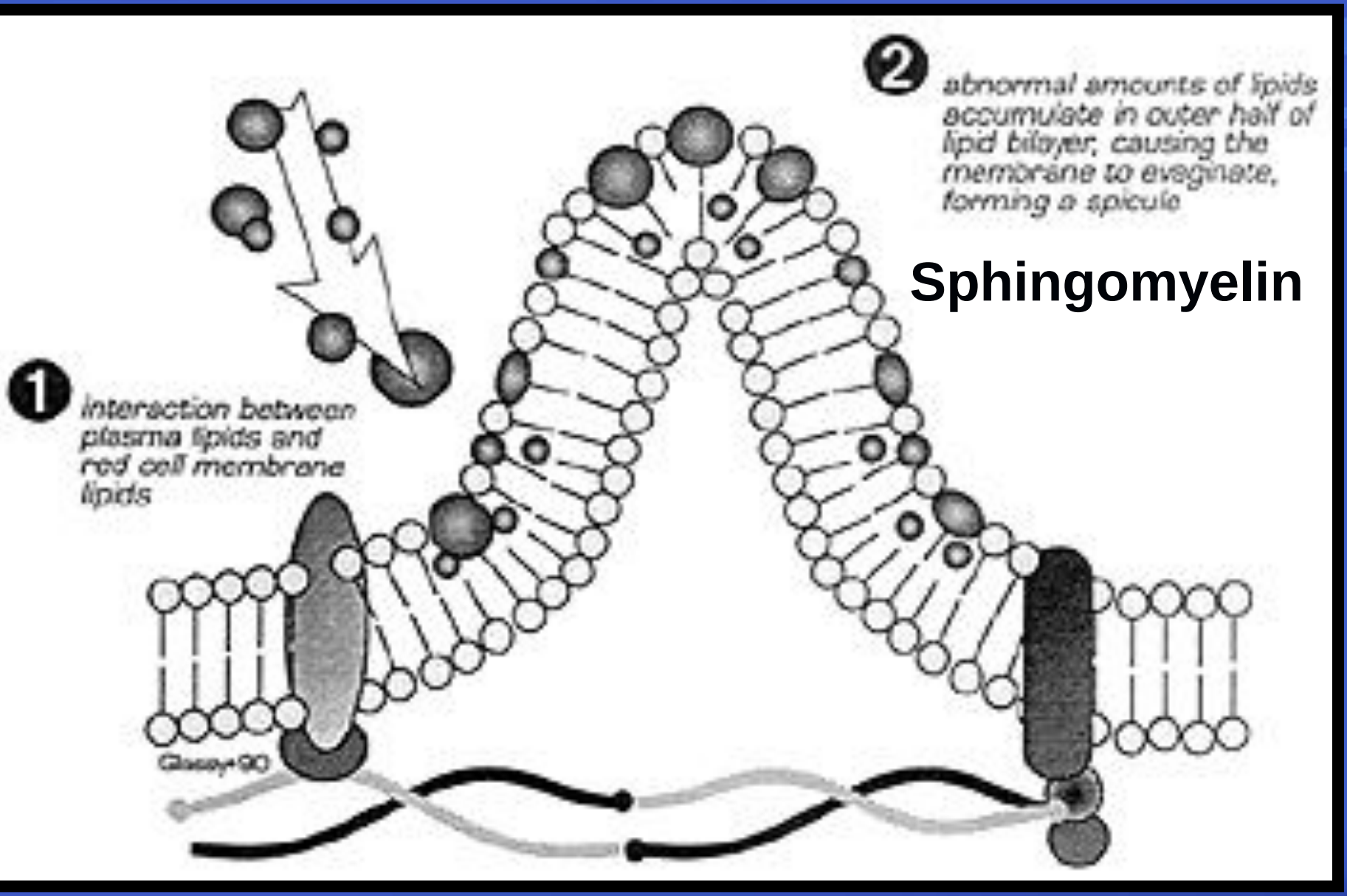
Type of Cell	Underlying Change	Disease States
Acanthocyte (spur cell)	Altered cell membrane lipids	Abetalipoproteinemia, liver disease, postsplenectomy
Bite Cell (degmacyte)	Heinz body pitting by spleen	G6PD deficiency, drug-induced oxidant hemolysis
Ovalocyte (elliptocyte)	Abnormal cytoskeletal proteins	Hereditary elliptocytosis
Rouleaux	Circulating paraprotein	Paraproteinemia
Schistocyte (helmet cell)	Mechanical destruction in microvasculature	TTP, HUS, prosthetic heart valves
Spherocyte	Decreased membrane redundancy	Hereditary sphereocytosis, immunohemolytic anemia
Stomatocyte	Membrane defect with abnormal cation perm	Hereditary stomatocytosis, immunohemolytic anemia
Target Cell (codocyte)	Increased redundancy of cell membrane	Liver disease, thalassemia postsplenectomy,HgbC dz, Fe deficiency
Burr Cell (echinocyte)	Altered membrane lipids	Usually artifactual but maybe uremia
Tear Drop Cell (dacrocyte)		Myelofibrosis

Acanthocytes (Spur cells)



- Irregular, long, sharply pointed and be spicules
- Absence of central pallor
- Most commonly seen in liver disease



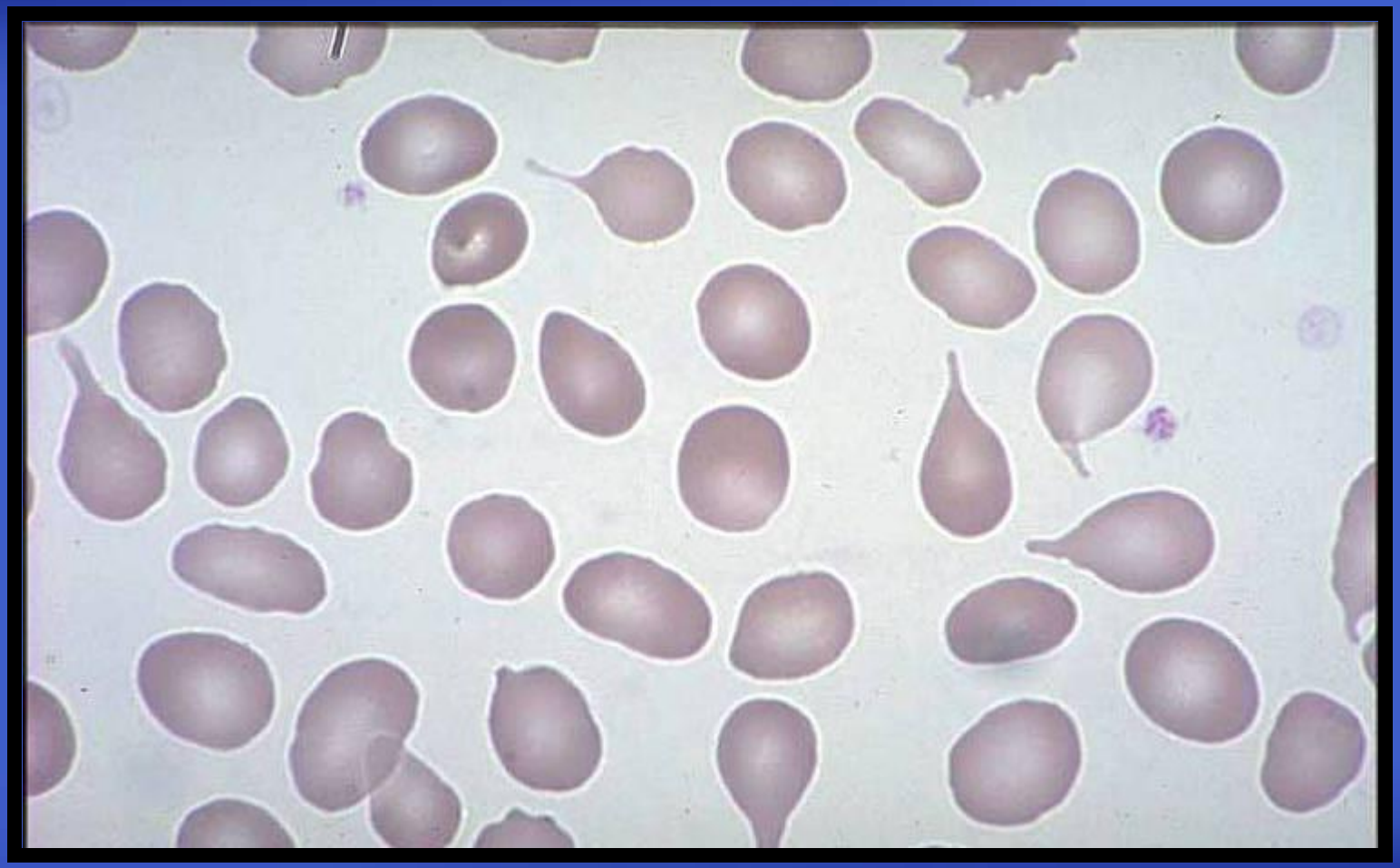


Absence of apolipoprotein B results in inability to transport triglycerides in the blood

McLeod phenotype/syndrome

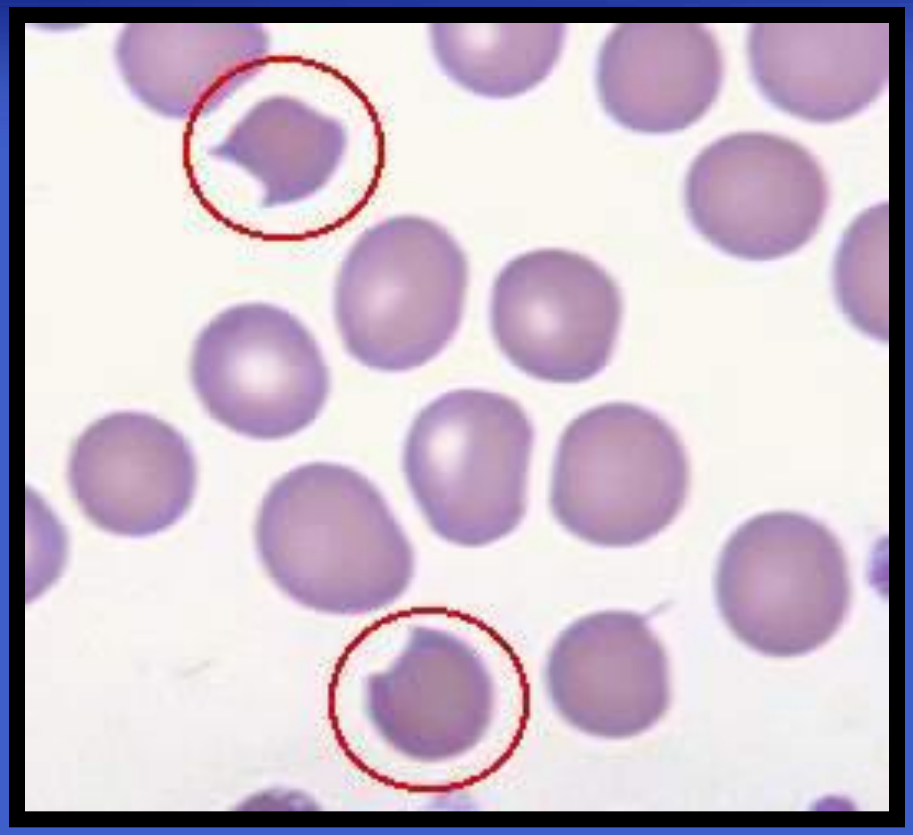
- Absence of erythrocyte surface Kx antigen (part of Kell antigen group)
- Acanthocytes with chronic but well compensated hemolytic anemia
- Disease processes include muscular dystrophy, cardiomyopathy, and “neuroacanthosis”
- May be associated with **chronic granulomatous disease**

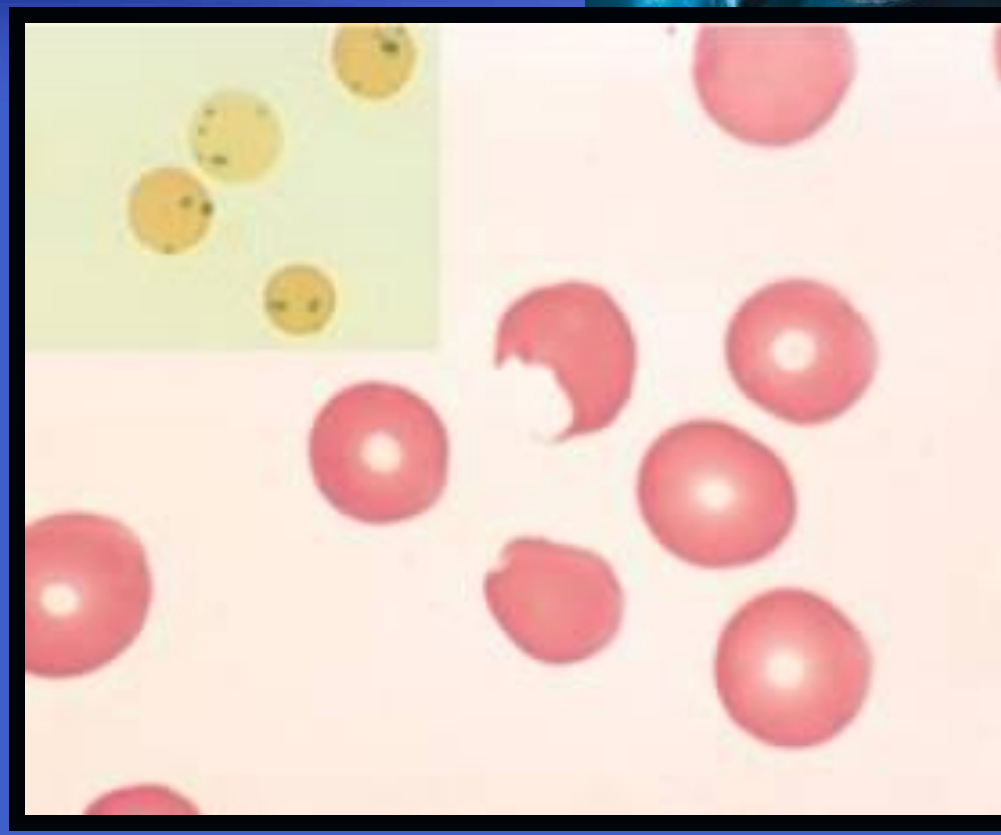
Tear Drop Cells (Dacrocytes)



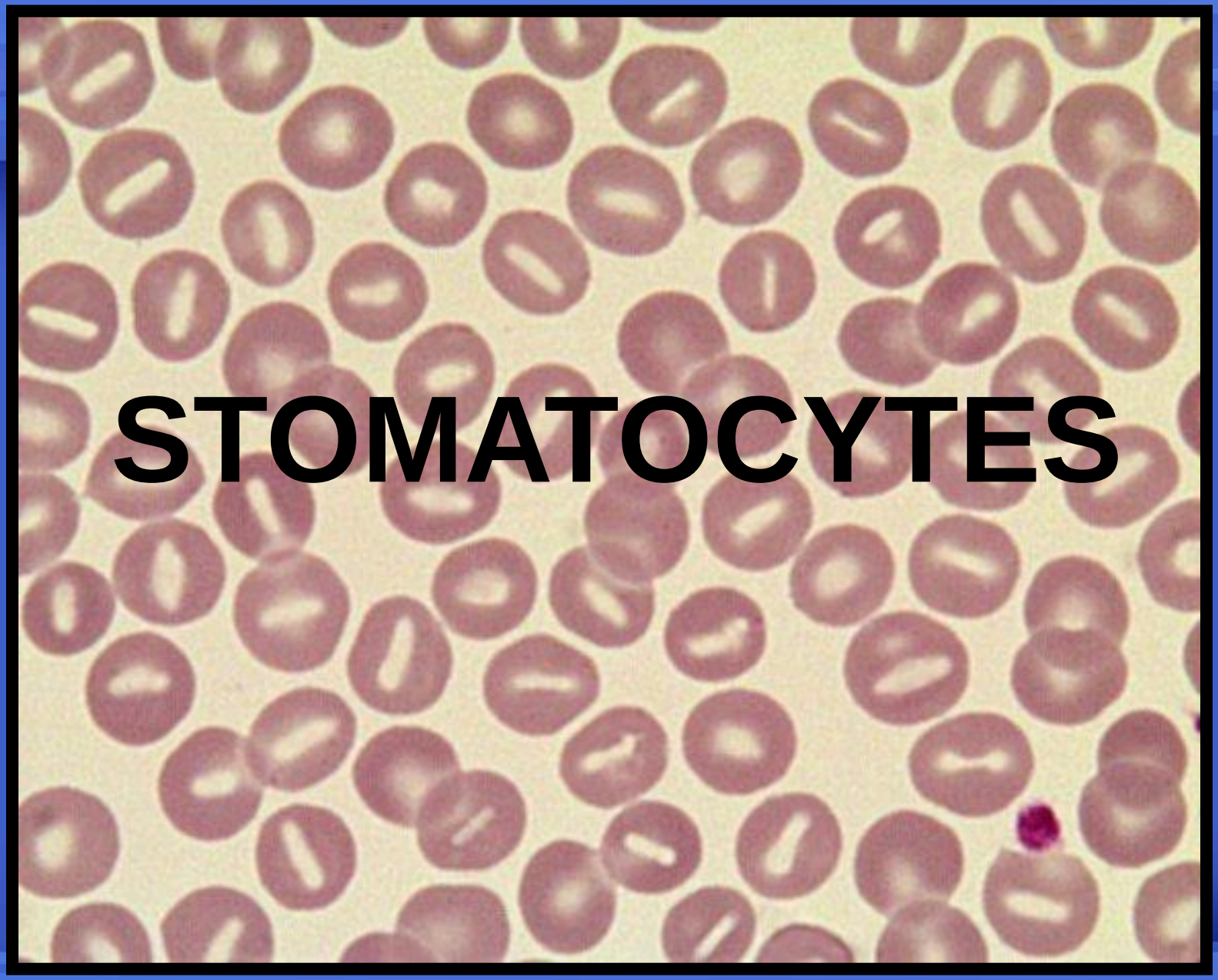
Myelofibrosis or bone marrow infiltrate

Bite Cells



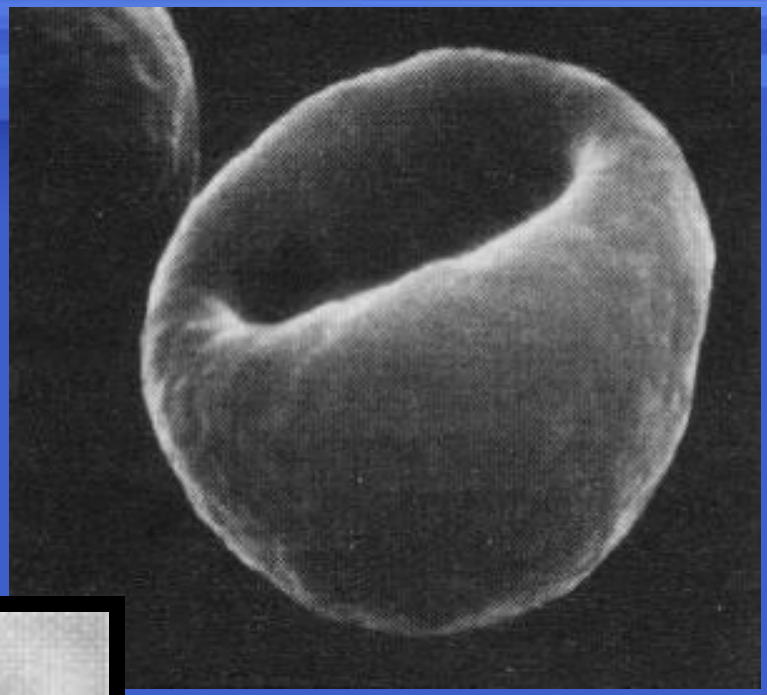


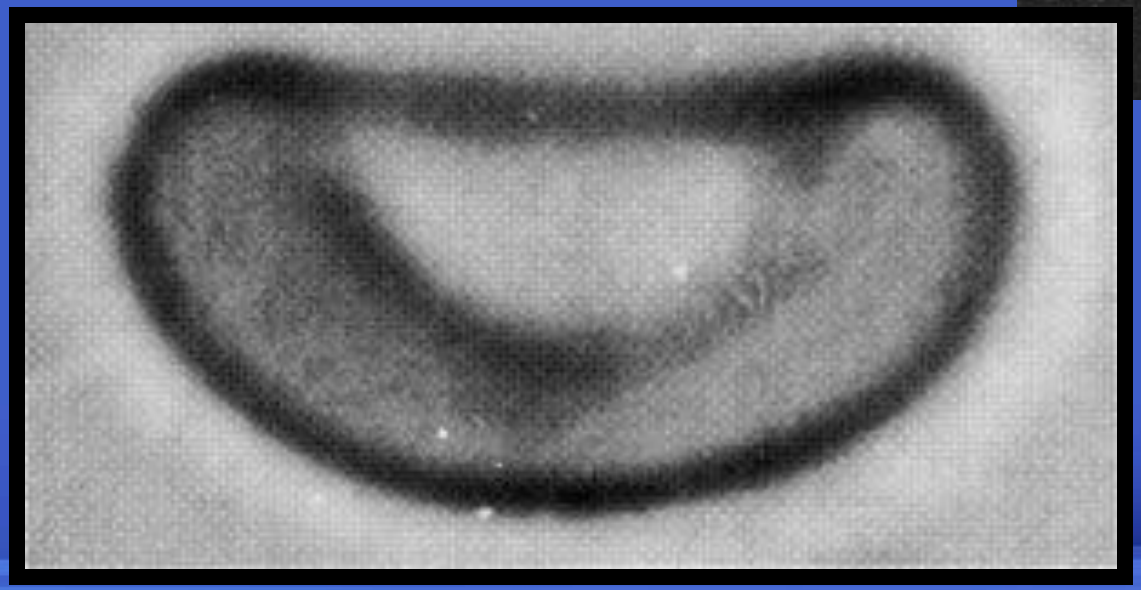
G6PD deficiency

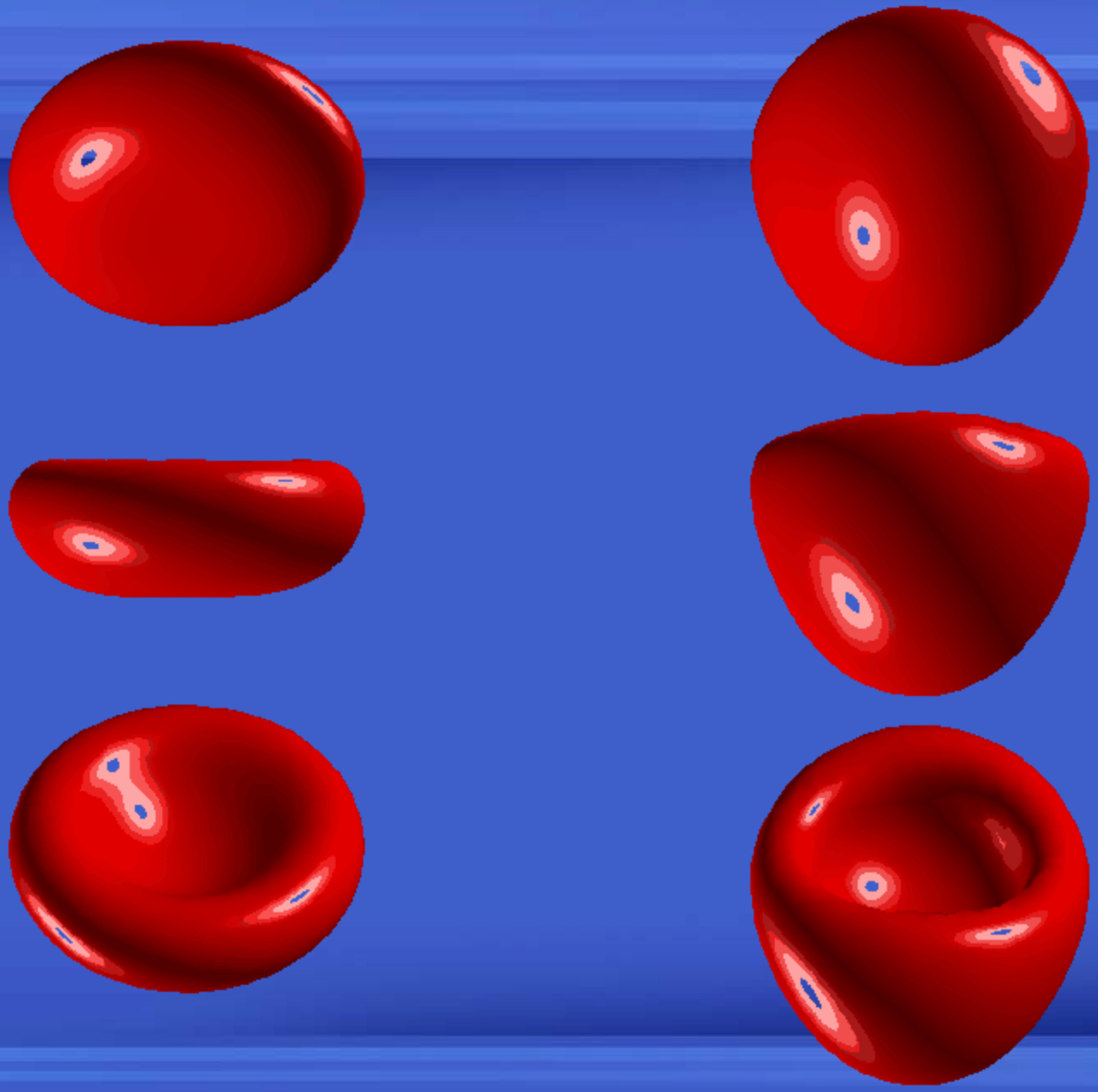

STOMATOCYTES

RBC with slit-like or rectangular area of central pallor, a mouth

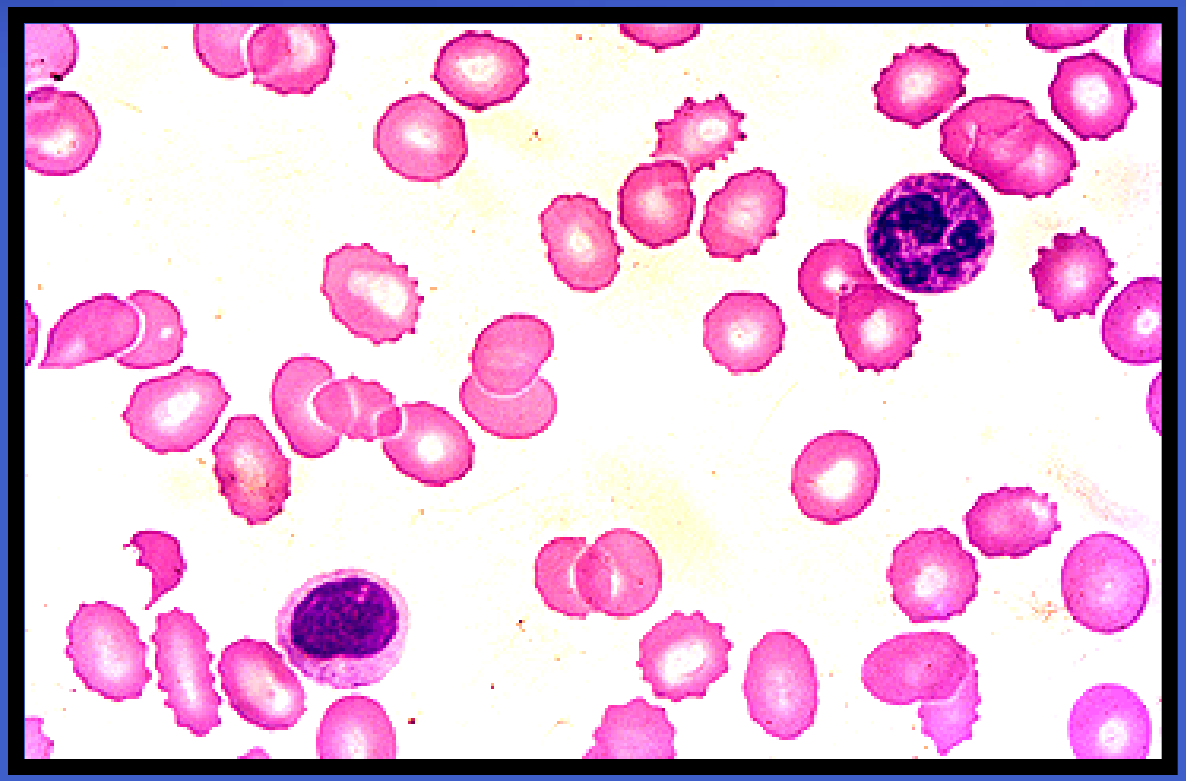
Most often seen in liver disease







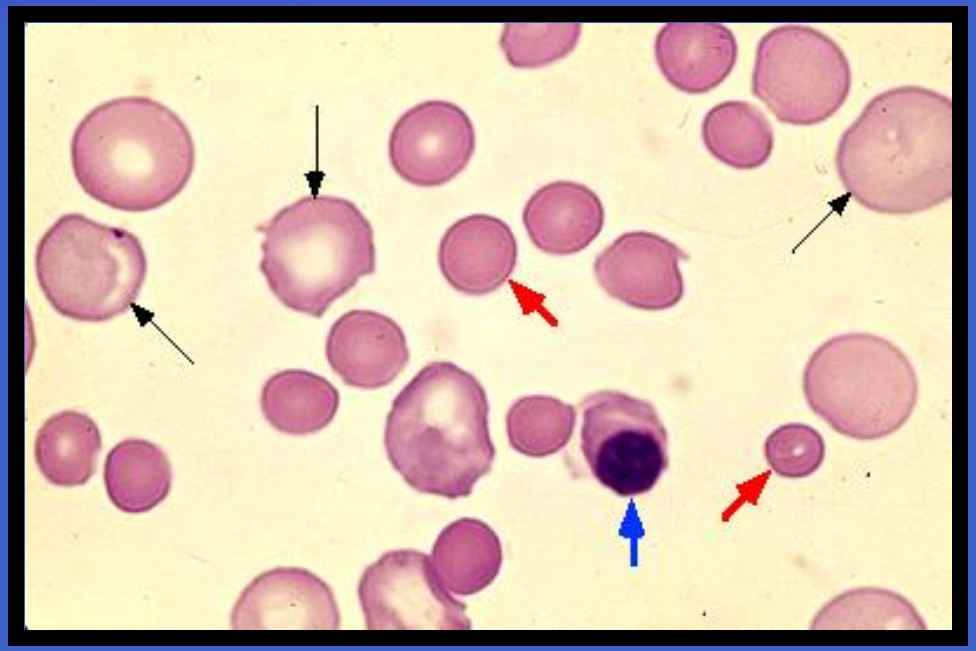
Burr Cells (Echinocytes)

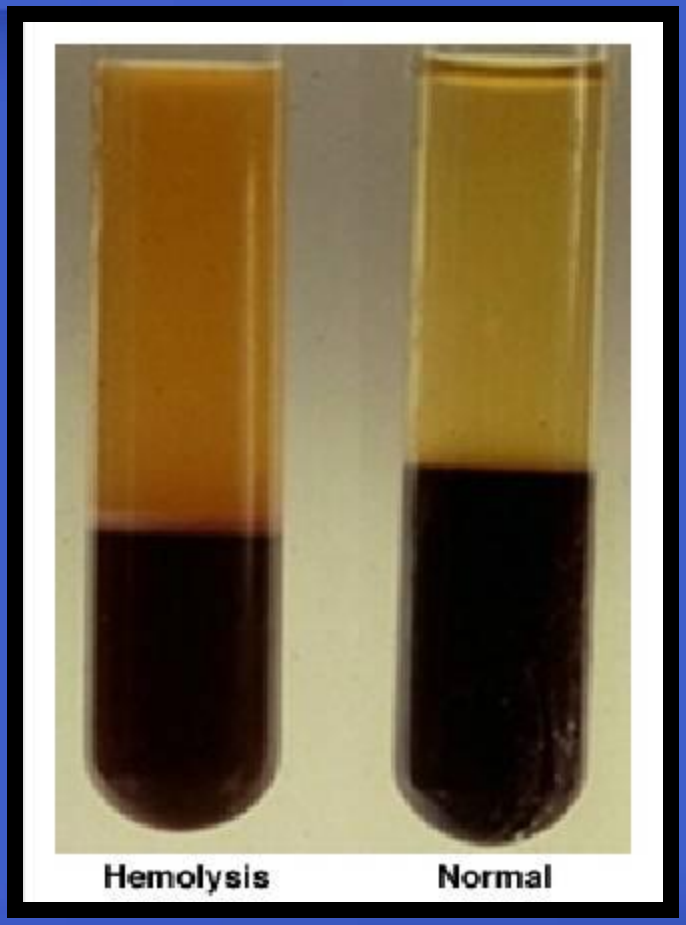


Projections- smaller more regular than acanthocytes

Often Artifactual but seen in **UREMIA**

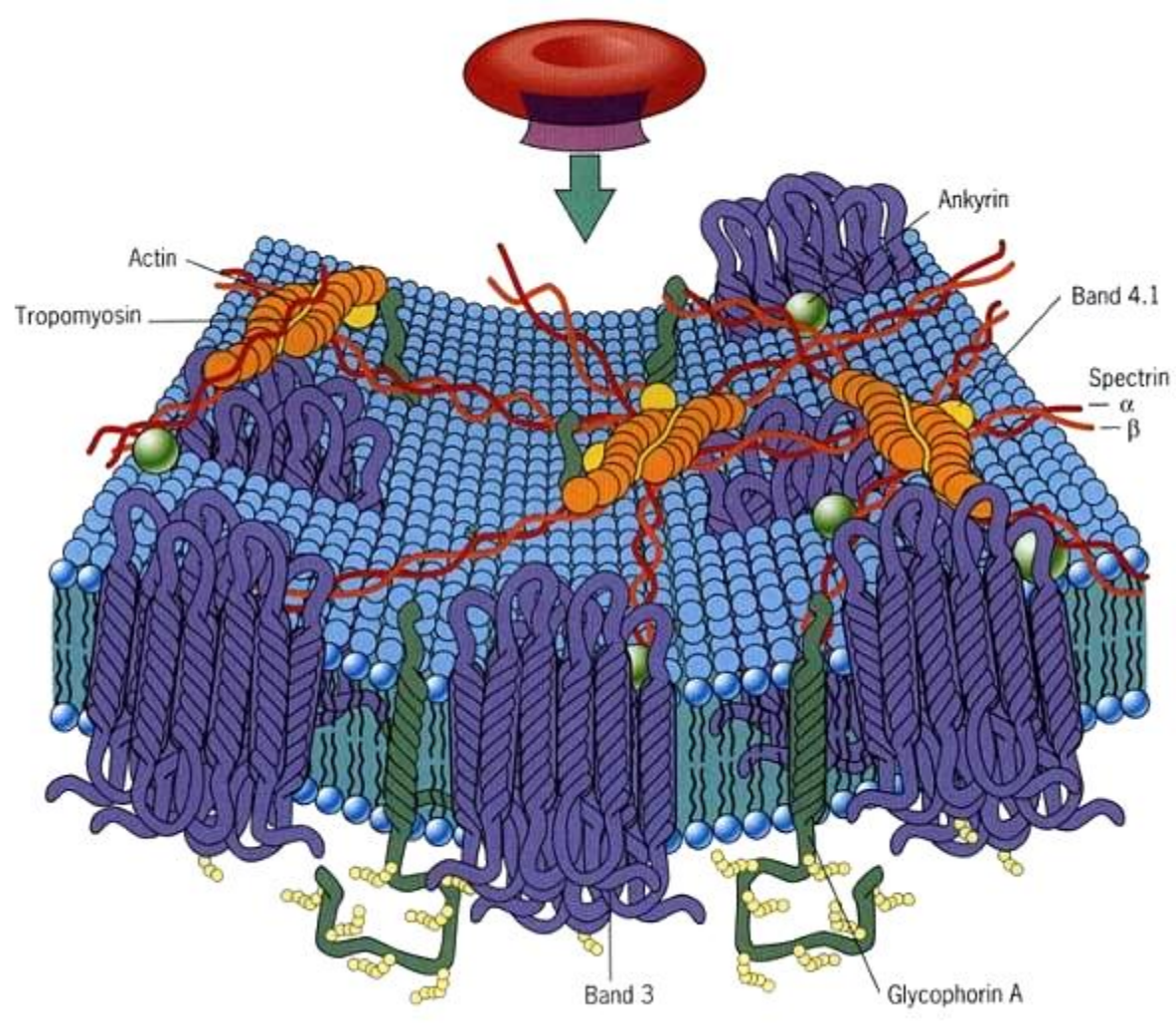
Spherocytes

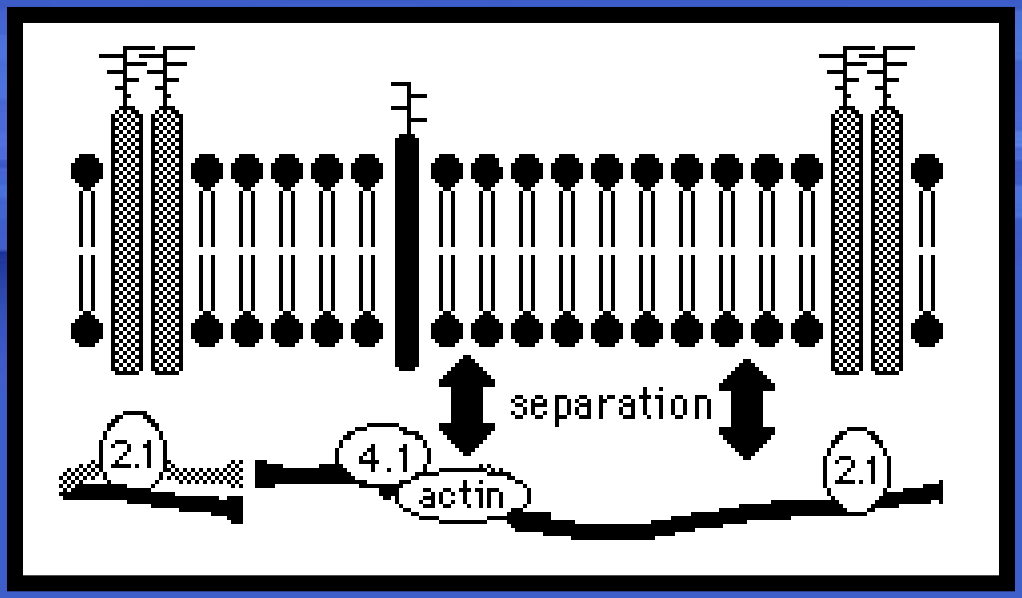




Hereditary spherocytosis

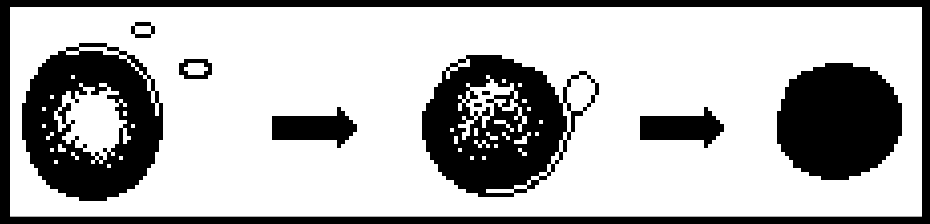
- Northern European ancestry
- Spectrin, ankyrin or band 3 or 4.1 deficiency

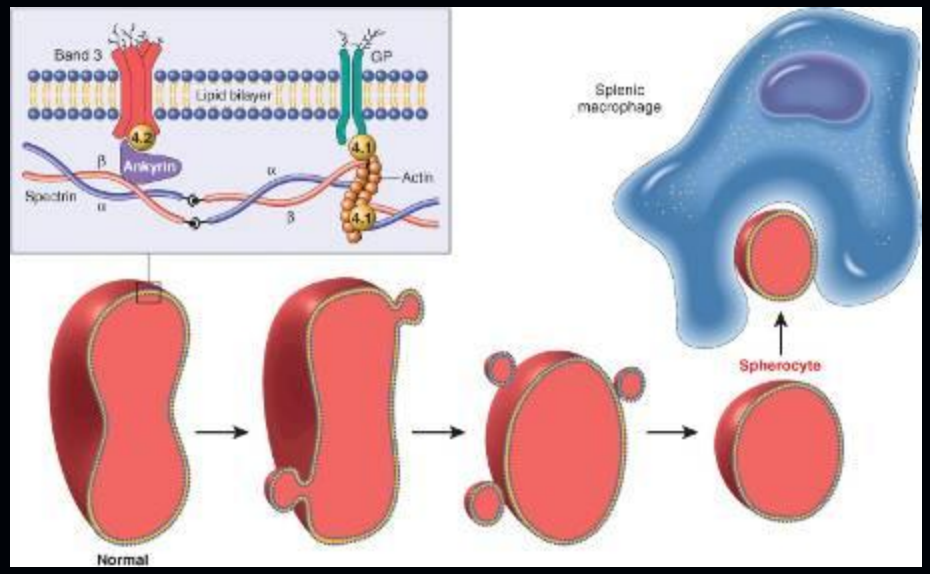


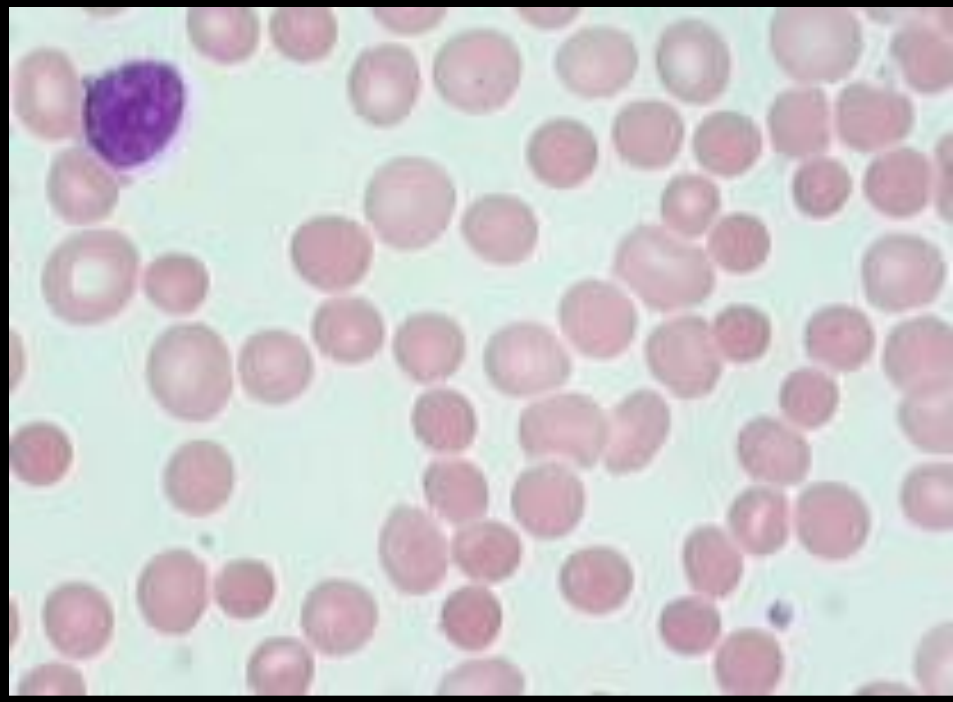


- Defects in vertical stabilization of the phospholipid bilayer of the RBC membrane cause separation of the spectrin - phospholipid bilayer

Hereditary spherocytosis

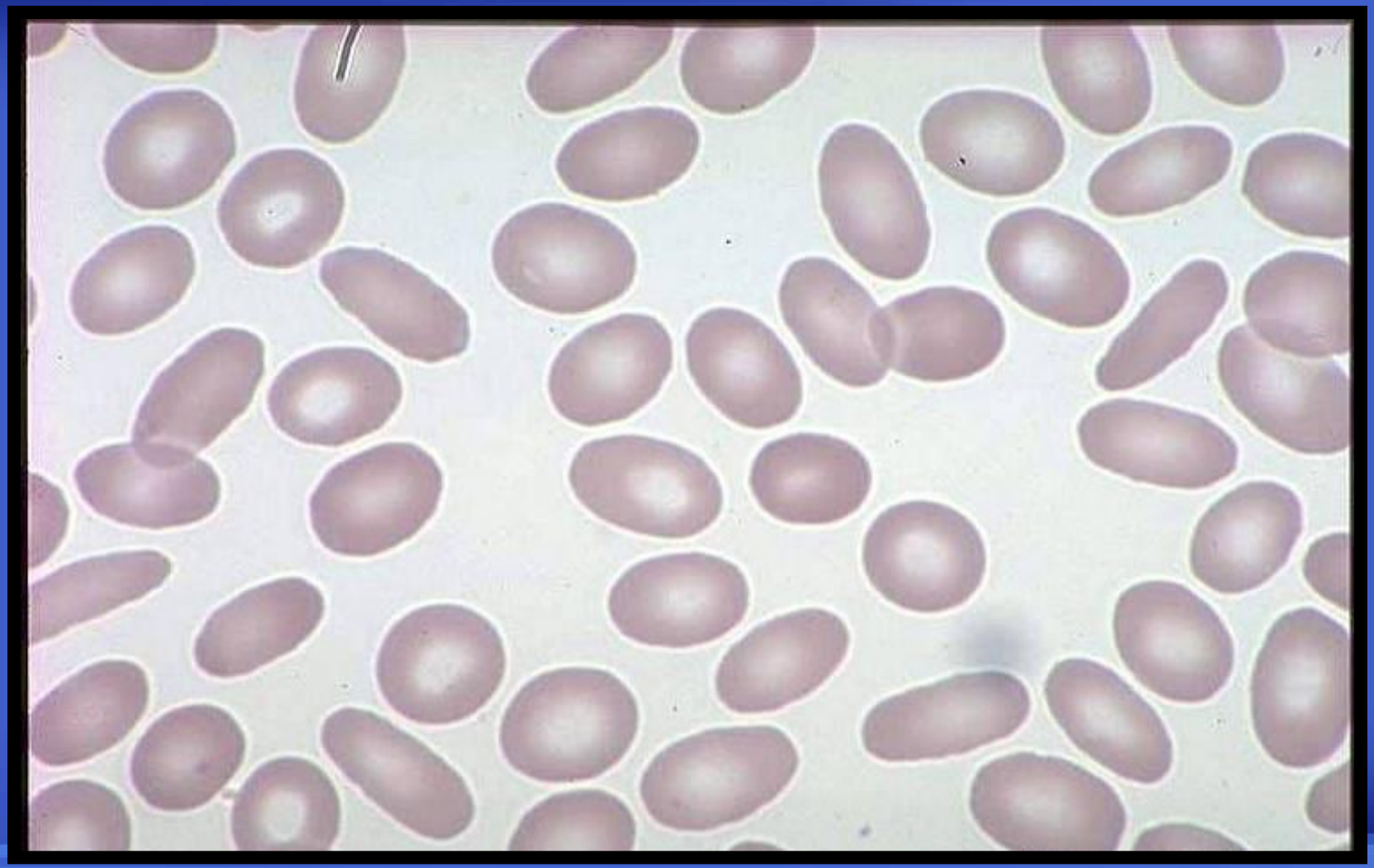






- Normal biconcave red cell loses membrane fragments and adopts a spherical shape
- Inflexible cells are trapped in the splenic cords, phagocytosed by macrophages

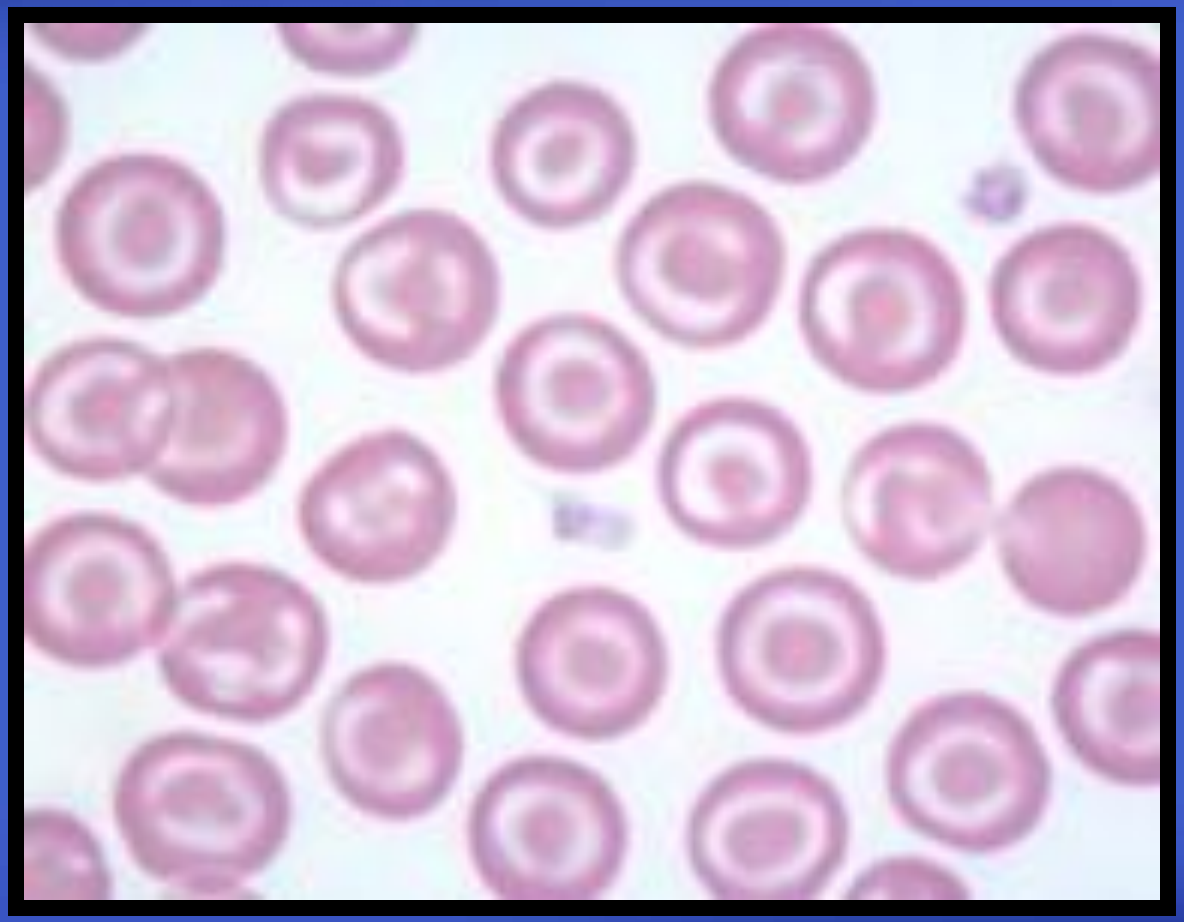
Elliptocytes



Hereditary elliptocytosis

- Autosomal dominant trait
- Spectrin abnormality or deficiency of protein 4.1
- Asymptomatic without anemia and usually with no splenomegaly and only mild hemolysis
- RBC hemolysis occurs in the spleen, thus splenectomy corrects the hemolysis, but not the RBC membrane defect.

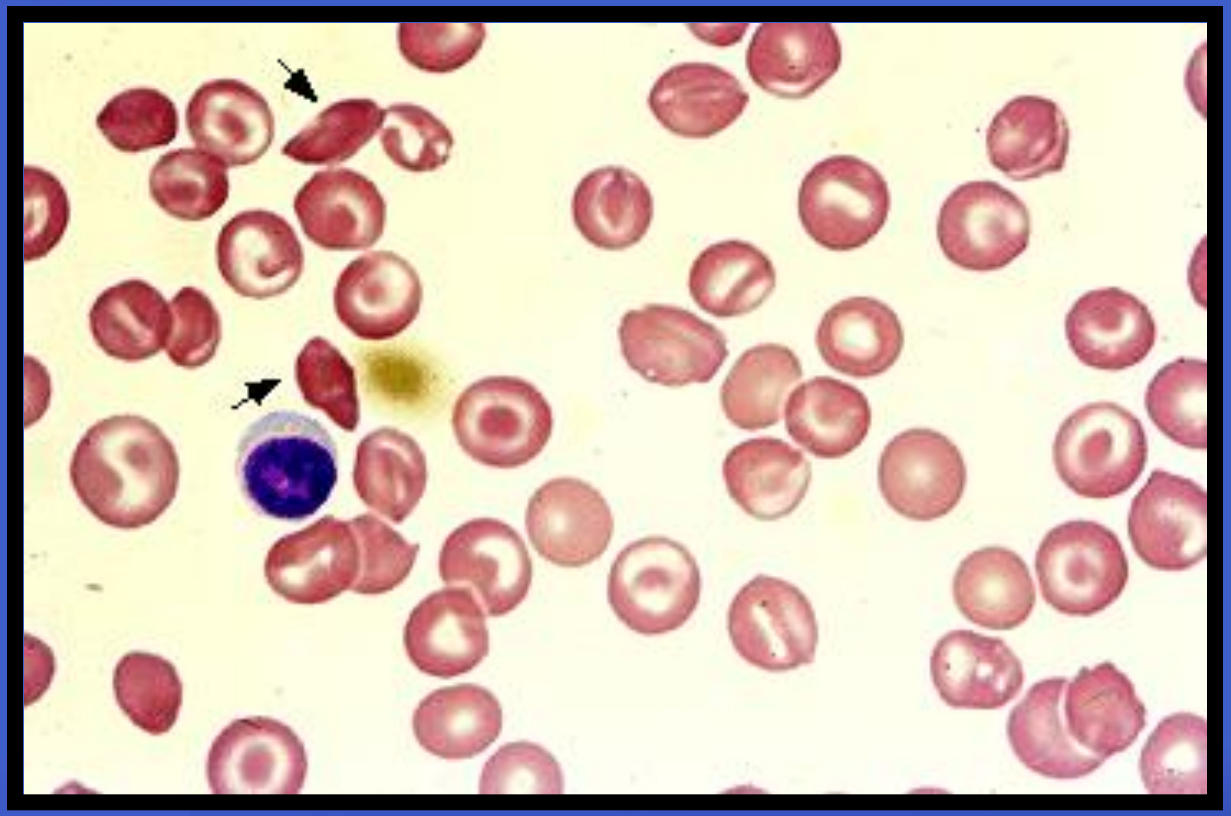
Target Cells

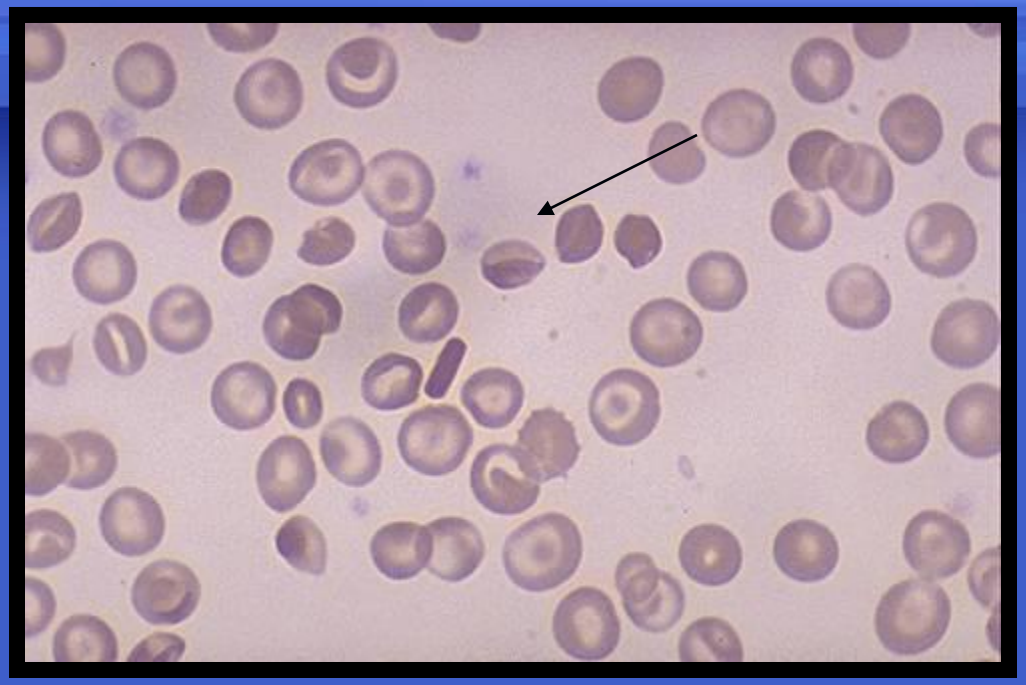


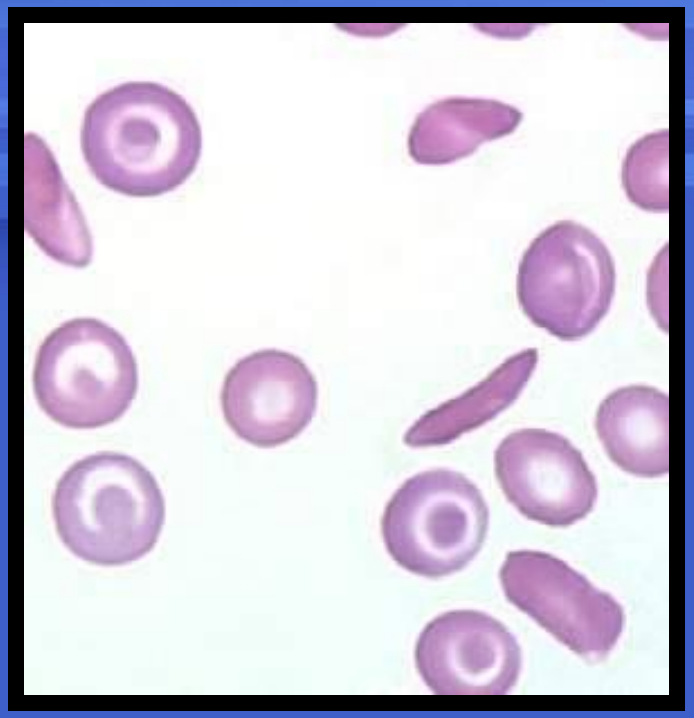
Characteristic of:

- Liver disease
- Post-splenectomy
- Hemoglobinopathies
  - Thalassemia
  - **Hb C,** D and E

Canoe cells (aka Taco cells, folded cells)



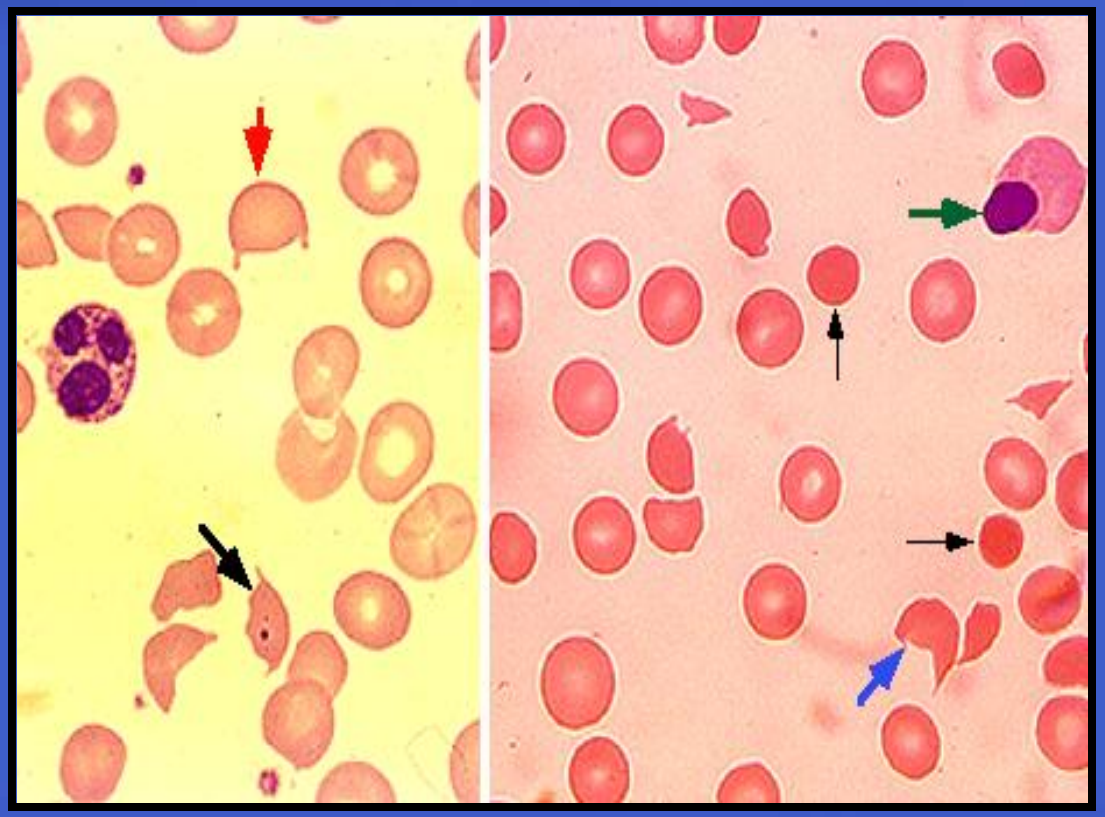




HgbSC disease with canoe and sickle cells

“Washington Monument crystals”

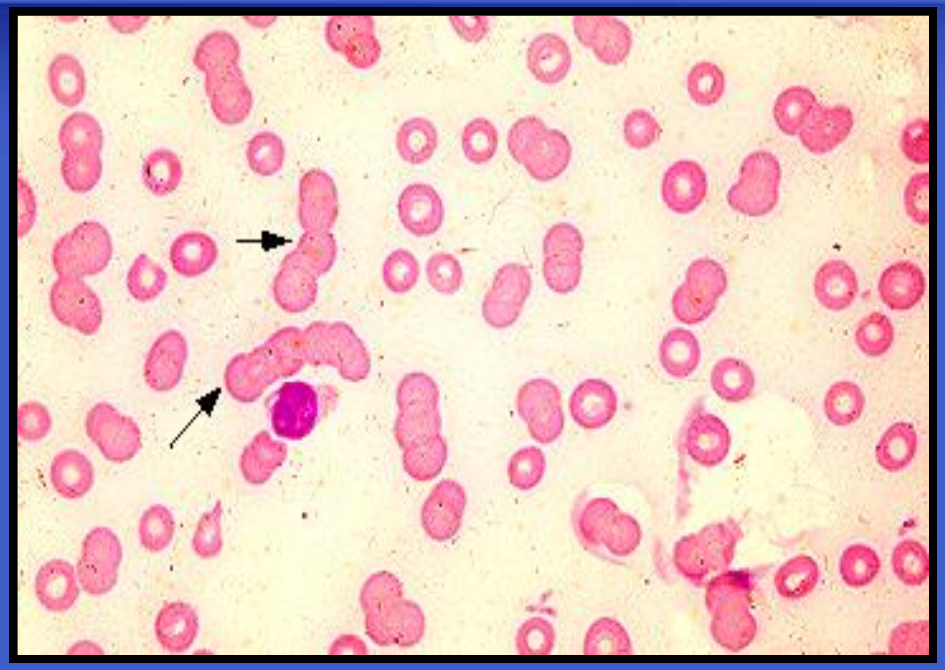
Schistocytes



Microangiopathic hemolytic anemia

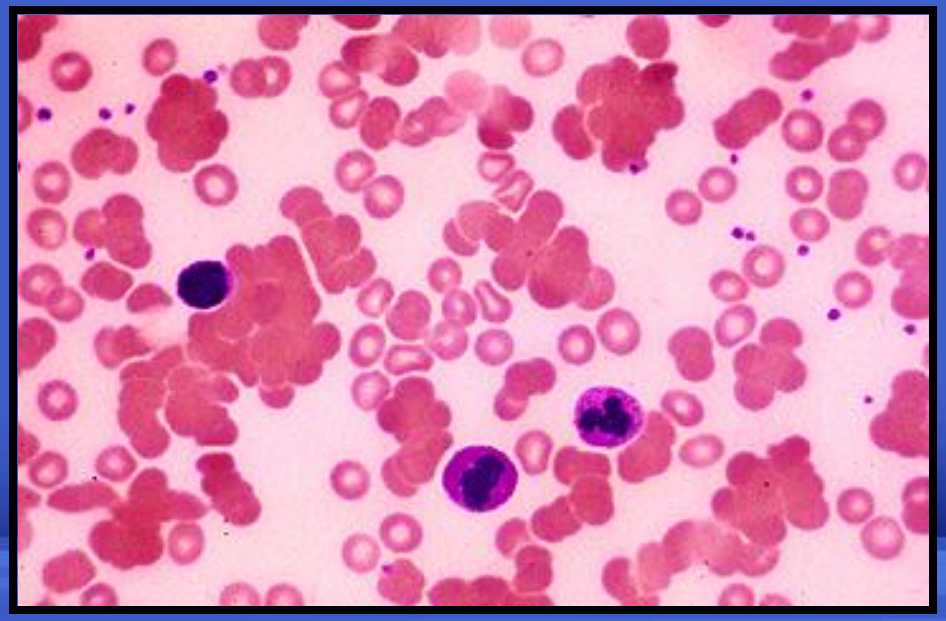
DIC, TTP, HUS

Rouleoux and Clumping



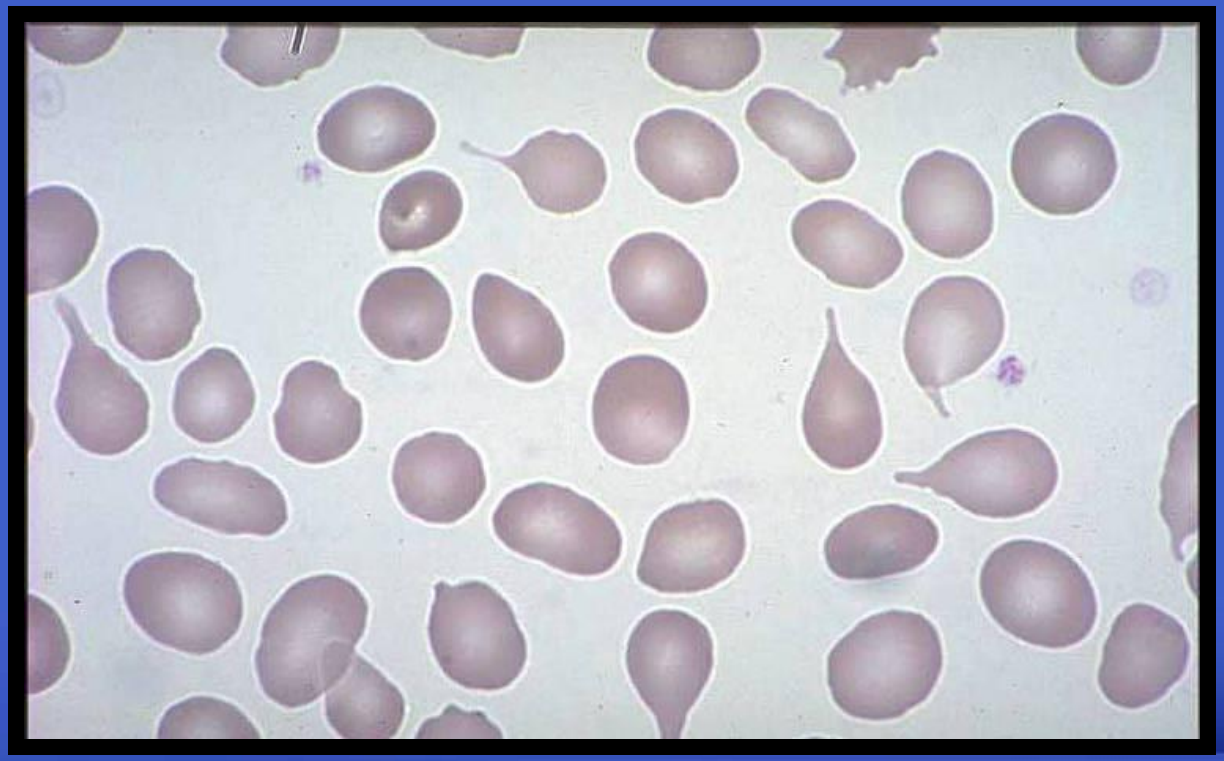
Rouleaux

RBC Clumping



Quiz!

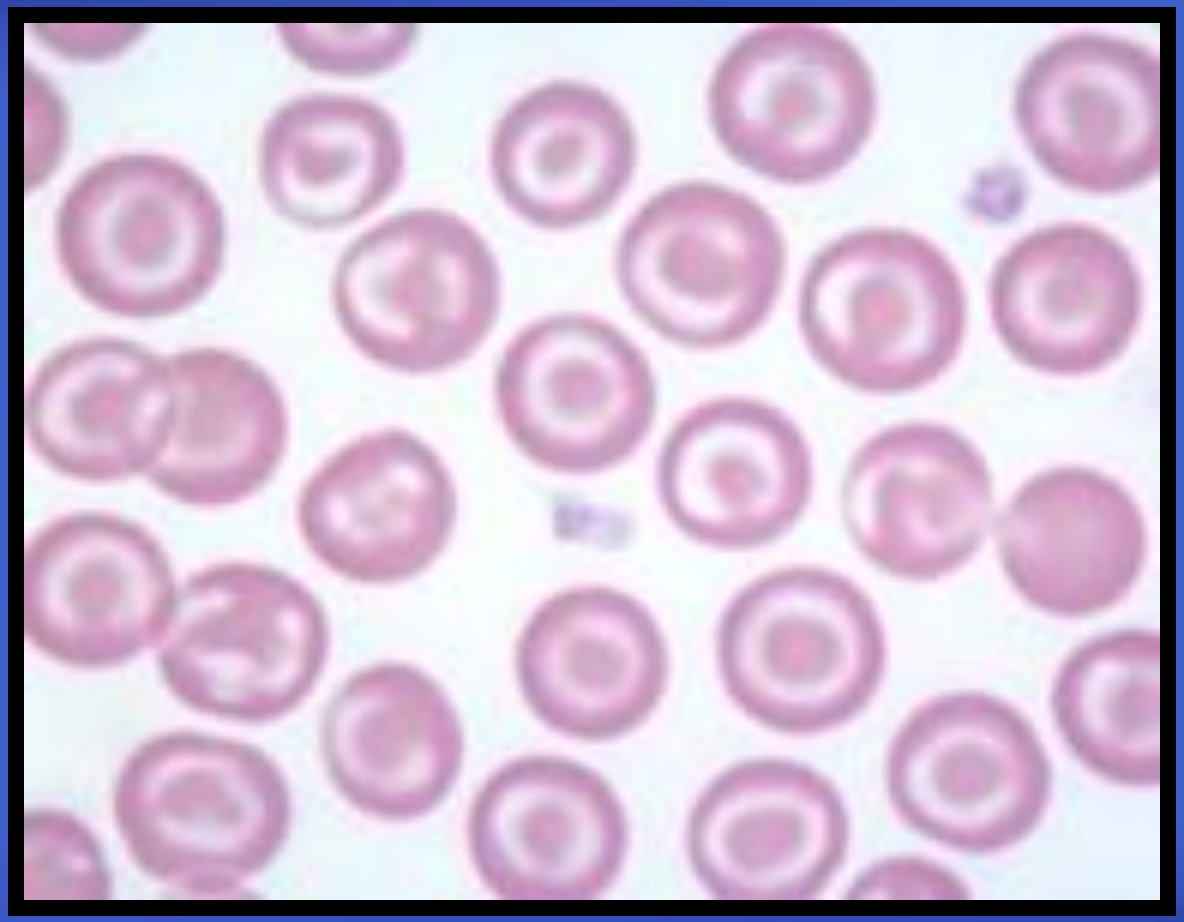
- 1. 60 yo woman with a history of treated breast cancer now presents with anemia



Most Likely Diagnosis?

- Tear drop cells 2^{o} to bone marrow infiltration with tumor

2. 45 yo man with macrocytic anemia



Most Likely Diagnosis?

- Liver disease

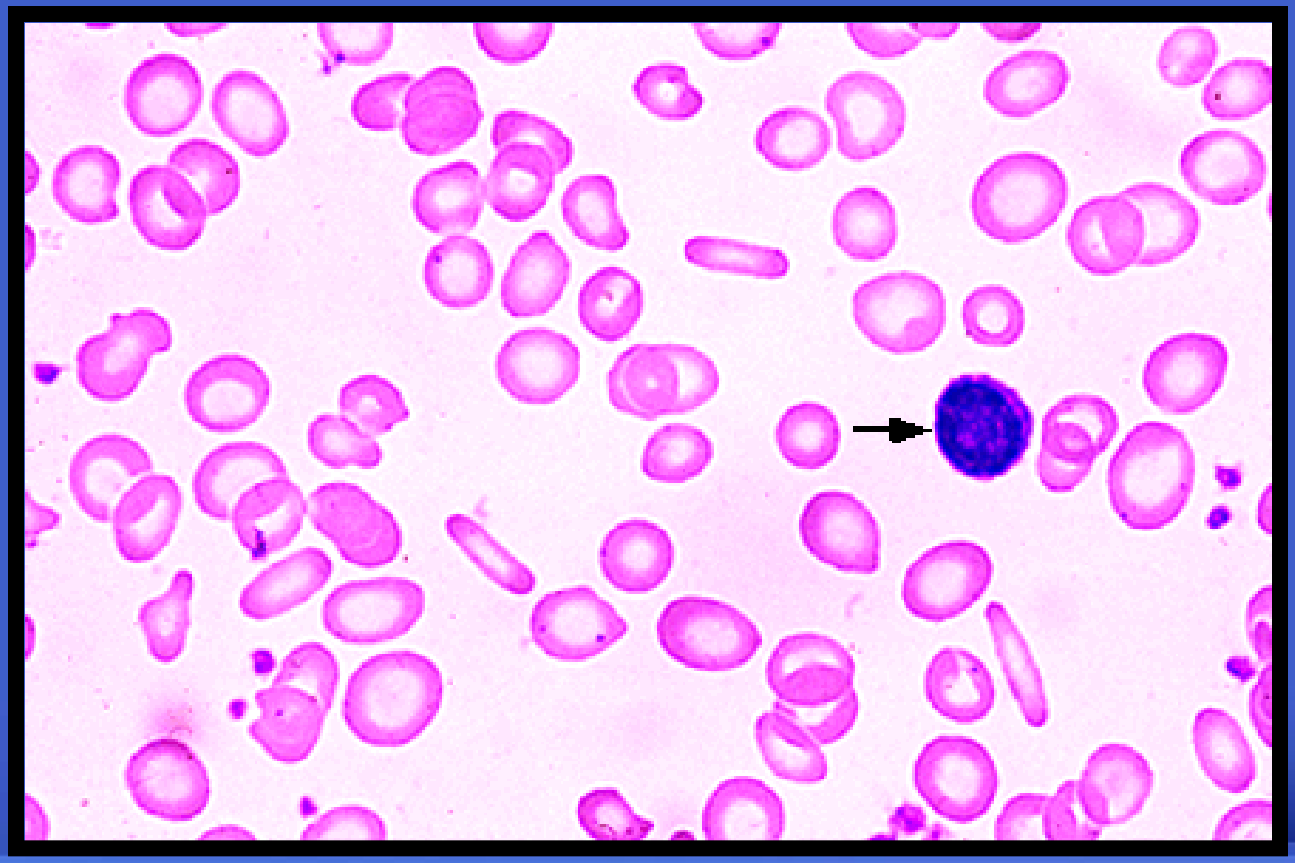
PALLOR

Hypochromic anemia

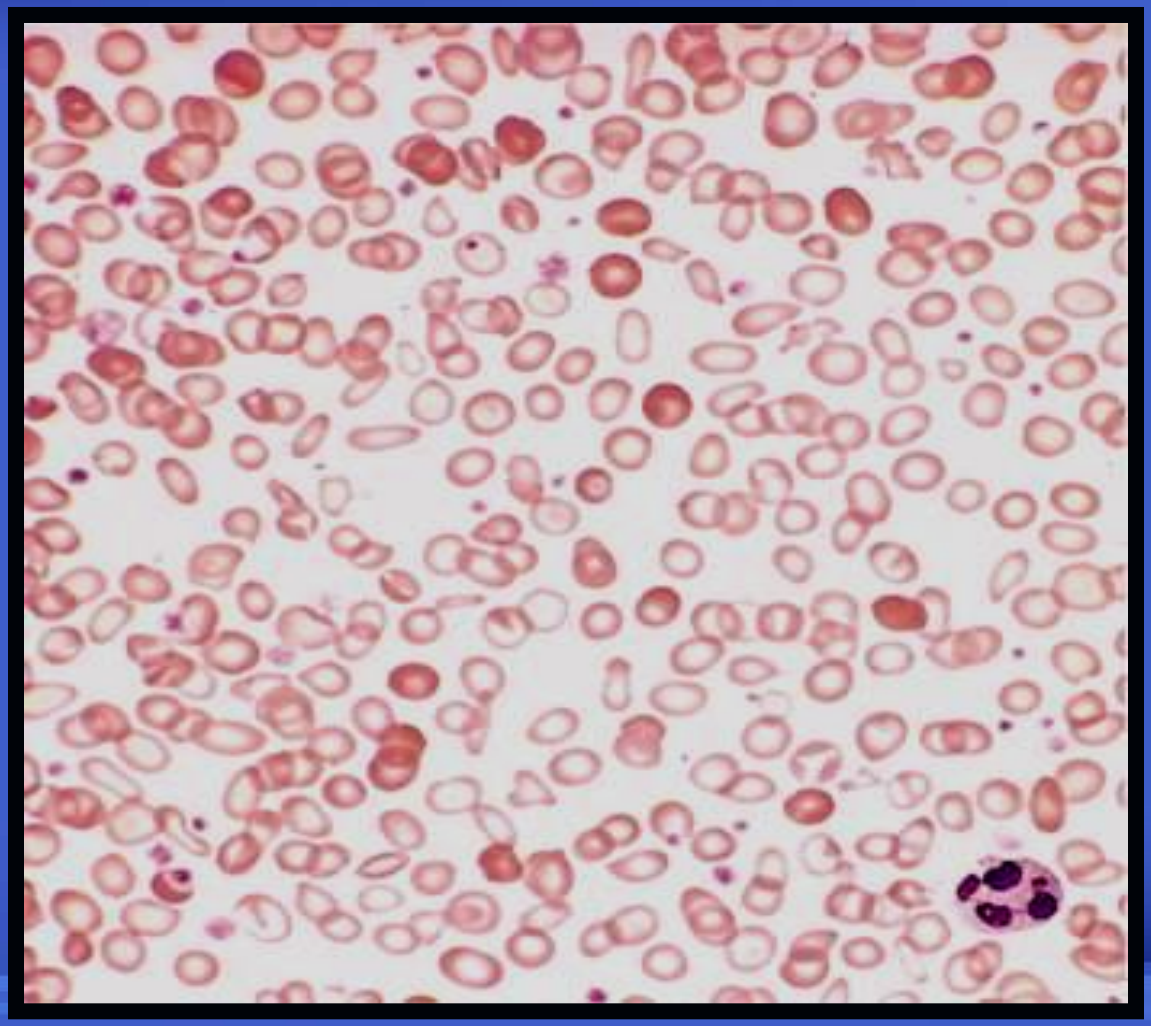
MCH < 27 pg

- Disorders of globin synthesis
  - Thalassemic syndromes
    - α-Thalassemia
    - B-Thalassemia
- Disorders of heme synthesis
  - Sideroblastic anemias
    - Hereditary (X-linked auto. Dominant)
    - Acquired idiopathic
    - Acquired toxic
- Disorders of Fe metabolism
  - Fe deficiency
  - Chronic disease
  - Neoplasia

Iron Deficiency



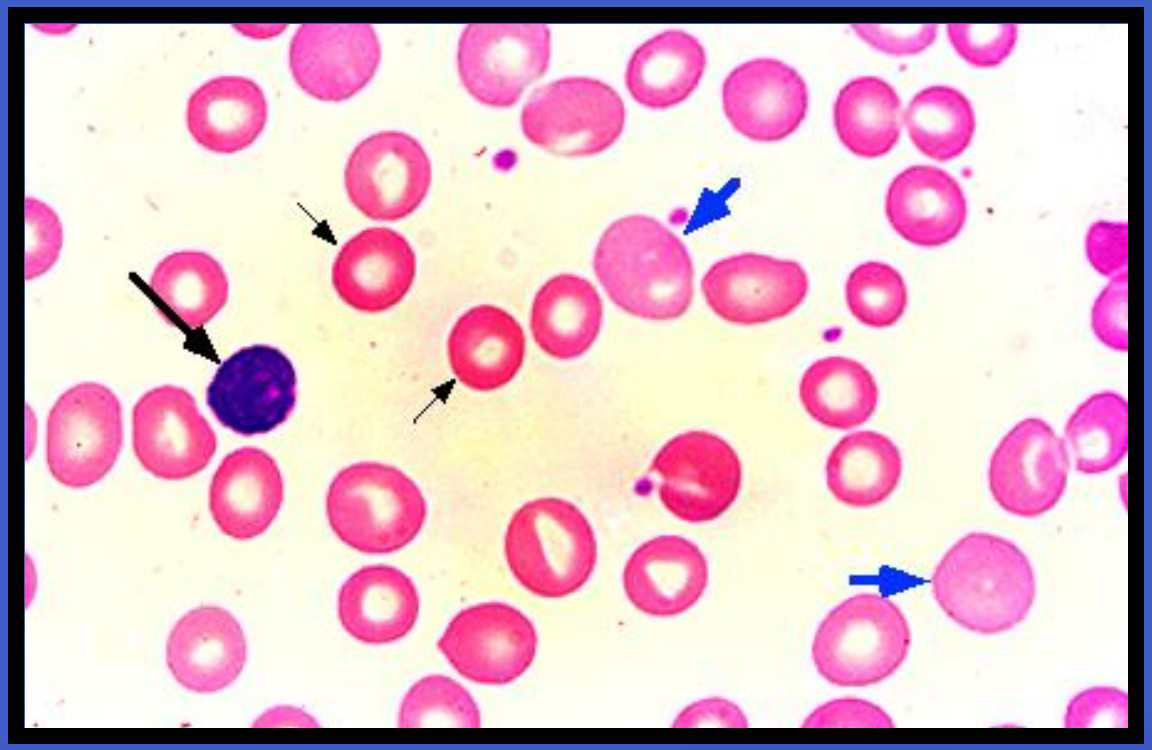
Iron deficiency s/p transfusion



PREMATURE RBCs

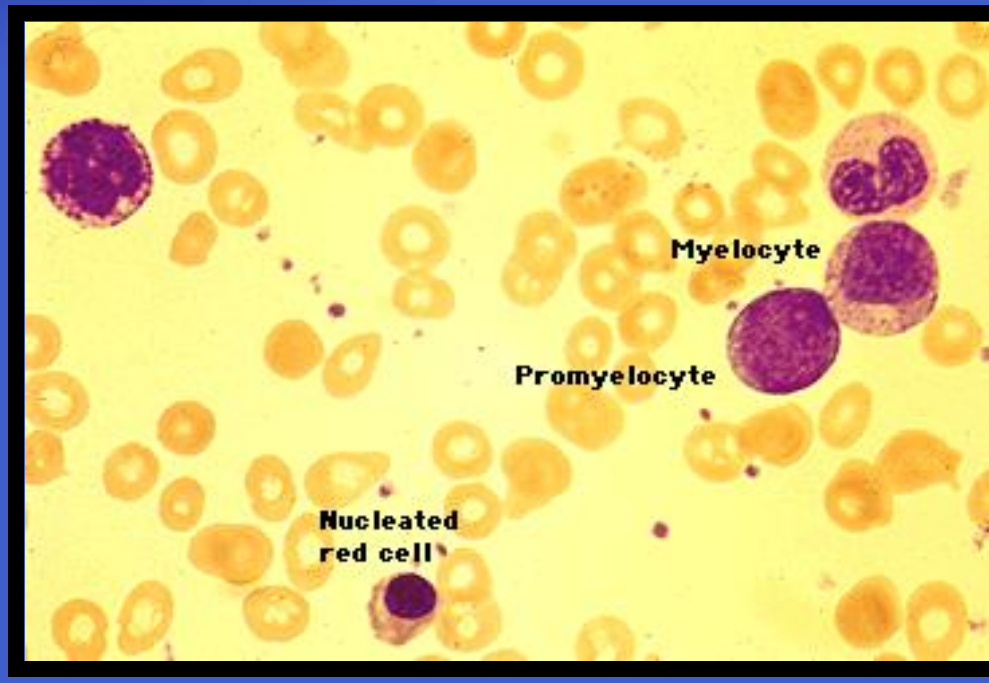
Reticulocytes

- Decreased cell survival
  - Blood loss
  - Autoimmune HA
  - Nonimmune HA
    - TTP, HUS, DIC
    - Spherocytes
    - G6PD
    - PNH
    - Hemoglobinopathy
    - Thalassemia



Nucleated RBCs

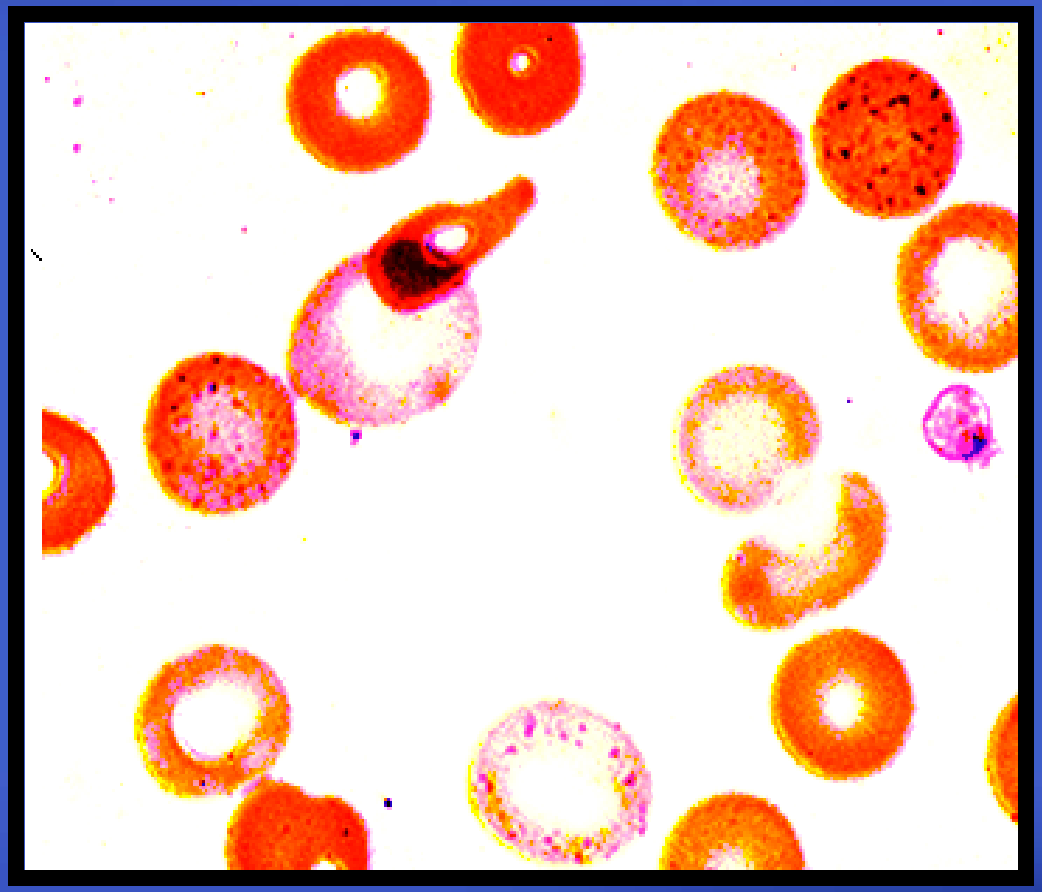
- Not normally present in adult patients PS
- Present in:
  - Severe hemolysis
  - Profound stress or hypoxemia
  - Myelophthisic condition
  - Leukoerythroblastic smear

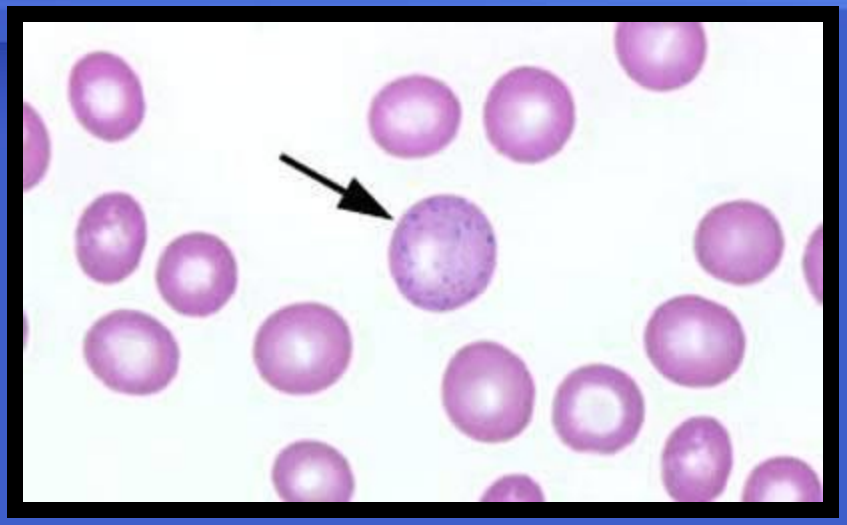


INCLUSIONS

Basophilic Stippling

Precipitated ribosomes (RNA)



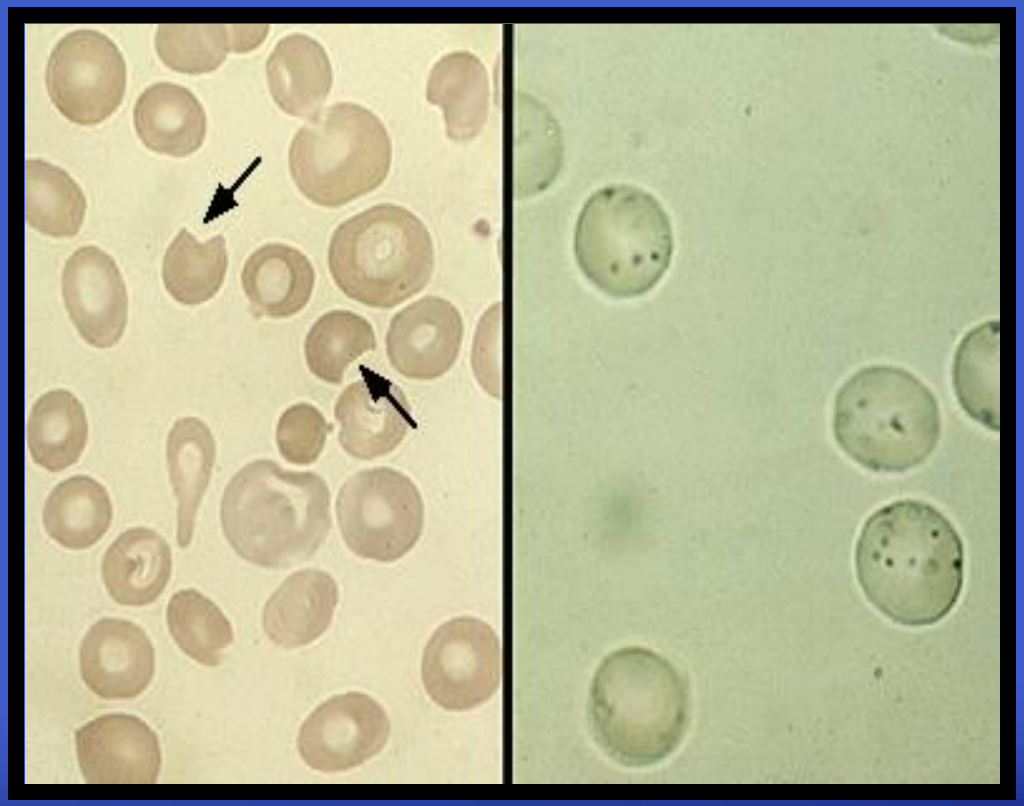


Fine – variety of anemias
Siderblastic, sickle cell, megaloblastic

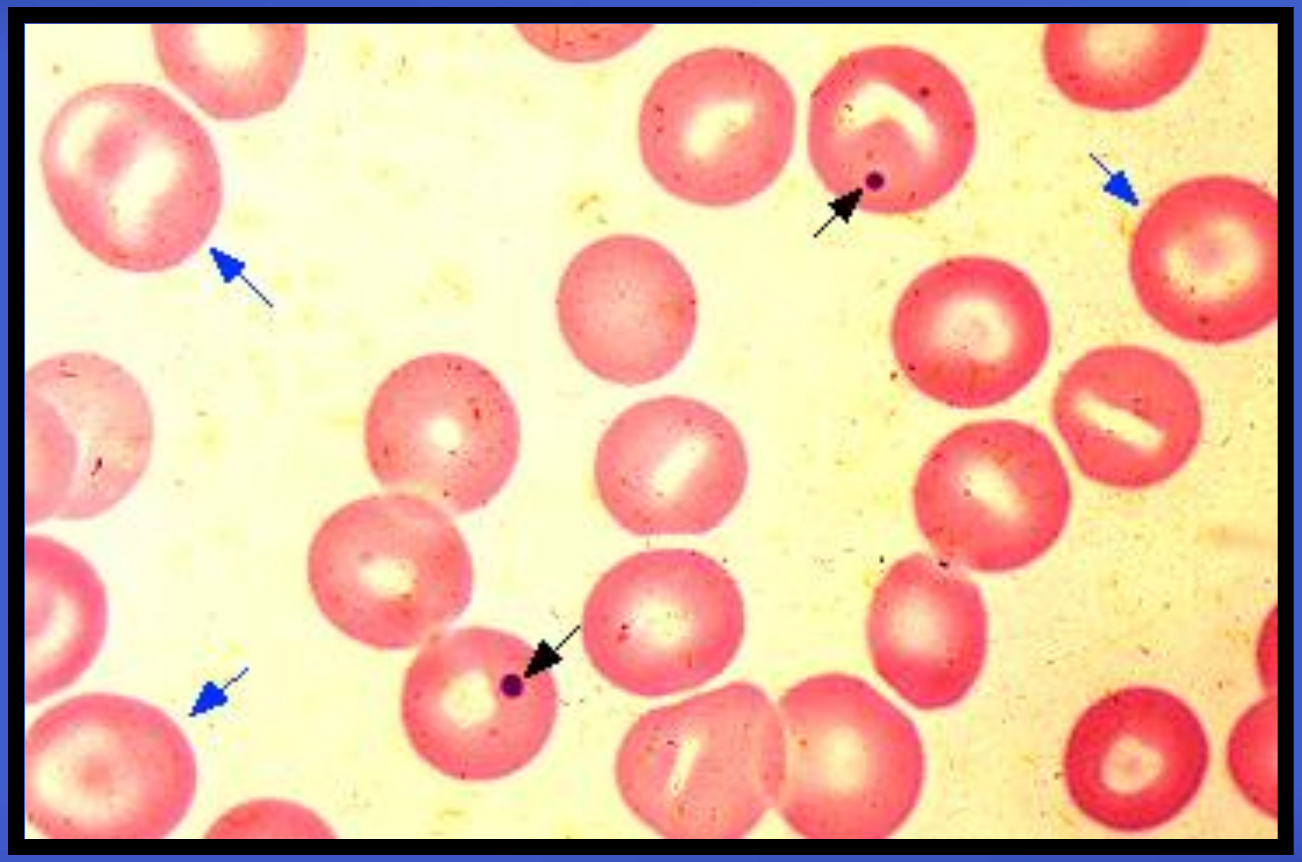
Coarse – Pb intoxication, thalassemia

Heinz Bodies

- Precipitated denatured Hgb
- Seen in G6PD deficiency
- Seen with supravital staining
  - Crystal violet
  - Brilliant cresyl blue



Howell Jolly Bodies



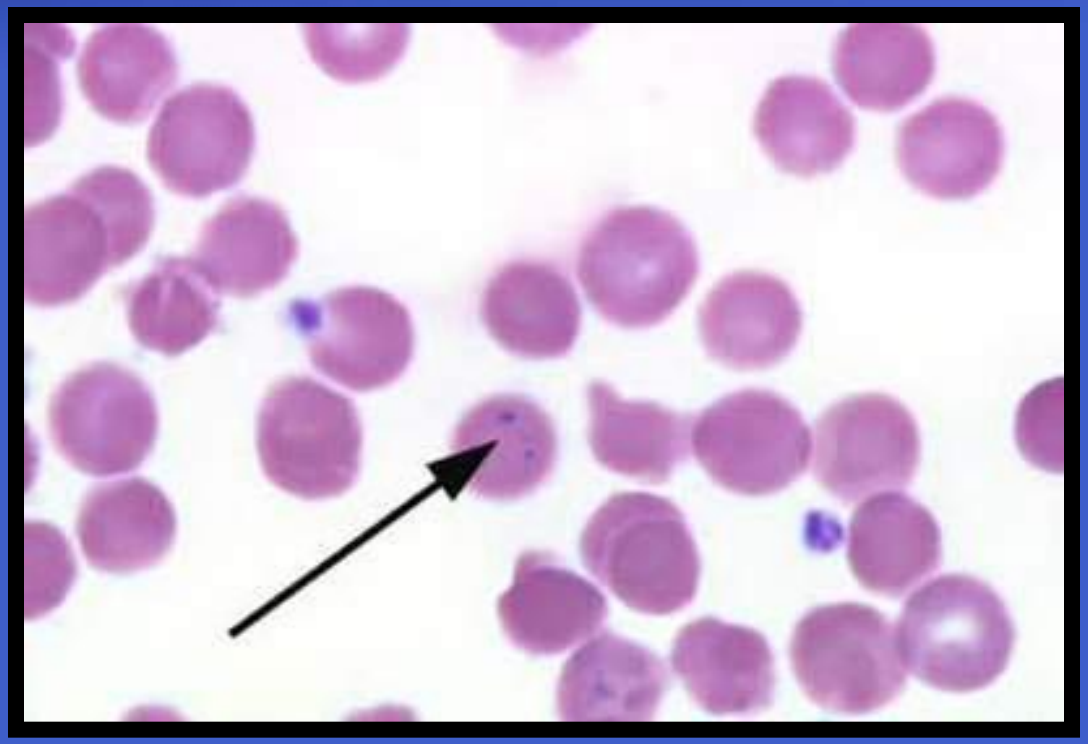
Dense,usually single

Nuclear remnant

Seen in:

- Postsplenectomy
- Hemolytic anemia
- Megaloblastic anemia

Pappenheimer bodies



Small, dense basophilic granules

Fe-containing mitochondrial remnant or sidersome

Seen in:

- Sideroblastic anemia

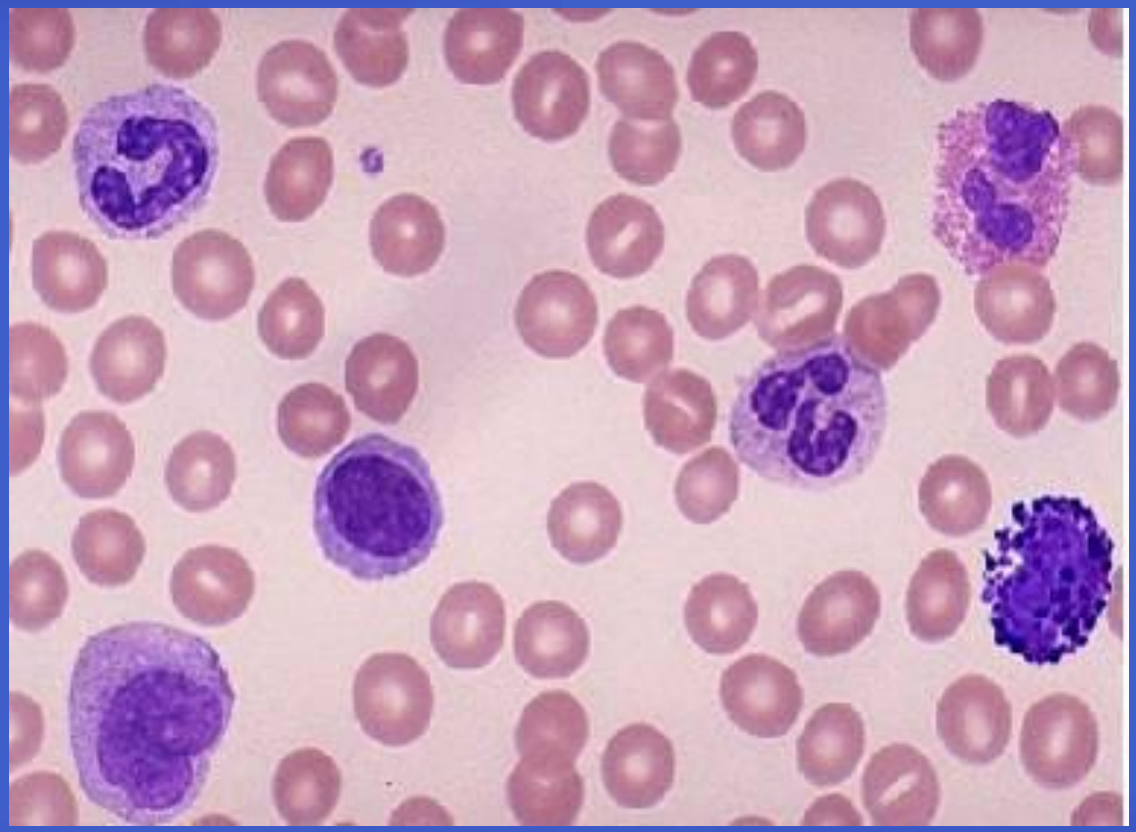
(Hereditary, idiopathic or secondary)

- Post-splenectomy

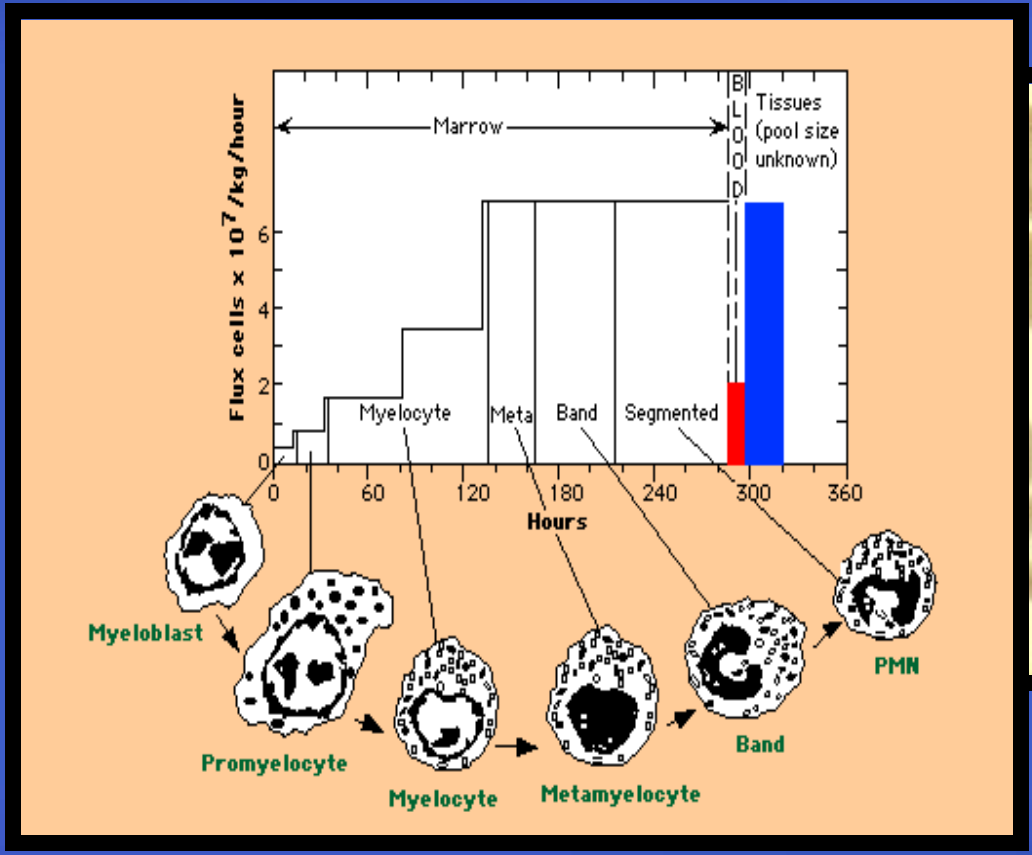
LEUKOCYTES

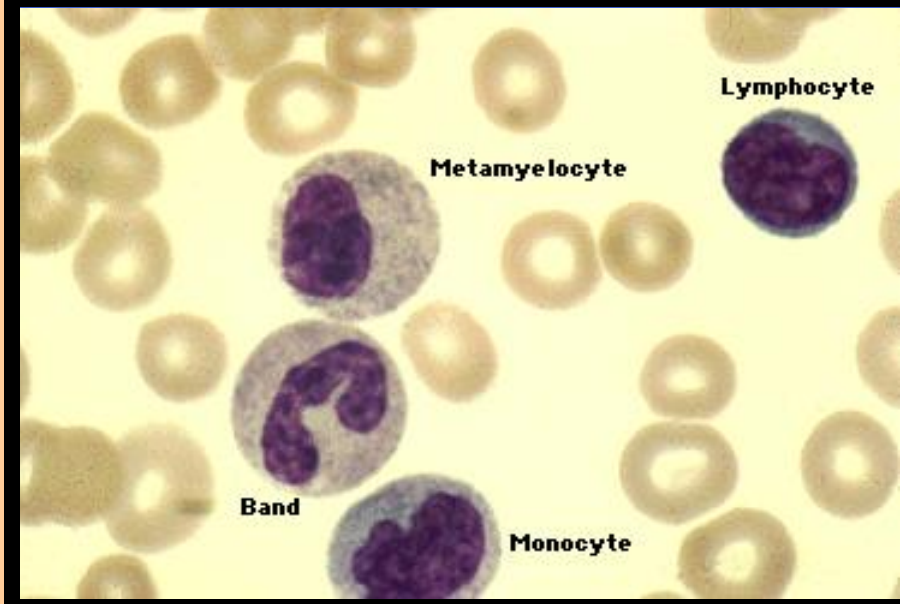
WBC

- Neutrophil
- Eosinophil
- Basophil
- Lymphocyte
- Monocyte



Polymorhponuclear Neutrophils (PMNs)





PMNs

- Normal range ANC: 1.5-7.0 $10^3/mm^3$
- Reflects only the CIRCULATING PMNs
- Does not include marginated PMNs or stored PMNs in the bone marrow
- Mechanisms for neutrophilia:
  - Demargination
  - Release of bone marrow component
  - Increased production

Mechanisms Causing Nonneoplastic Neutrophilia

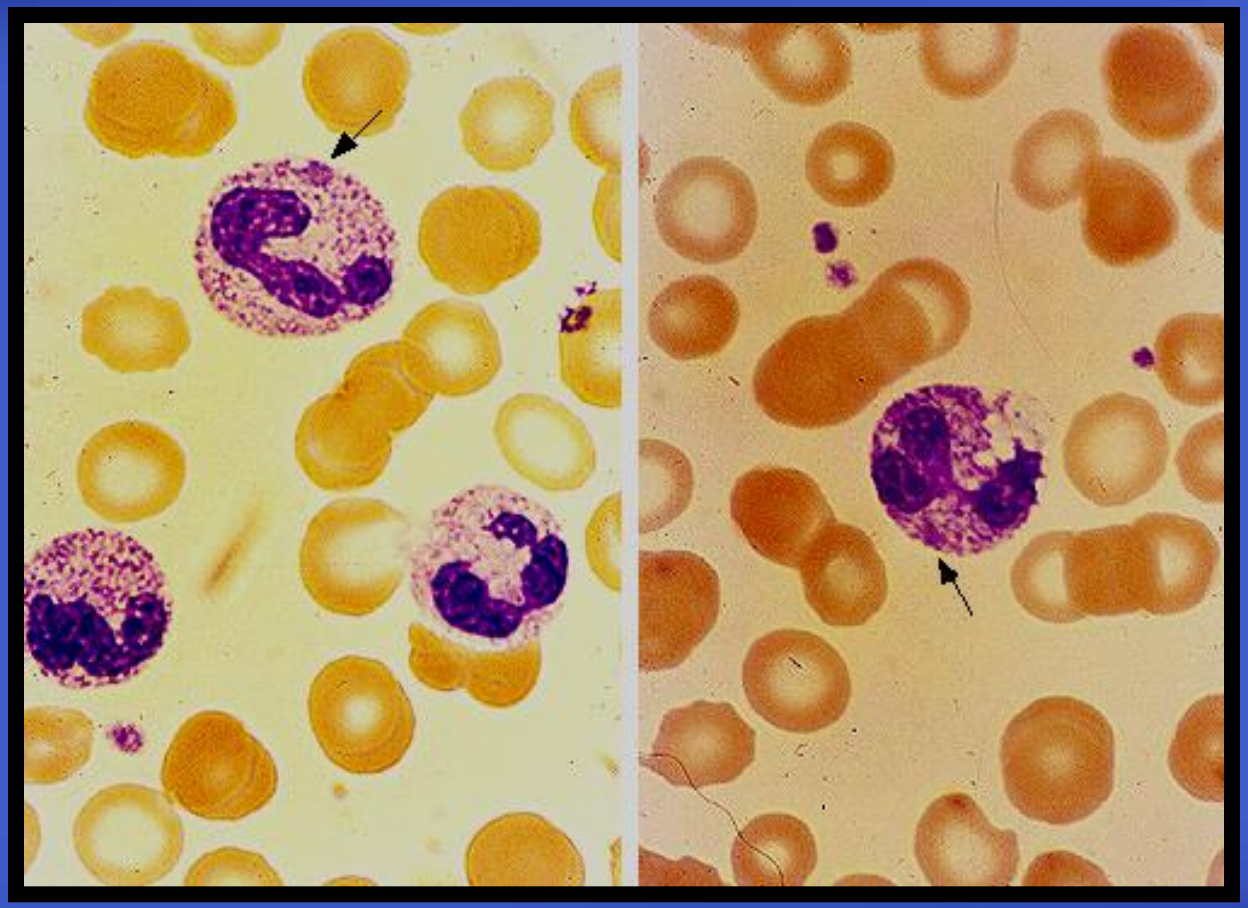
Mechanism	Time Course	Causes
Demargination	Minutes	Epinephrine release, acute stress, exercise
Mobilization of bone marrow	Hours	Corticosteroids, infections, inflammation
Increased production	Days	Sustained infection, chronic inflammation, CSF-producing tumors, CSF therapy

Distinguishing Between Reactive Changes and Neoplasia

Parameter	Reactive	Neoplastic
WBC	<30 x $10^3/mm^3$	>50 x $10^3/mm^3$
Toxic Neutrophils	Present	Absent*
Left Shift	Includes myelocytes	Includes blasts
Basophilia	Absent	Present
Platelet count	Variable, decreased in sepsis	Increased
Platelet Morphology	Unremarkable	Abnormal
Nucleated RBC	Absent	Present

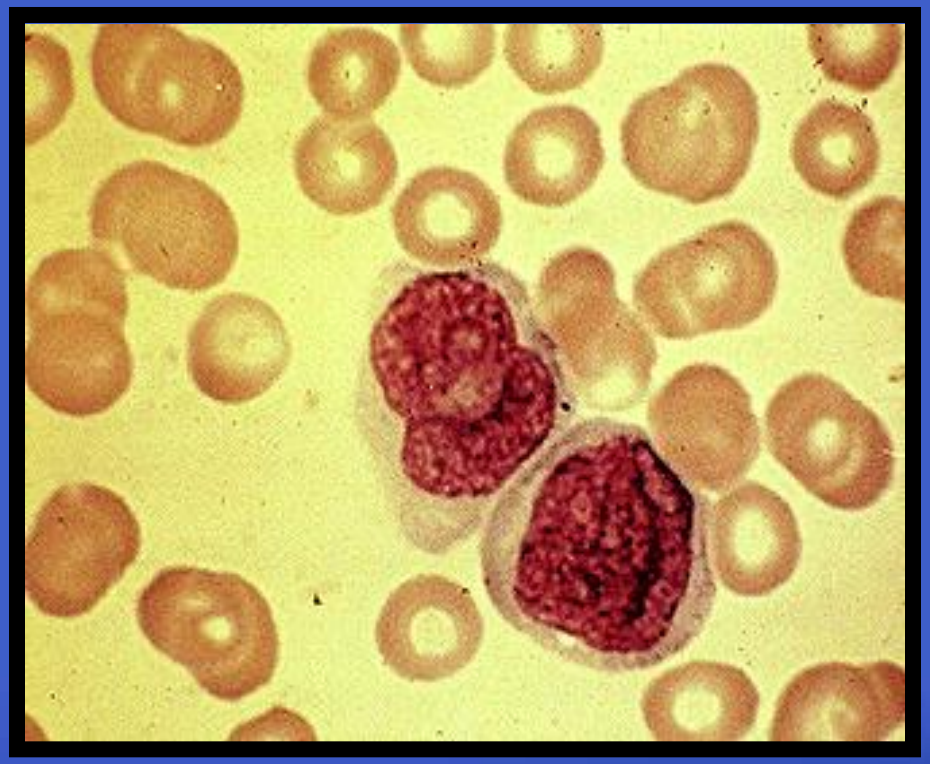
*Except in patients with infection

Reactive Neutrophil

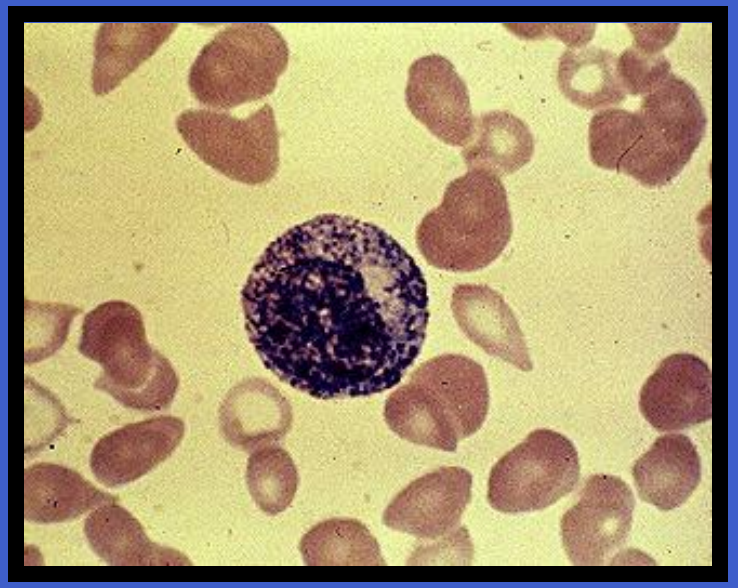


3 Features:
Toxic granulations
Dohle bodies
Cytoplasmic vaculoes

Immature Granulocyte Suggesting Neoplasia

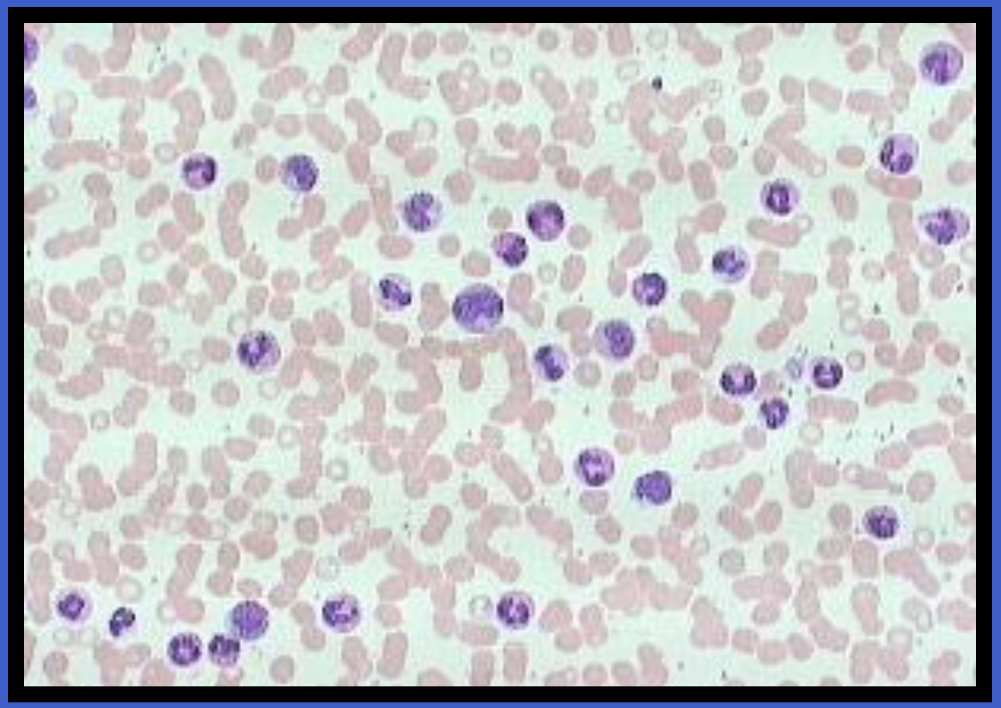


Myeloblast



Promyelocyte

The Malignant Mimicker: Leukemoid Reaction

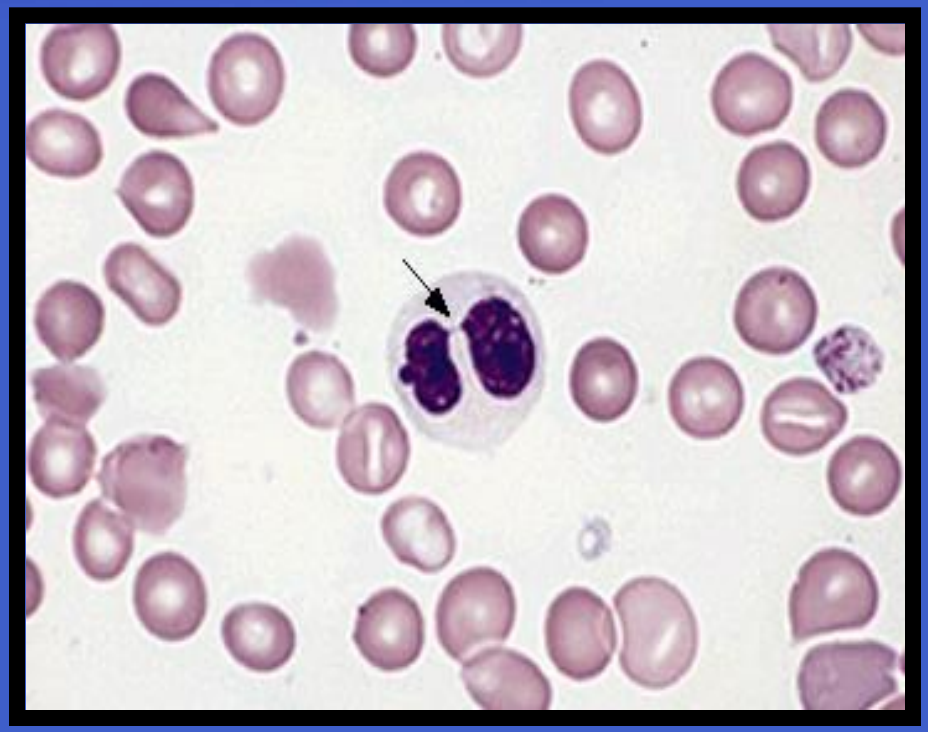


- All precursor granulocytes in the PBS
- WBC in the range up to 100K
- Response to severe stress or infection
- Other signs of malignancy not present

Neutrophil Disorders with Abnormal Morphology

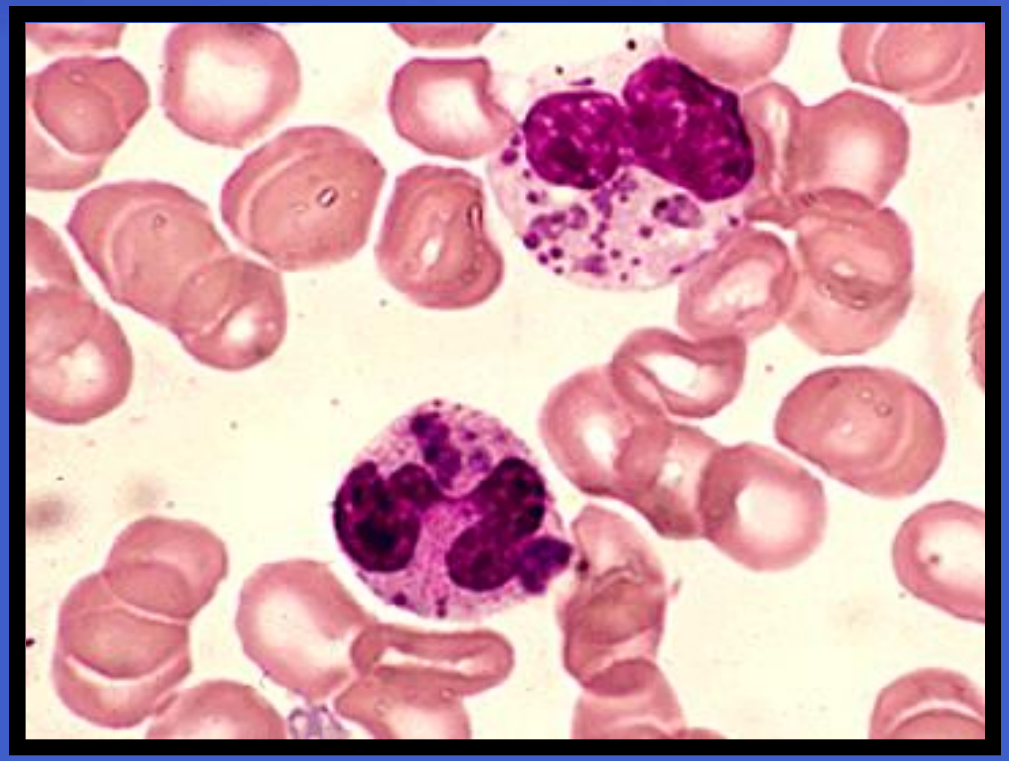
- Pelger-Huet anomaly
  - Bilobed or nonsegmented nucleus
  - asymptomatic
- May-Hegglin anomaly
  - Cytoplasmic inclusions resembling Dohle bodies
  - Many asymptomatic
- Chediak-Higashi syndrome
  - Giant cytoplasmic granules in all granulocytes
  - Immunodeficiency
- Hypersegmentation
  - B12/Folate deficiency, myelodysplasia, myeloid leukemia, chemotherapy, or renal failure

Pelger-Huet Anomaly



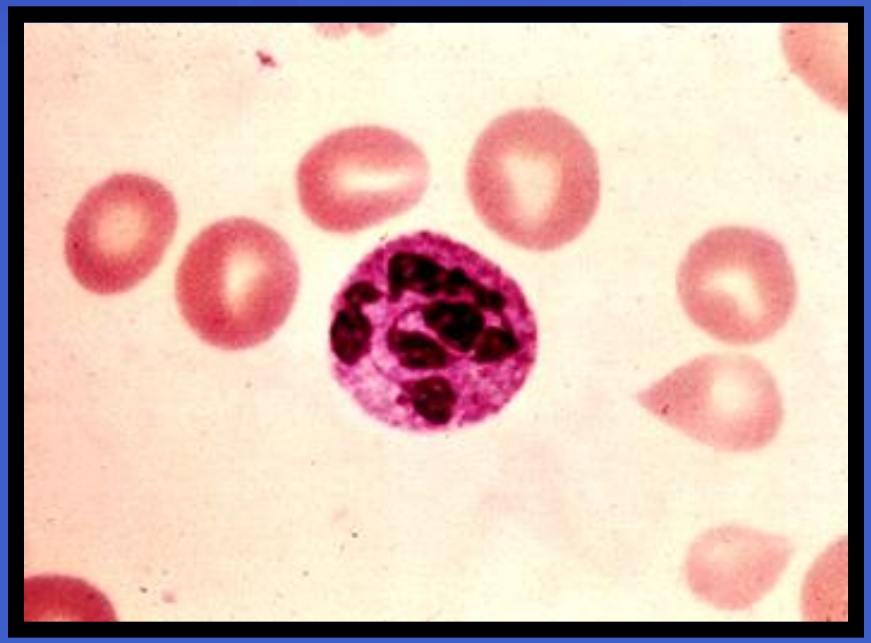
- Inherited, AD or acquired
- Acquired = “pseudo” Pelger-Huet as in MDS

Chediak-Higashi Syndrome



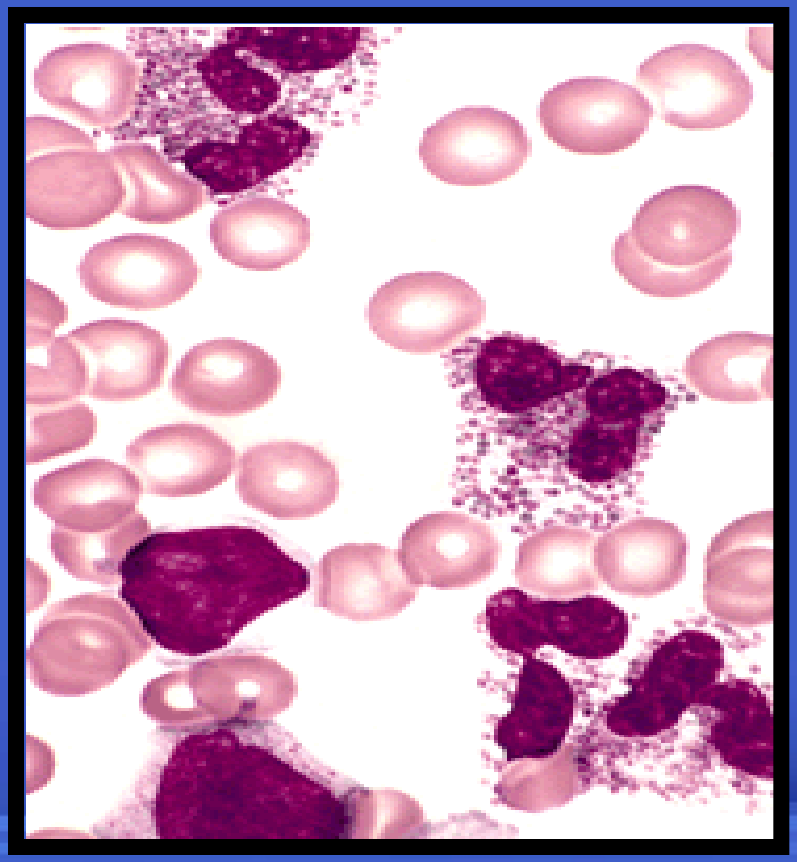
- Autosomal recessive
- Giant granules
- Severe immunodeficiency

Hypersegmentation



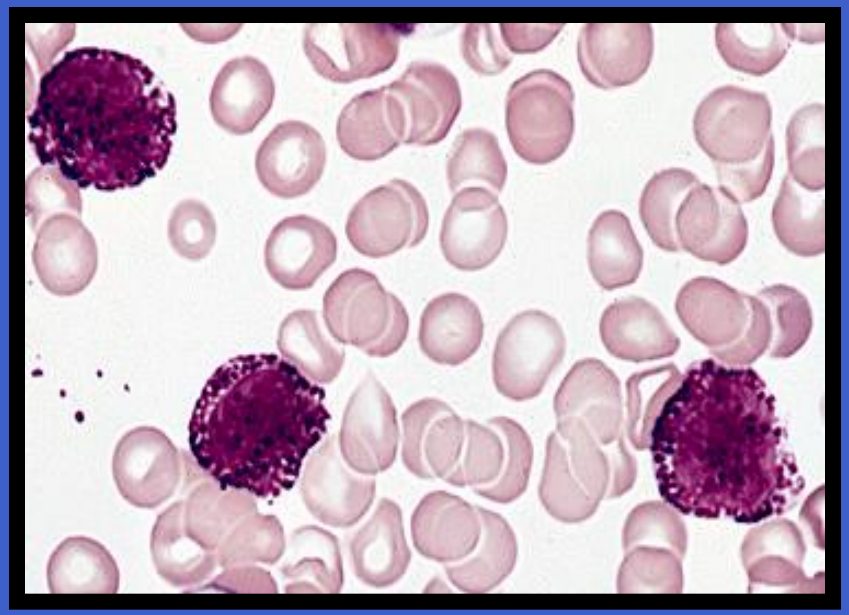
- Normal to have 4-5 lobes
- Seen most commonly in B12/folate def
- Uremia
- Chemotherapy
- Also seen in MDS and other myeloid neoplasms
- Can be inherited

Eosinophilia



- Allergic/hypersensitivity reactions
- Drug allergies
- Parasitic infections
- Connective tissue/collagen vascular disease
- Neoplasms
  - T-cell lymphoma
  - Hodgkin lymphoma
- Sarcoidosis
- Hypereosinophilic syndrome/Chronic eosinophilic leukemia

Basophilia

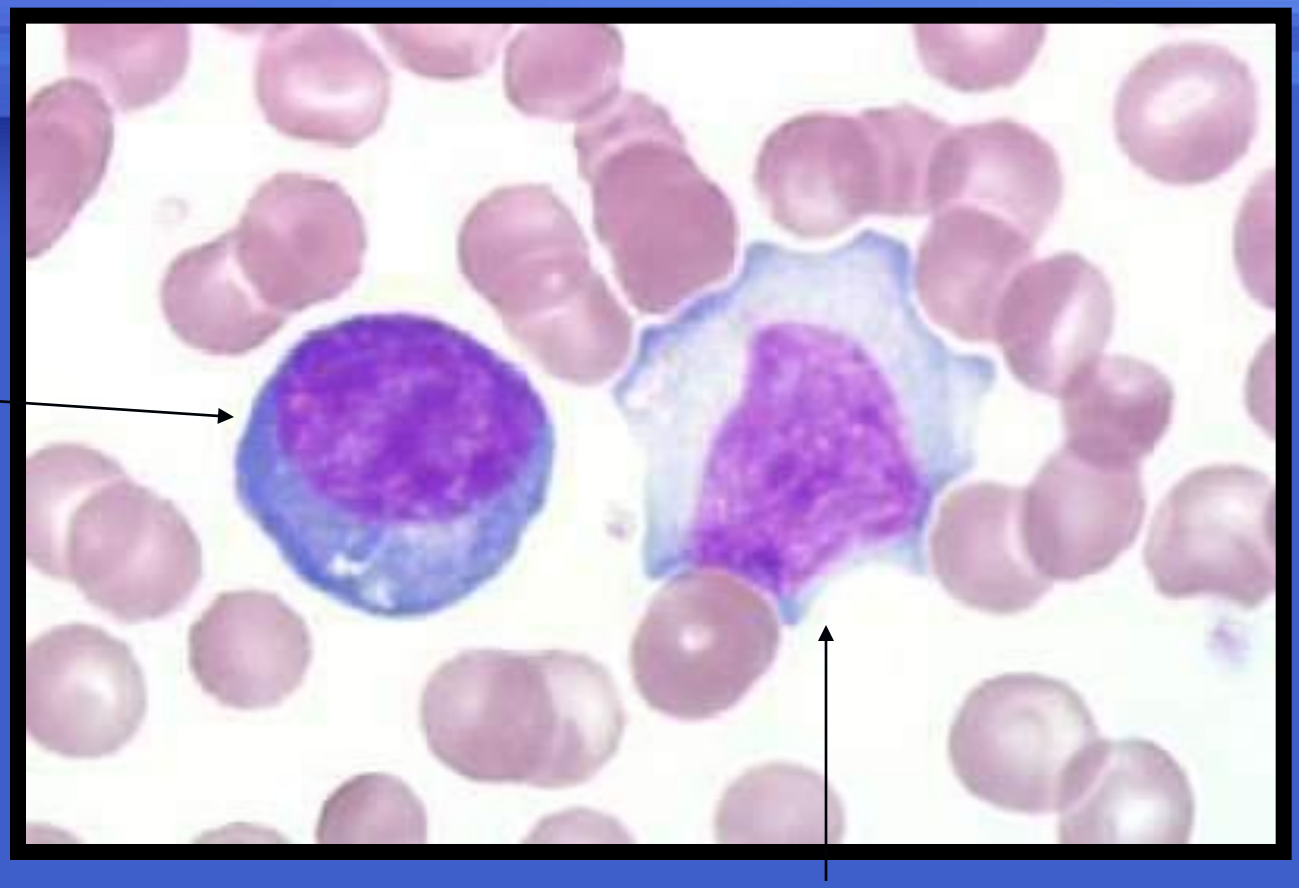


- Much more common in malignancies like CML vs. reactive

Reactive Lymphocytosis

- Diseases with nonreactive morphology
  - Infectious lymphocytosis (Whooping cough)
  - Transient stress lymphocytosis
- Diseases with reactive morphology
  - EBV, IM, CMV, Toxo, adenovirus, HHV-6, viral hepatitis

Plasmacytoid lymphocyte

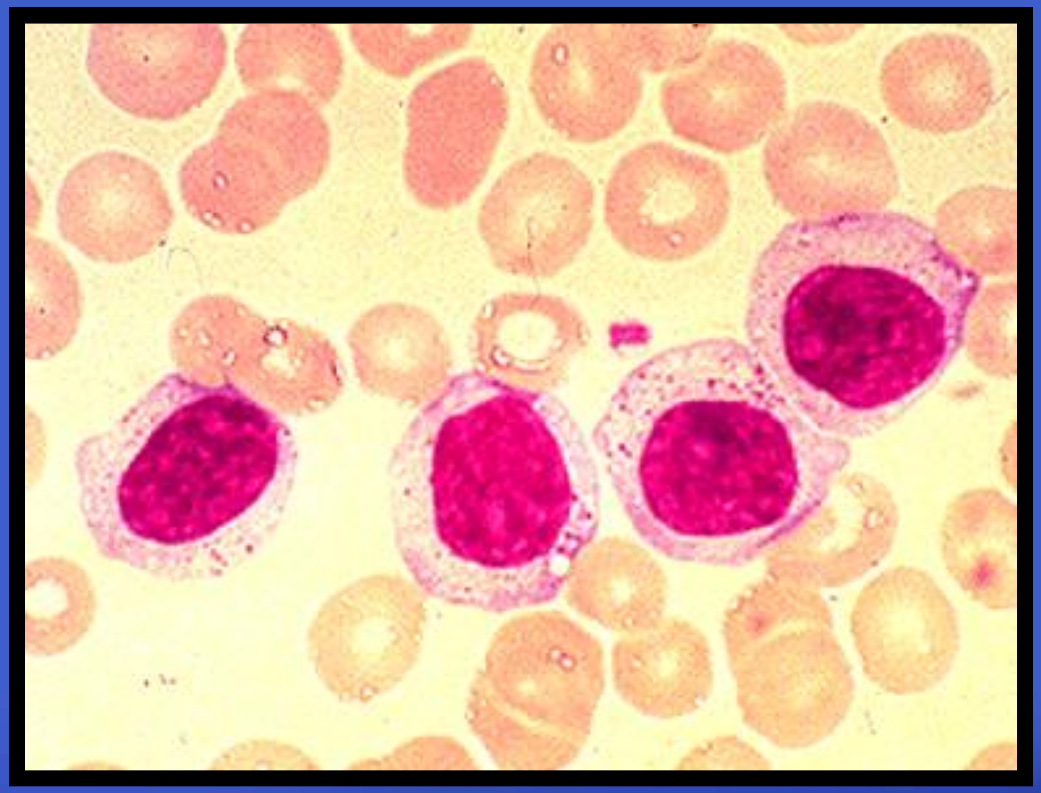


Atypical/reactive lymphocytes

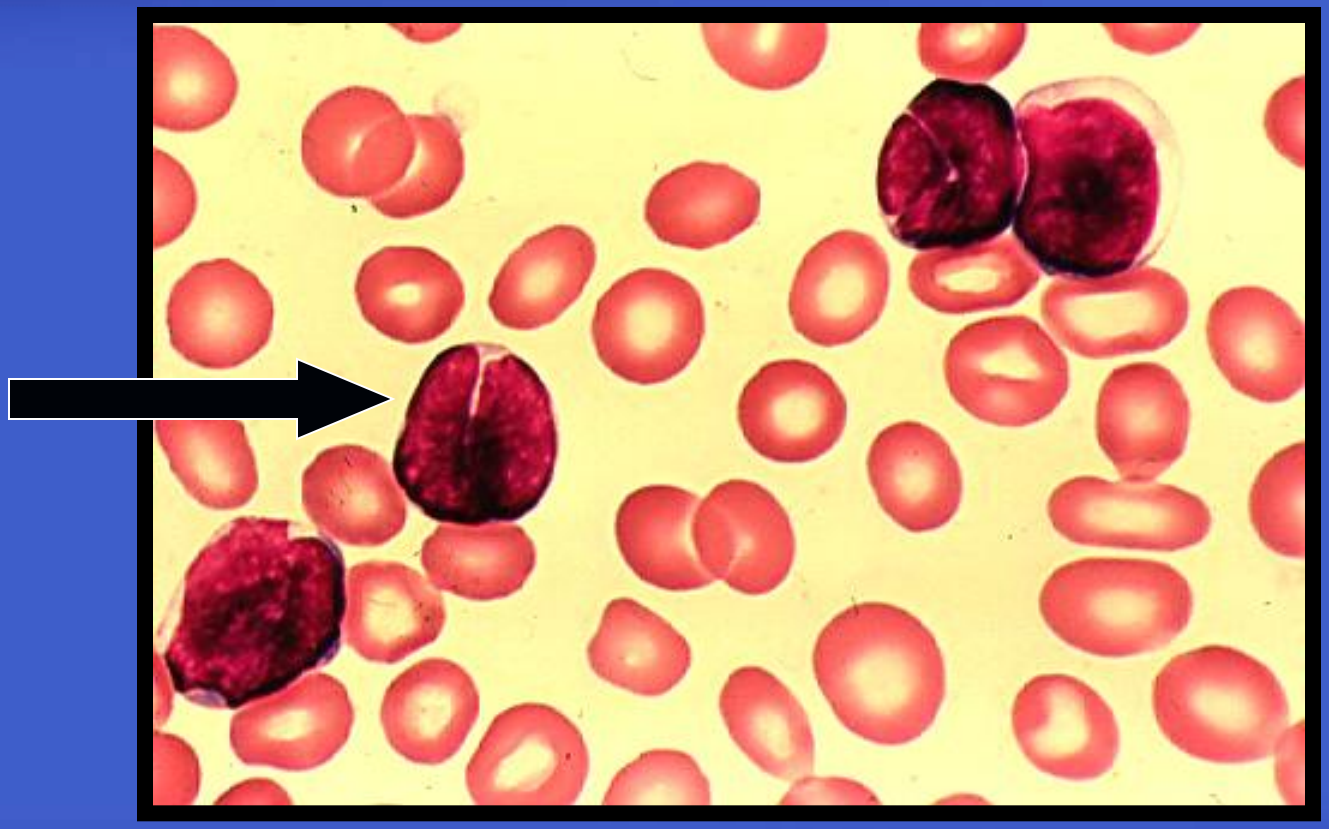
Features of Leukemias/Lymphomas

- T-cell large granular lymphocyte leukemia/NK Cell Leukemia
- Blasts with and without Auer rods
- Hairy cells
- Cleaved cells
- Smudge cells
- “Clover cells”

T-cell LGL Leukemia/NK Cell Leukemia

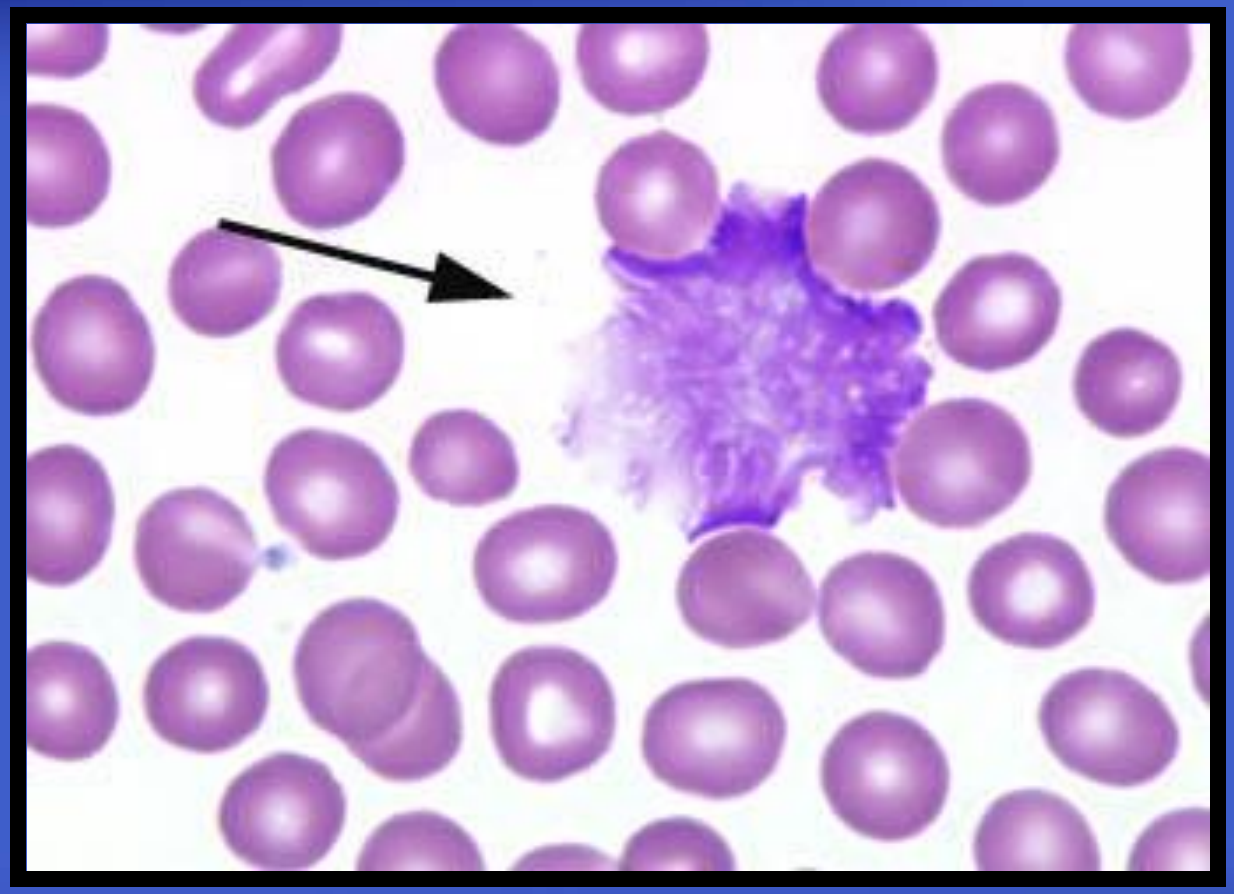


Small Cleaved Cells



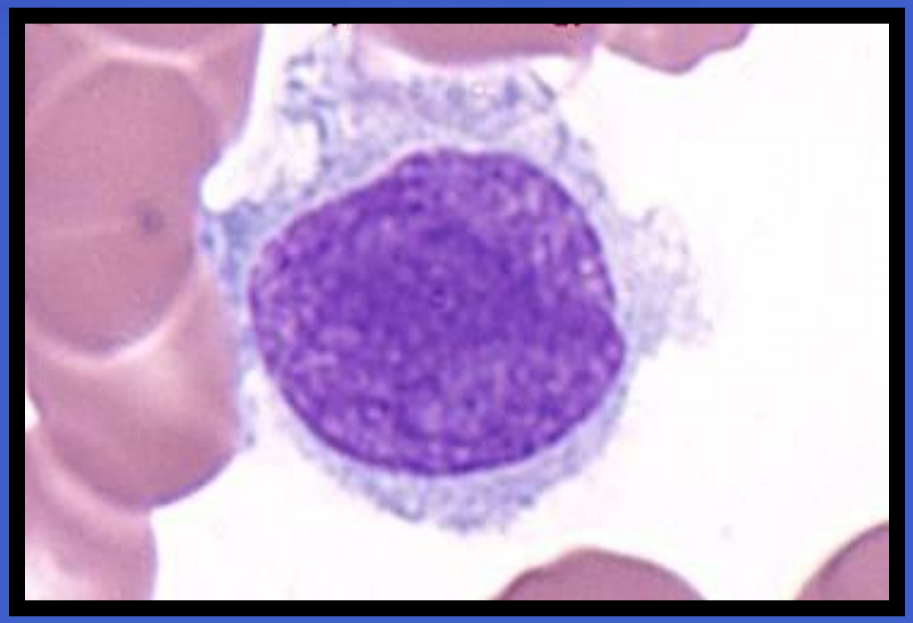
Follicular lymphoma

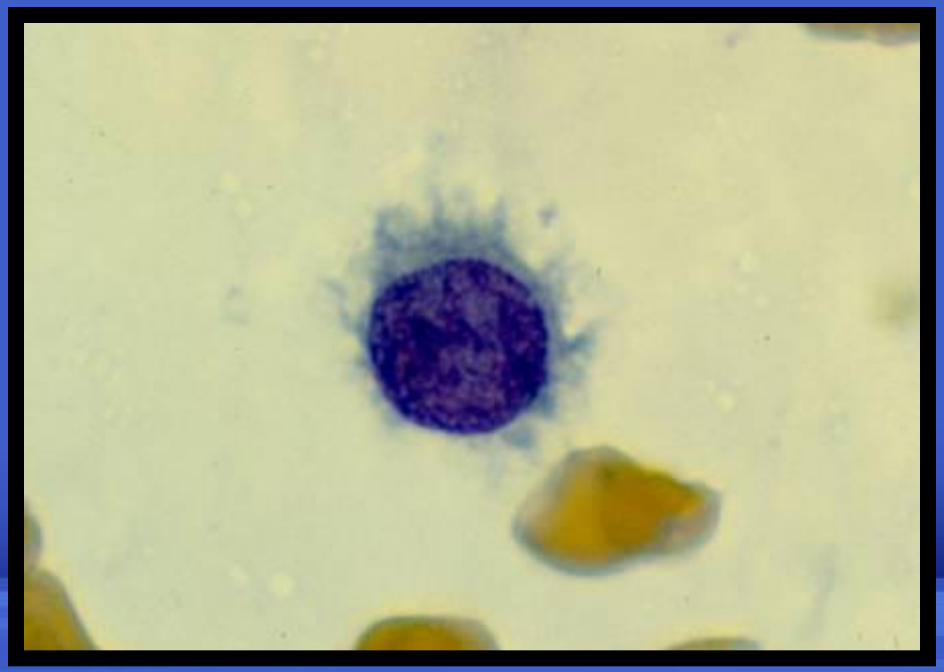
Smudge Cells



CLL

Hairy Cell Leukemia



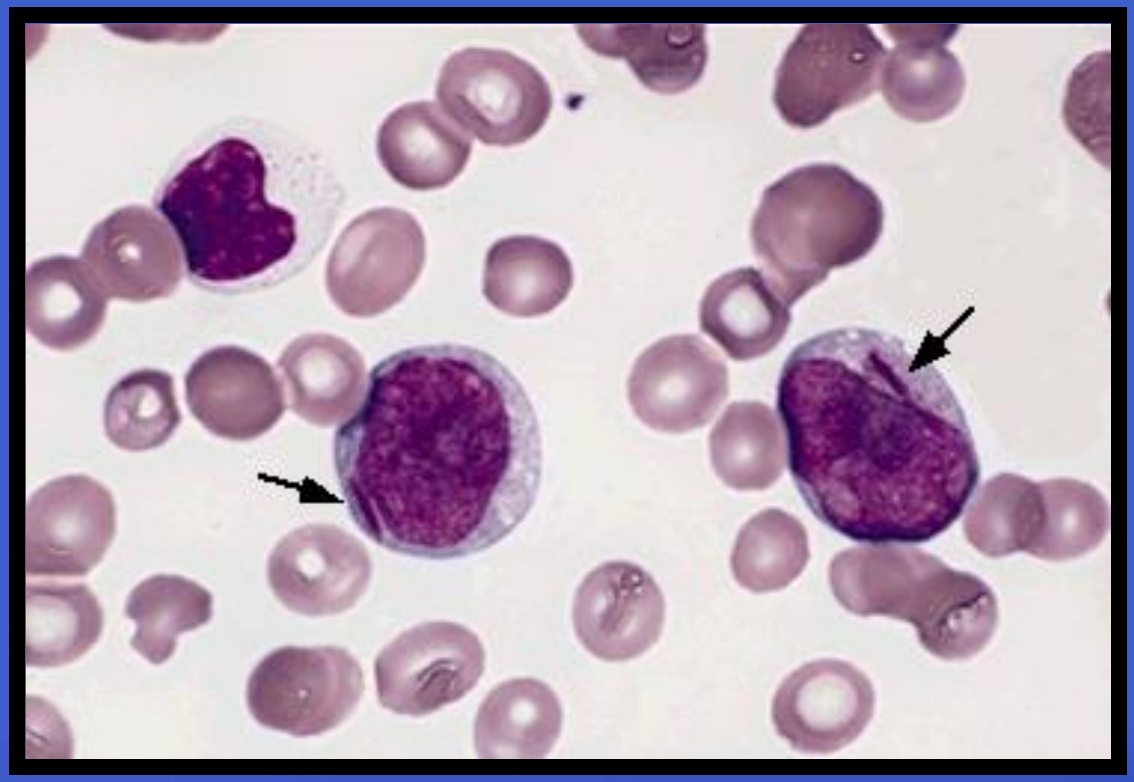


Blasts with Auer Rods (Myeloblasts)

Auer Rod=

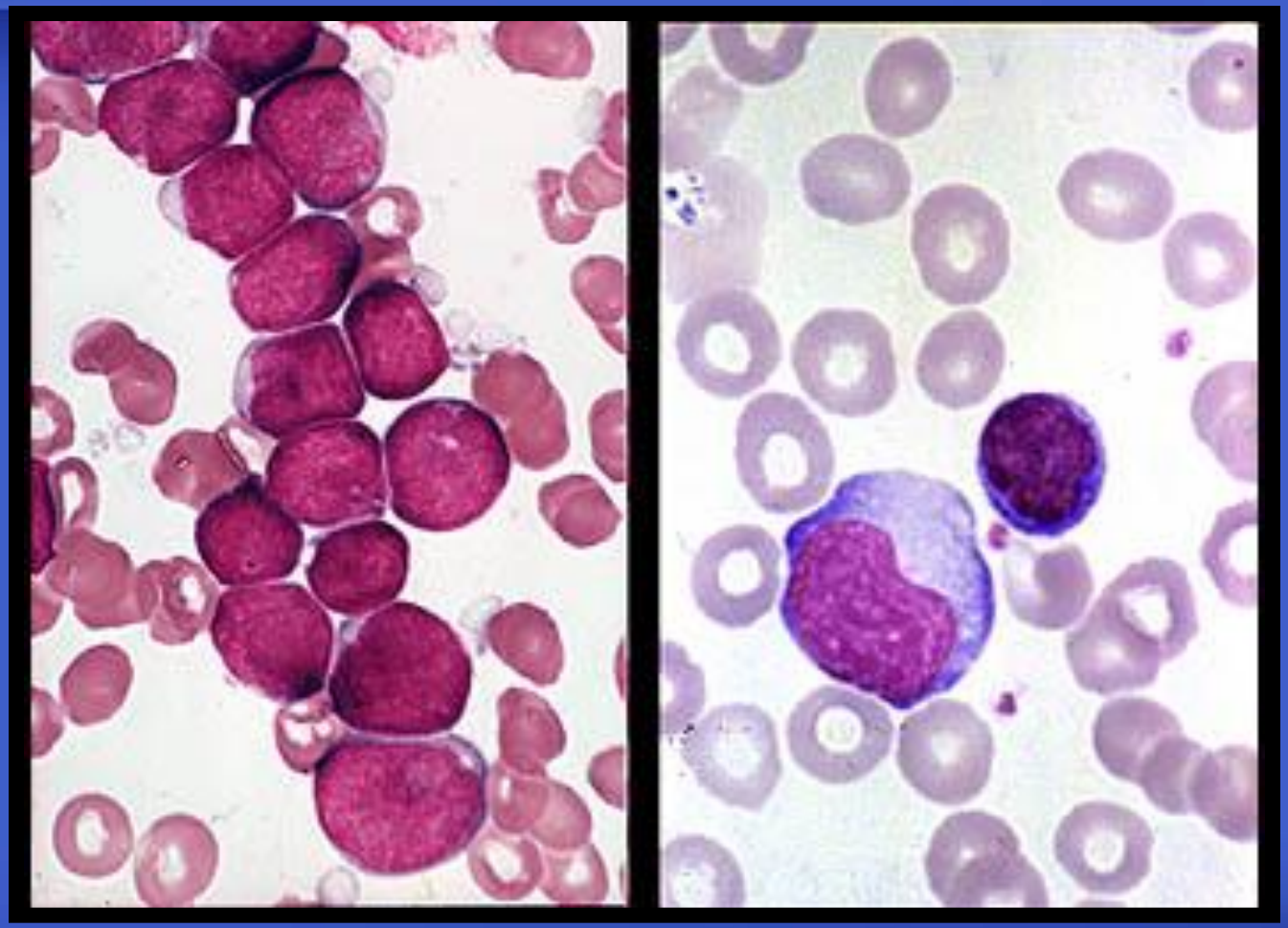
Fused lysosomal

granules



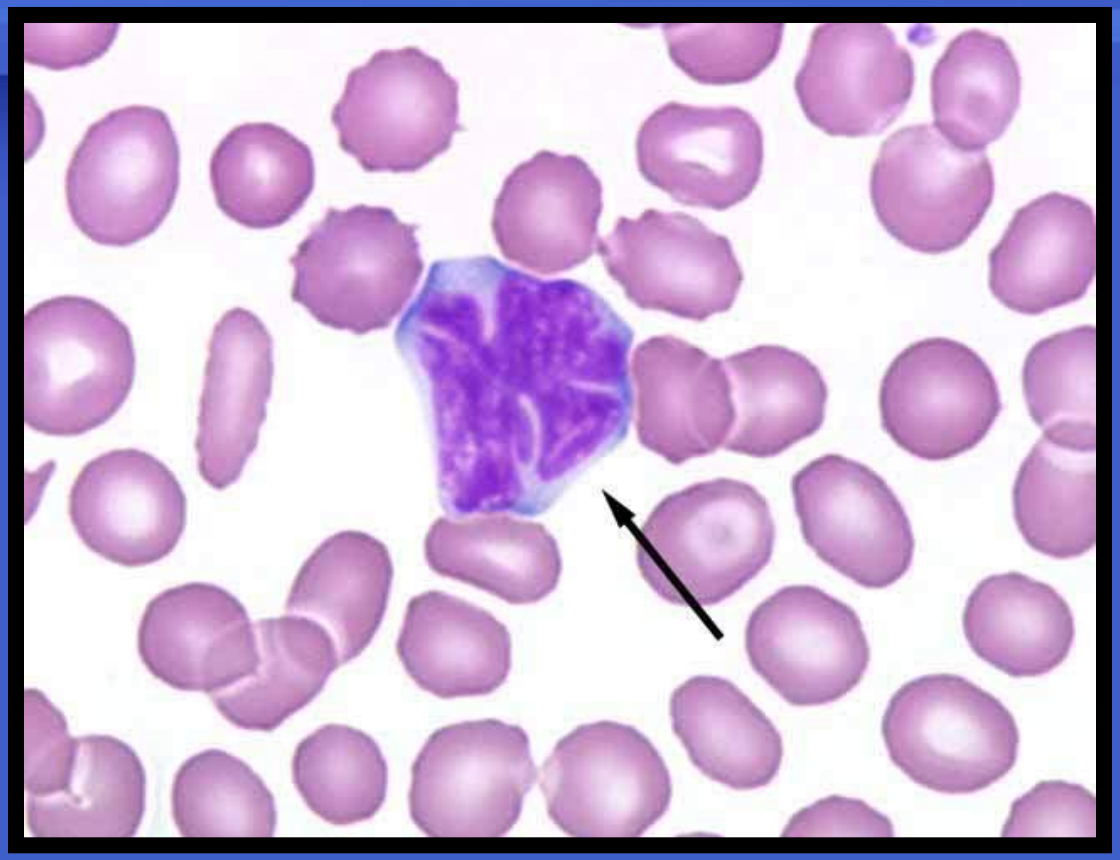
AML

Blasts without Auer Rods



Lymphoblasts (ALL) vs. Atypical lymphs

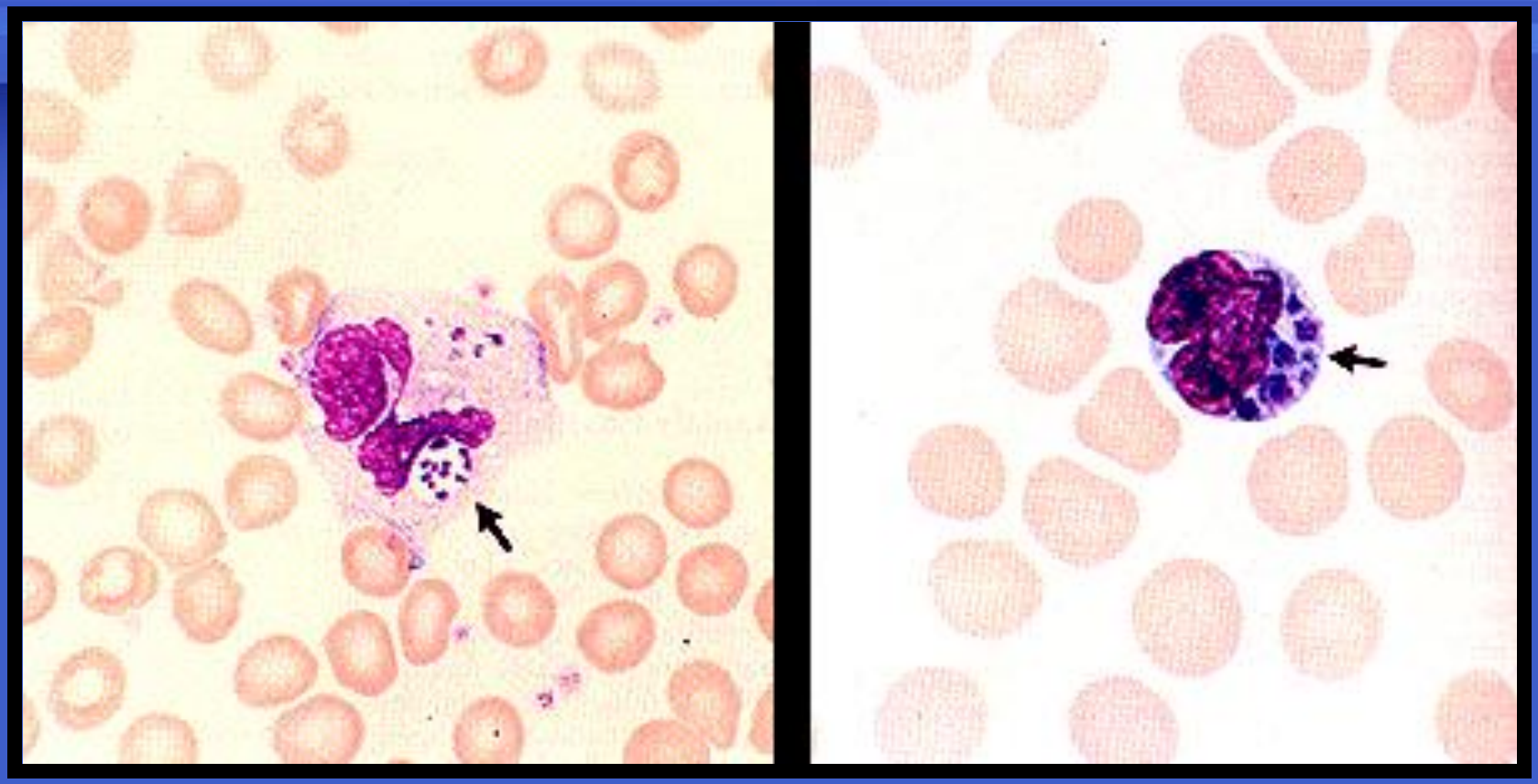
Clover Cells



Adult T-Cell Leukemia/Lymphoma

ORGANISMS

Ehrlichiosis

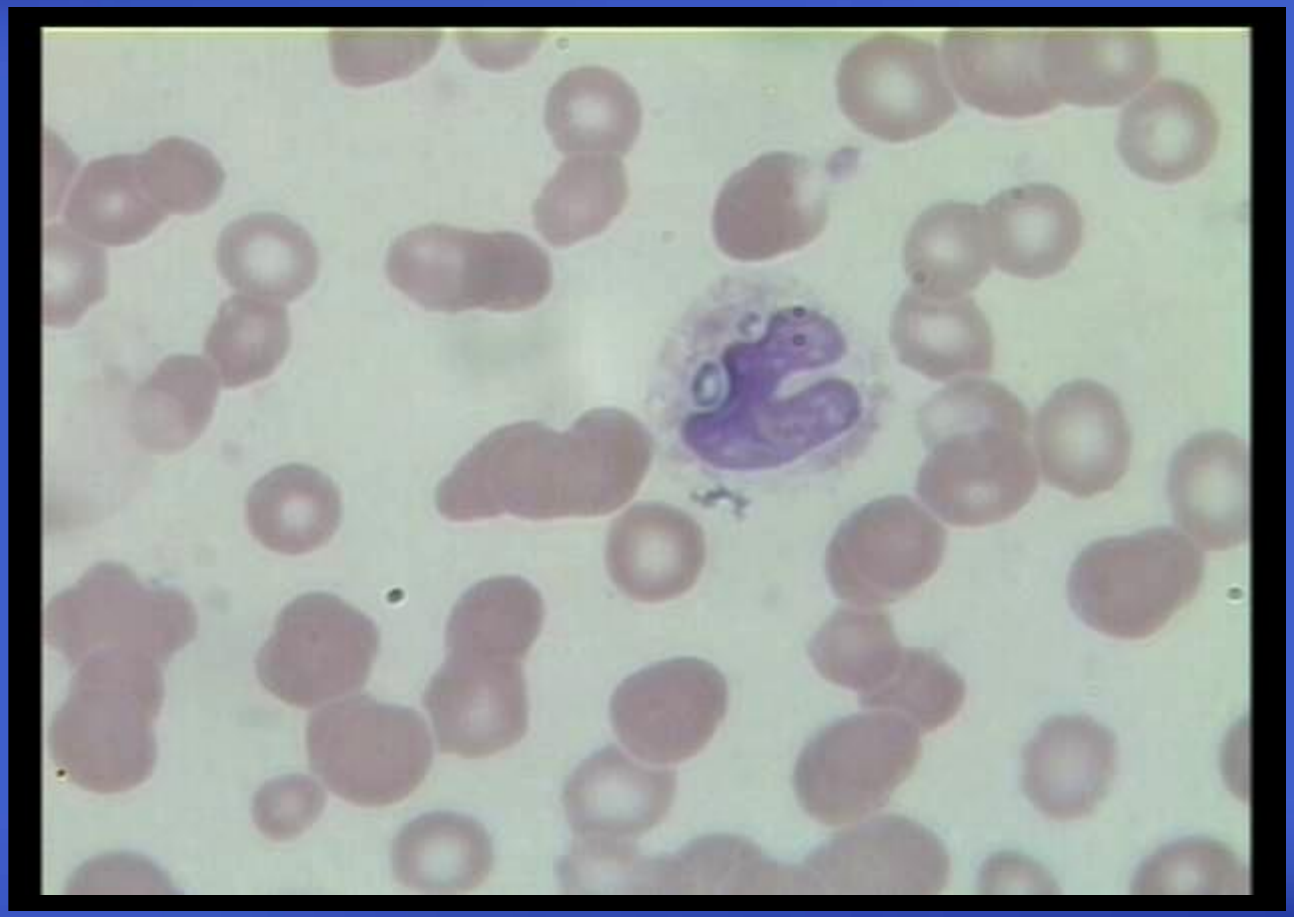


Found in the SE and S. Central US

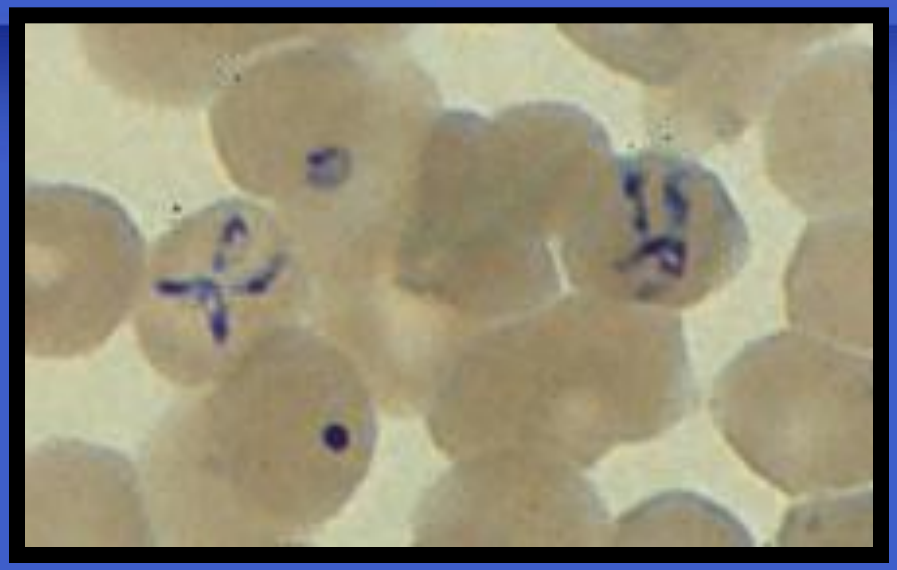
Transmitted by ticks

Rickettsial organism

Histoplasma

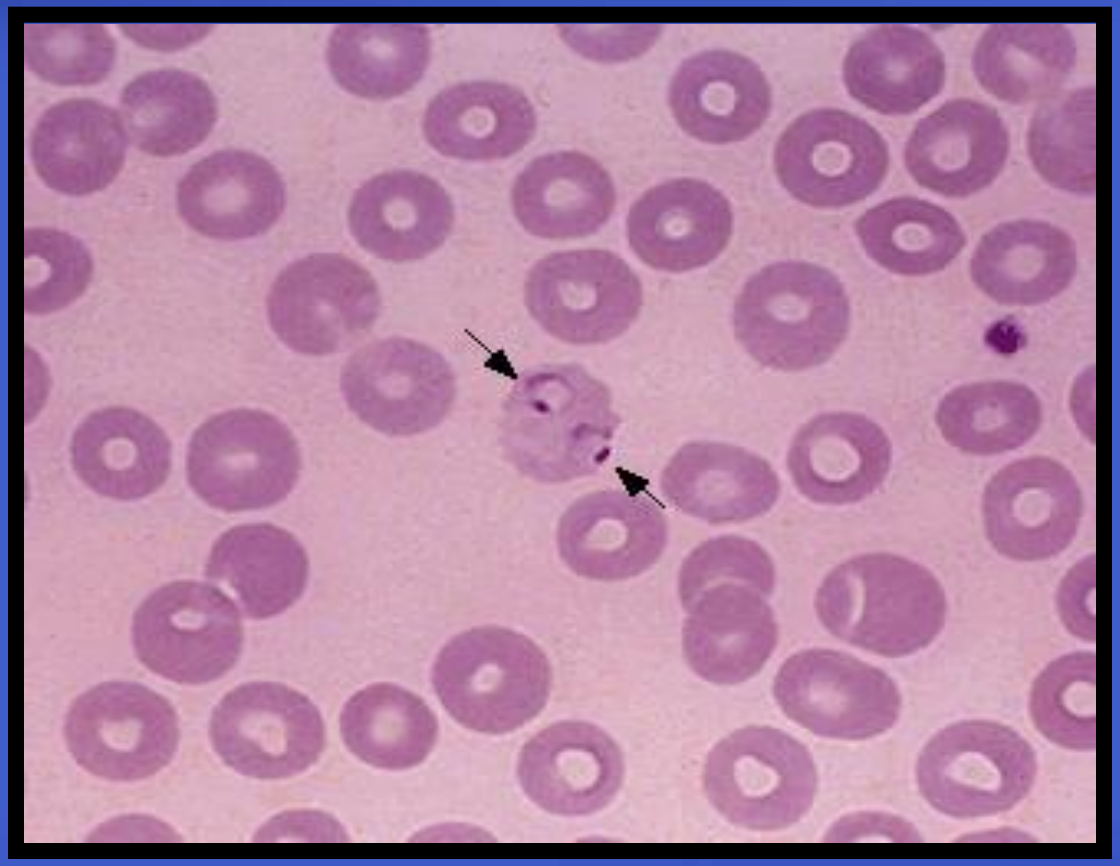


Babesiosis



- Protozoa
- Endemic in the NE US
- Transmitted by the Ixodidae tick
- Similar to Malaria
- Tetrad form is pathonogmonic
- Risk Factors:
  - Post-splenectomy
  - Immunocompromised

Malaria



- Ringed stage (trophozoite)
- Can see other stages within RBCs

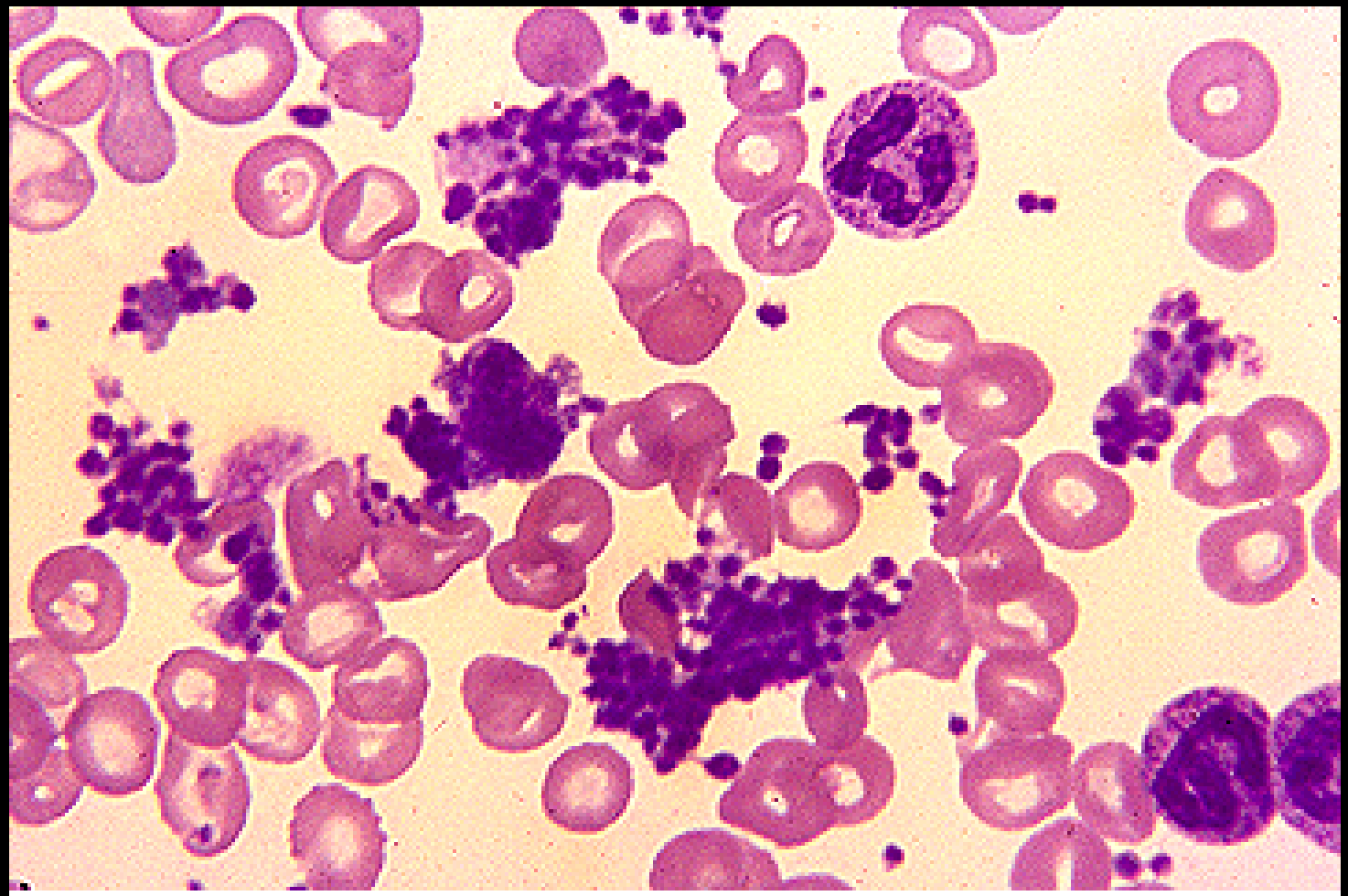
PLATELETS

Estimate platelet count on PBS

- 100x oil immersion
- Minimum of 5 fields
- Average # platelets, then X by 20,000

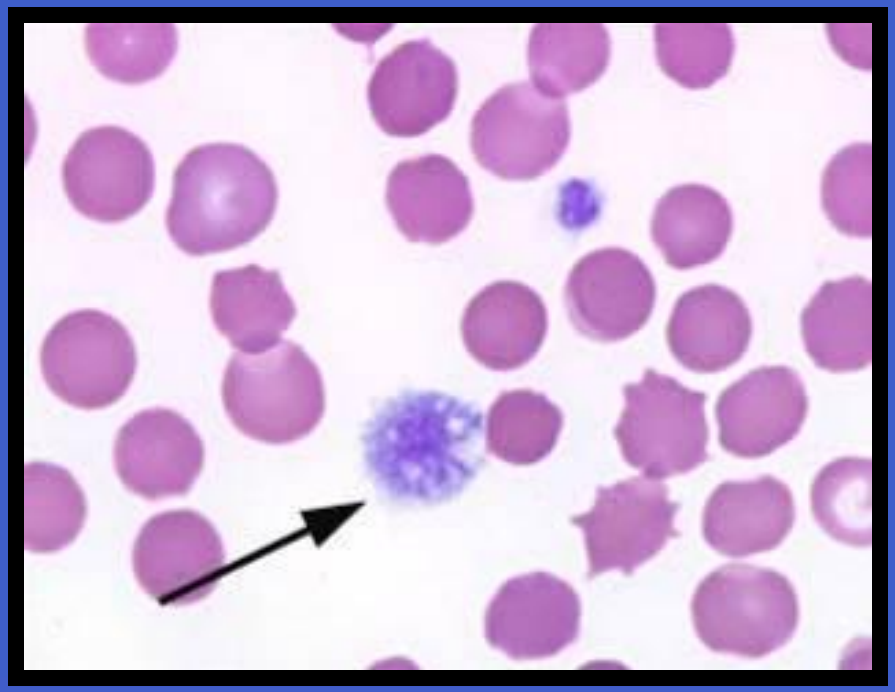
Platelet Count

Increased	Normal	Decreased
Over 20 platelets per HPF > 400,000	7 – 20 platelets per HPF	Under 7 platelets per HPF < 140,000



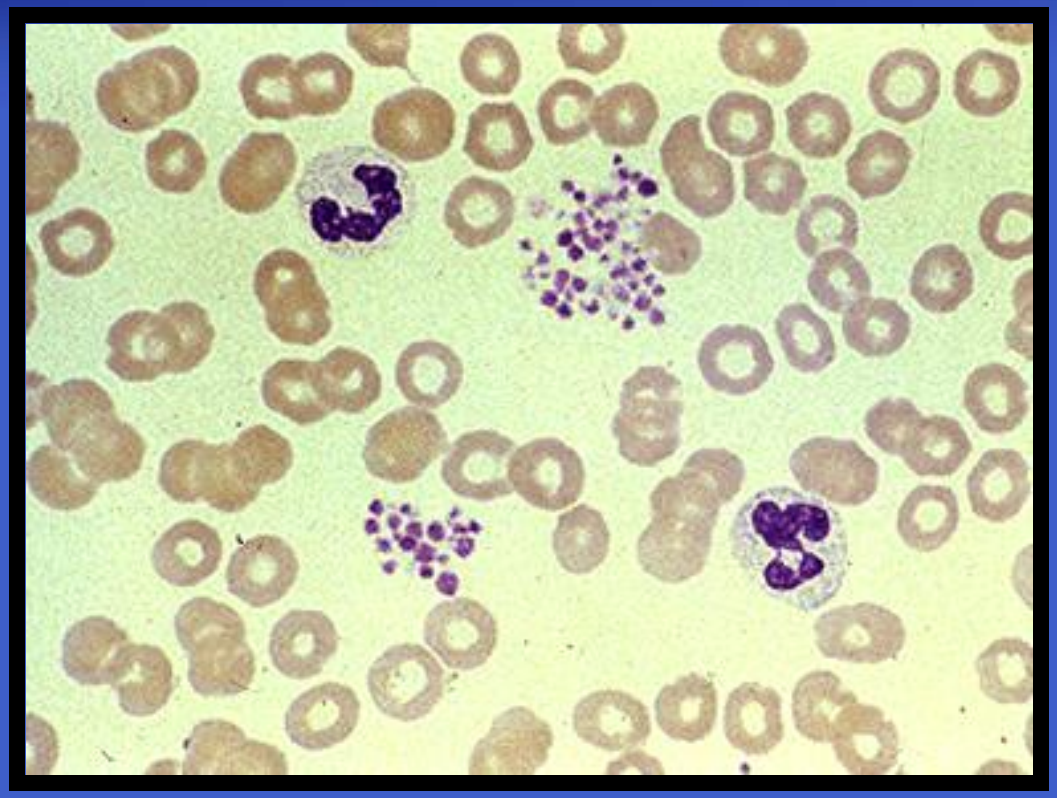
Essential thrombocythemia Peripheral smear from a patient with essential thrombocythemia shows an increased platelet number and clumps of large, abnormal platelets. Courtesy of Carola von Kapff, SH (ASCP).

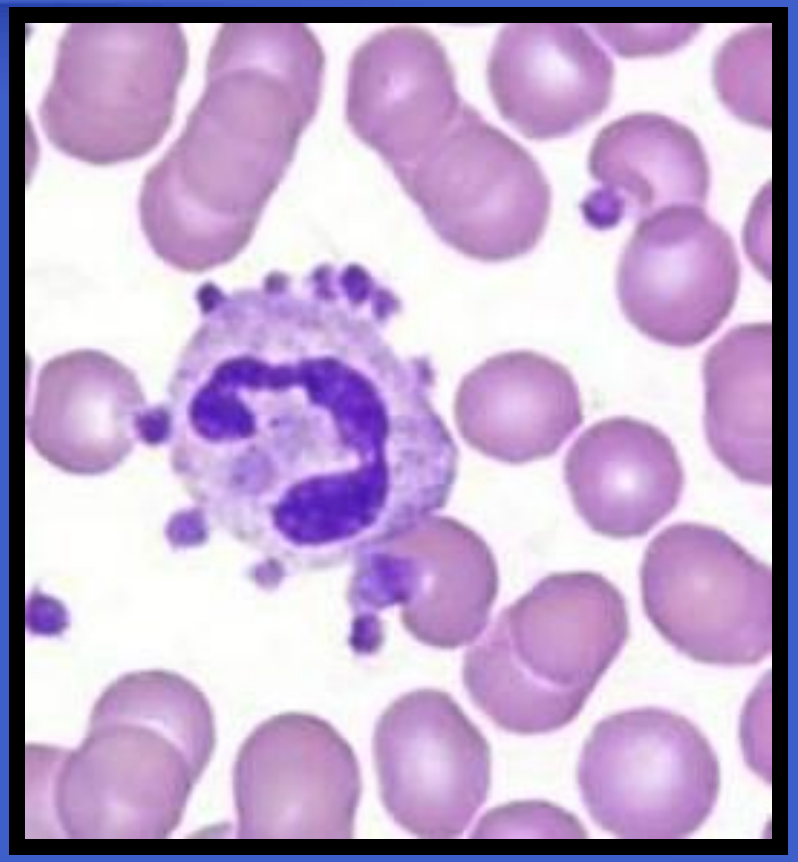
Giant Platelets



- Size of an RBC
- Usually indicates a hyperreactive bone marrow 2^{o} to underlying condition
  - ITP, TTP, DIC
- Can be inherited in the form of Bernard-Soulier syndrome, platelet dysfunction

Platelet Clumping and Satellitelosis



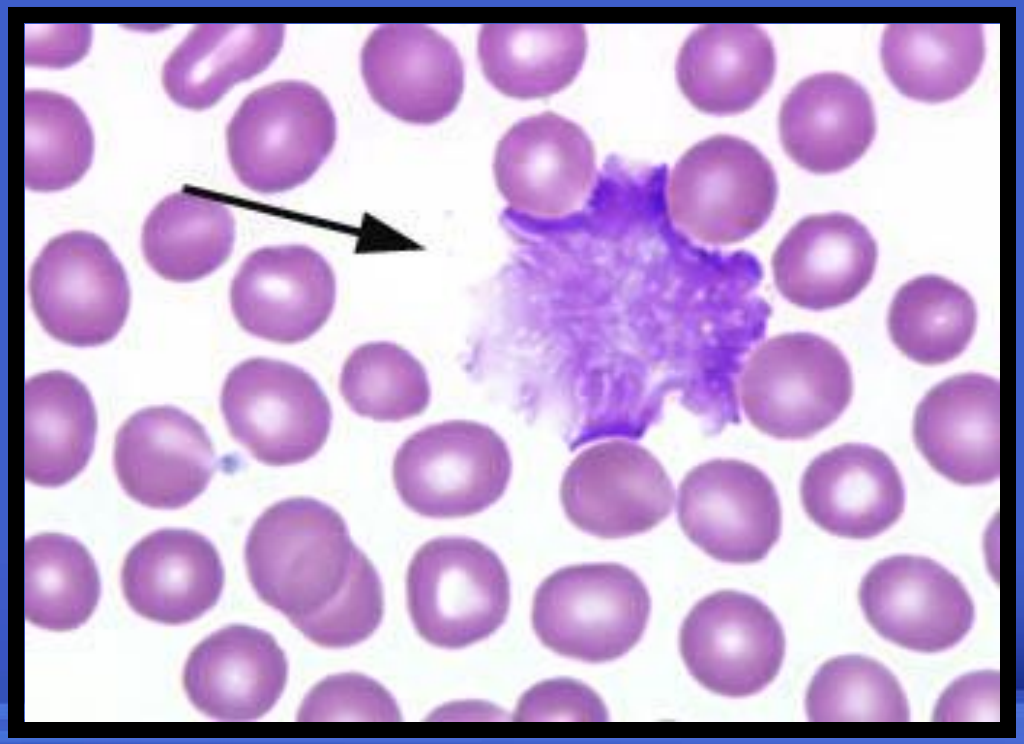


- Causes artificially low platelet counts
- 2^{o} EDTA used in collection tubes

Solution is to use sodium citrate instead of EDTA

Quiz!

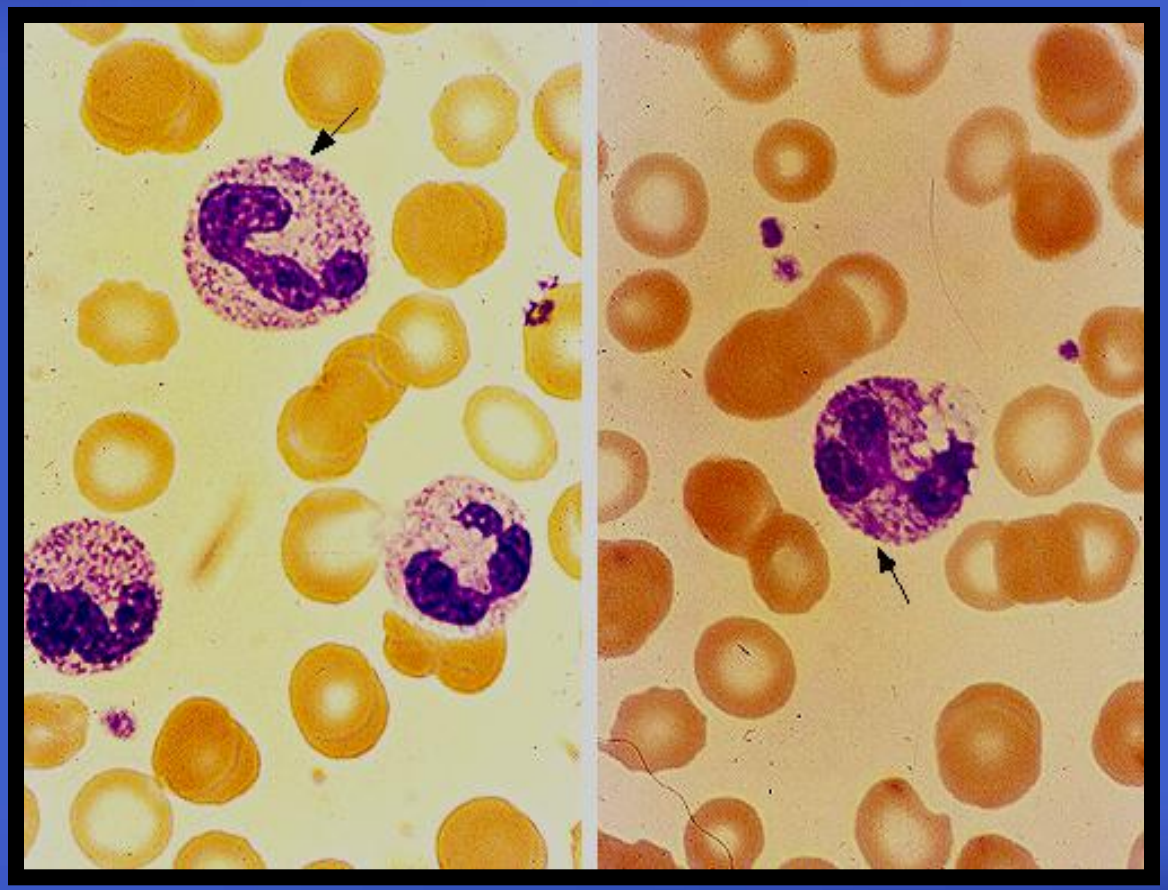
- 75 yo asymptomatic man with WBC 60,000



Diagnosis?

- CLL (chronic lymphocytic leukemia)

- 35 yo man with WBC 33,000 and thrombocytopenia



Diagnosis

- Reactive neutrophilia, sepsis

Conclusion

- Systematic approach to reviewing a peripheral blood smear
- All cell lines are evaluated
- MUST take into account the clinical history
- Integrate the information to make a differential diagnosis
- Most peripheral smears are non-specific and have a constellation of findings

White Blood Cell Morphologic Abnormalities Related to Hereditary Disorders

(Part 2)

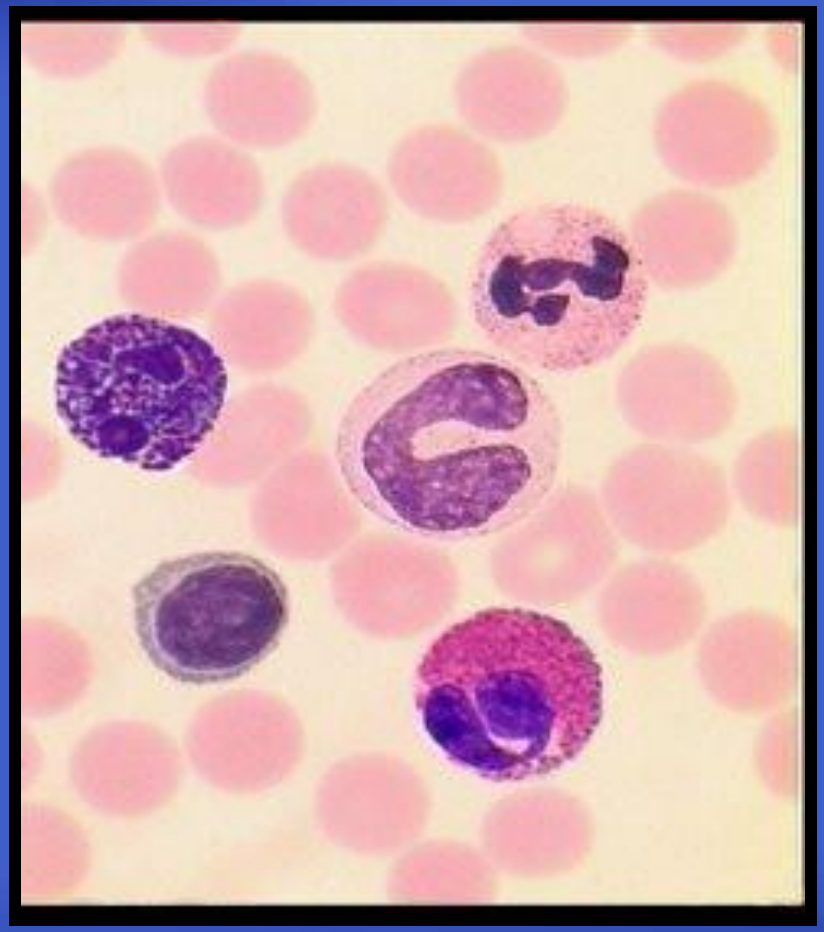
CP Talk

Jacqueline Nguyen, DO

April 2, 2007 8am

White Blood Cells

- Qualitative abnormalities
  - Hereditary disorders
    - Morphologically abnormal
      - Neutrophil inclusions
      - Abnormal neutrophil nuclei
      - Macrophage/histiocytic abnormalities



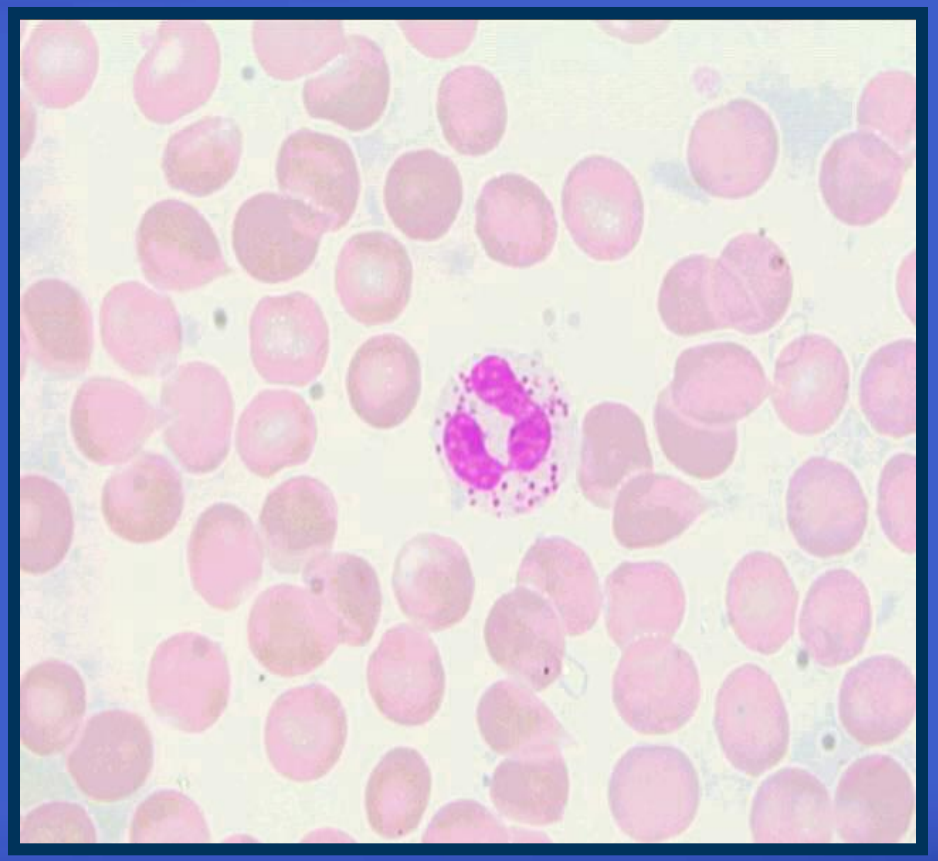
http://www.mhhe.com/biosci/ap/histology_mh/wbccomp.jpg

Neutrophil Inclusions

- Neutrophil inclusions-inherited
  - Alder Reilley anomaly
  - May Hegglin anomaly
  - Chediak-Higashi Syndrome

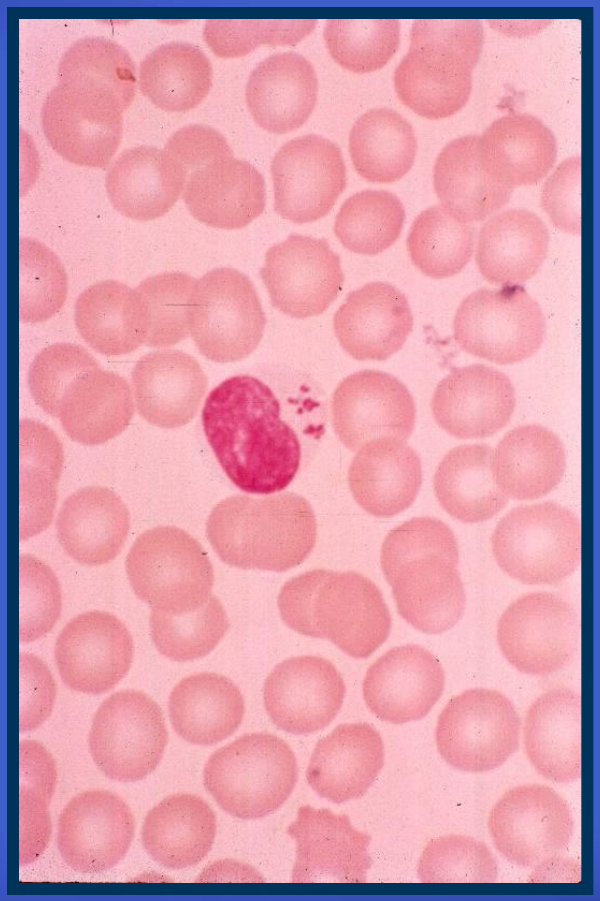
Alder Reilley Anomaly

- Resemble the large primary granules of promyelocytes
- Large, purple to purplish-black, coarse azurophilic granules
- No impaired PMN function
- Inclusion is a mucopolysaccharide (PAS+)



Alder Reilley Anomaly

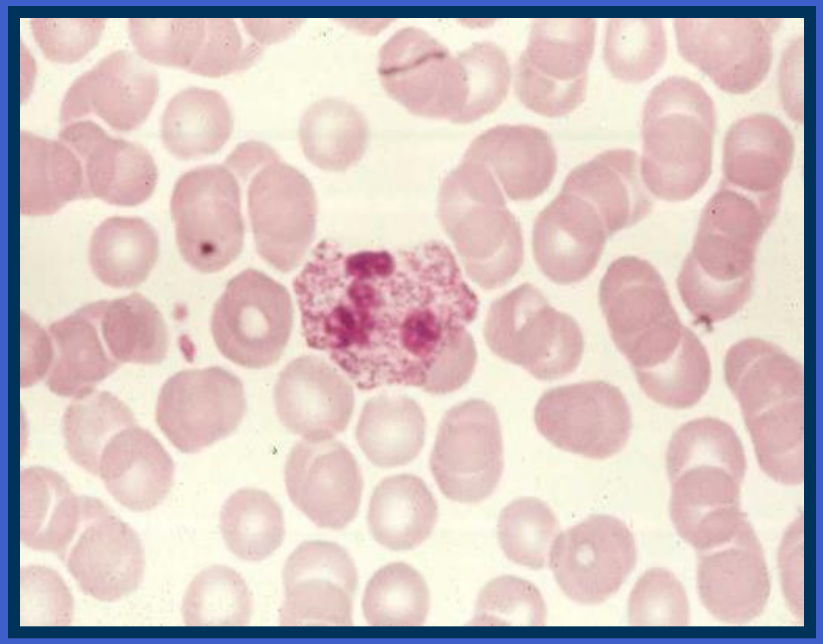
- Vacuolated/abnormally granulated lymphocytes in some case
- Eosinophils and basophils contain large basophilic granules



www.academic.marist.edu/.../HematologyI/7-24.jpg

Alder Reilley Anomaly

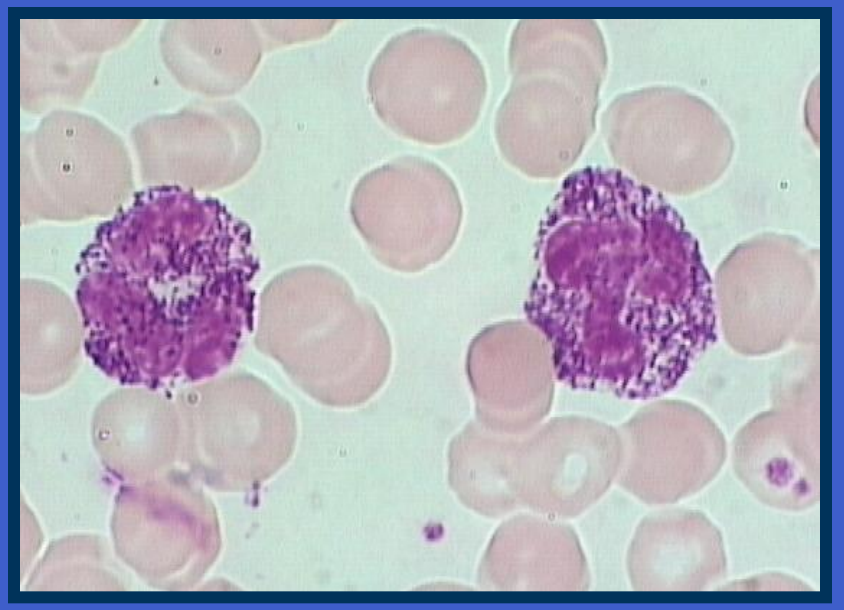
- Autosomal recessive
- Associated with several different types of genetic mucopolysaccharide disorders (Hurler, Hunter, San Fillipo, Maroteaux-Lamy, but not Moriquo)



www.academic.marist.edu/.../tn_bloodsmears28.jpg

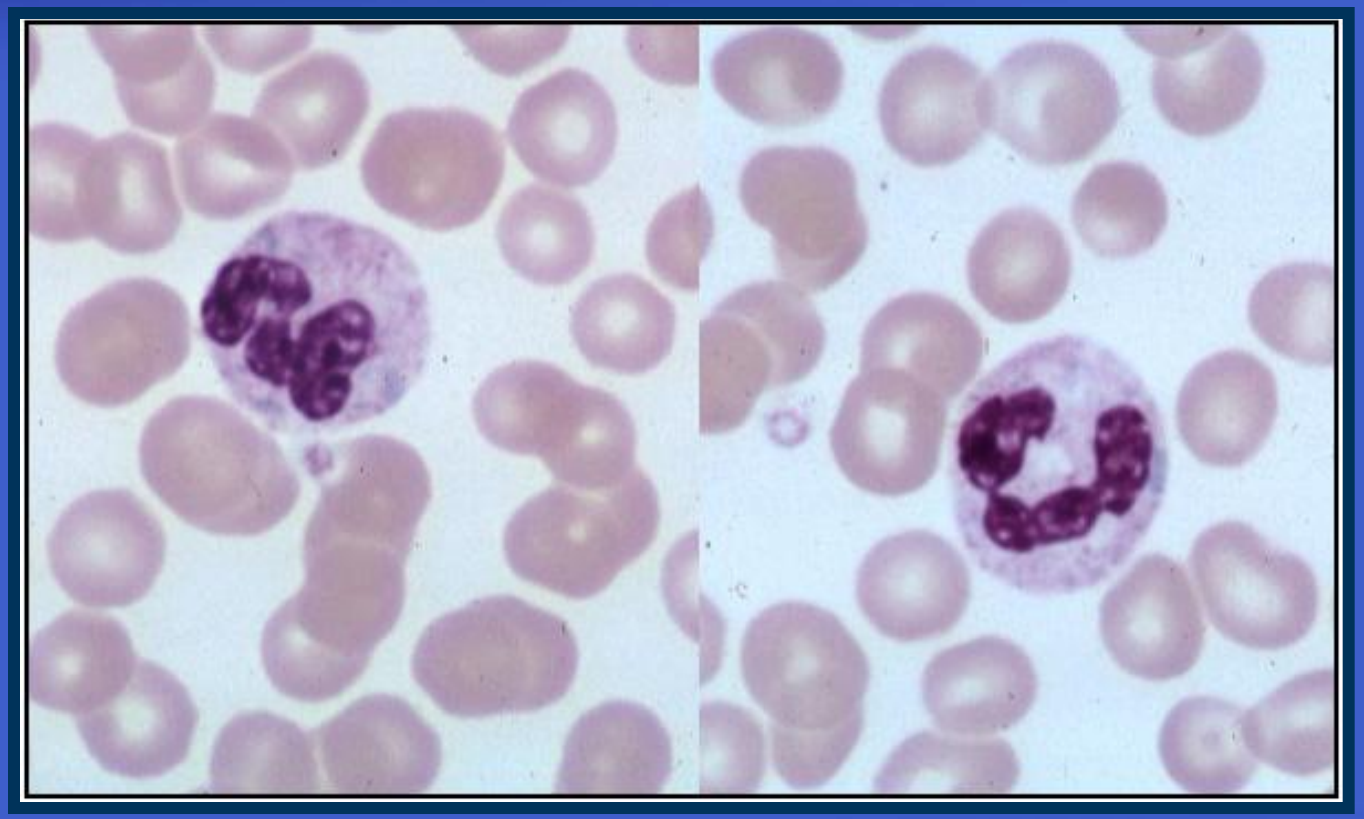
Alder Reilley Anomaly

- Not specific for one of the mucopolysaccharidoses
- First discovered in Hurler's syndrome
- May be seen following bone marrow transplants and chemotherapy



www.med-ed.virginia.edu/.../wcd/qualitative.cfm

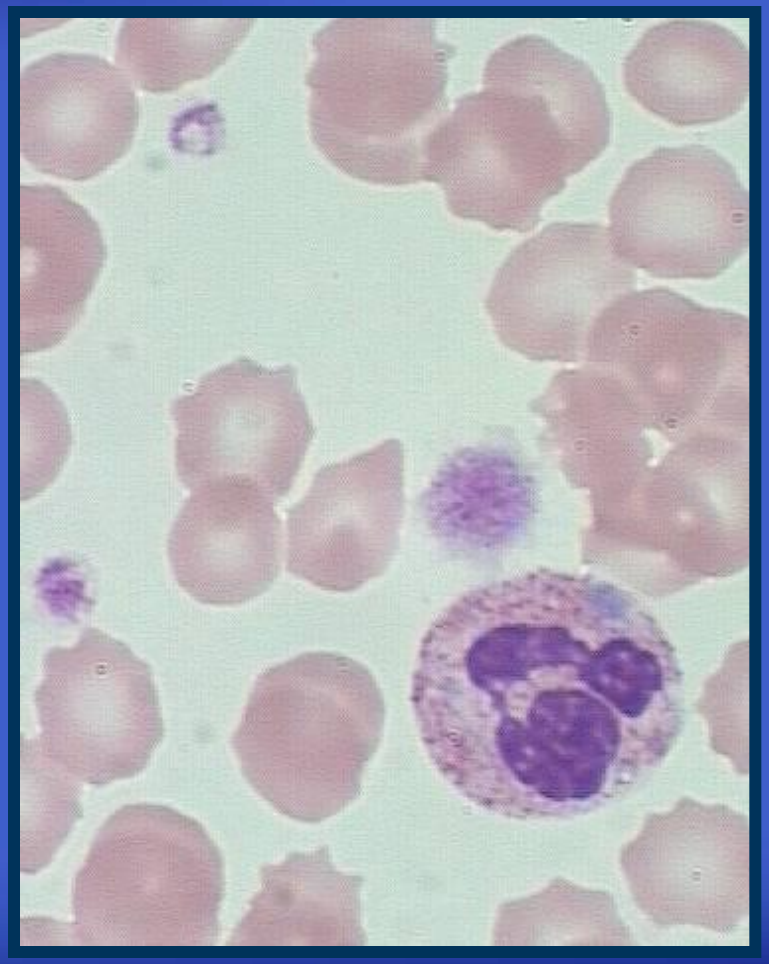
May Hegglin Anomaly



hsc.unm.edu/Pathology/MedLab/images/mhegglin.jpg

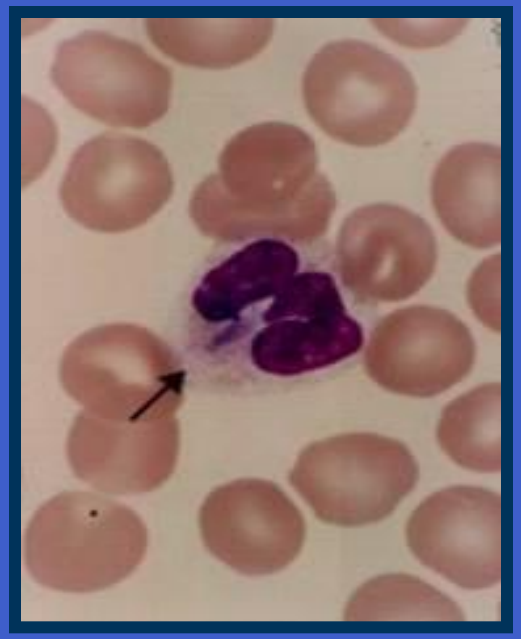
May Hegglin Anomaly

- Thrombocytopenia
- Enlarged platelets
- Variable neutropenia
- Inclusions also seen in eosinophils, basophils, and monocytes



May Hegglin Anomaly

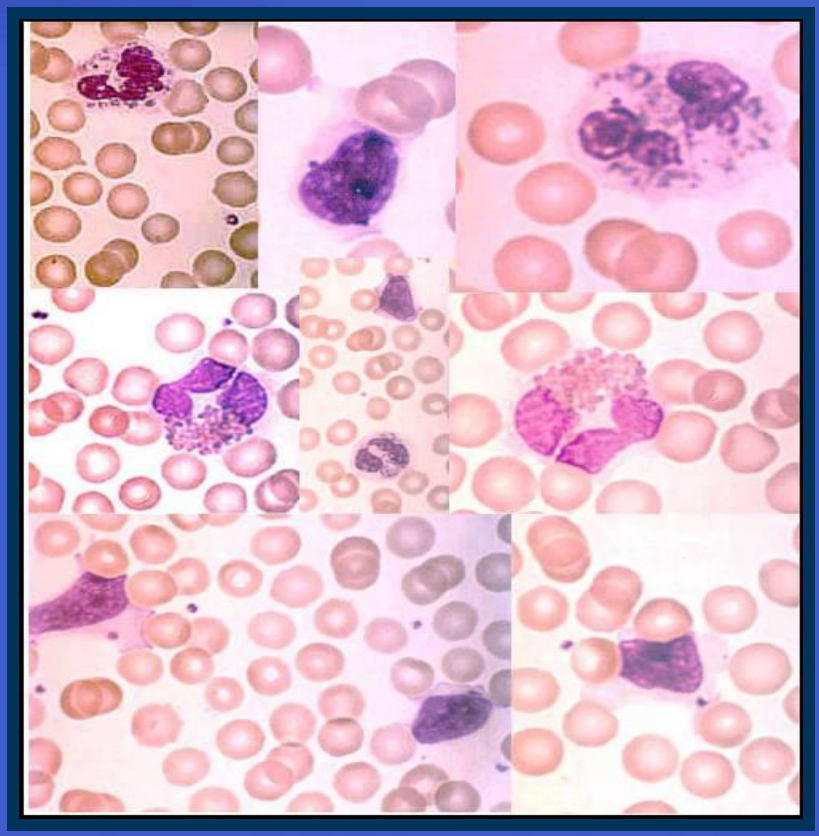
- Autosomal dominant
- Many patients are asymptomatic
- Non-muscle myosin heavy chain A (MYH9) mutation
- No impairment on PMN function



www.bekkoame.ne.jp/.../WBC/photo/MayHeggrin3.jpg

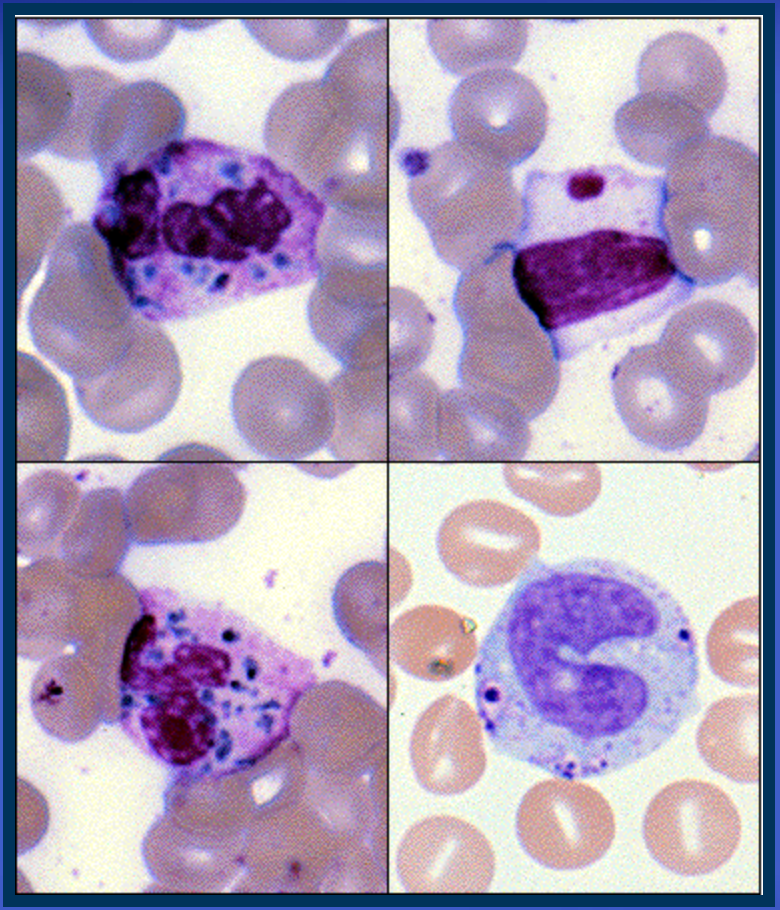
Chédiak-Higashi Syndrome

- Large, well-defined, round to irregular, blue to green-gray cytoplasmic granules (MPO+)
- All granulated cells and even lymphocytes/natural killer cells affected



hsc.unm.edu/pathology/MedLab/images/chediak.jpg

Chédiak-Higashi Syndrome

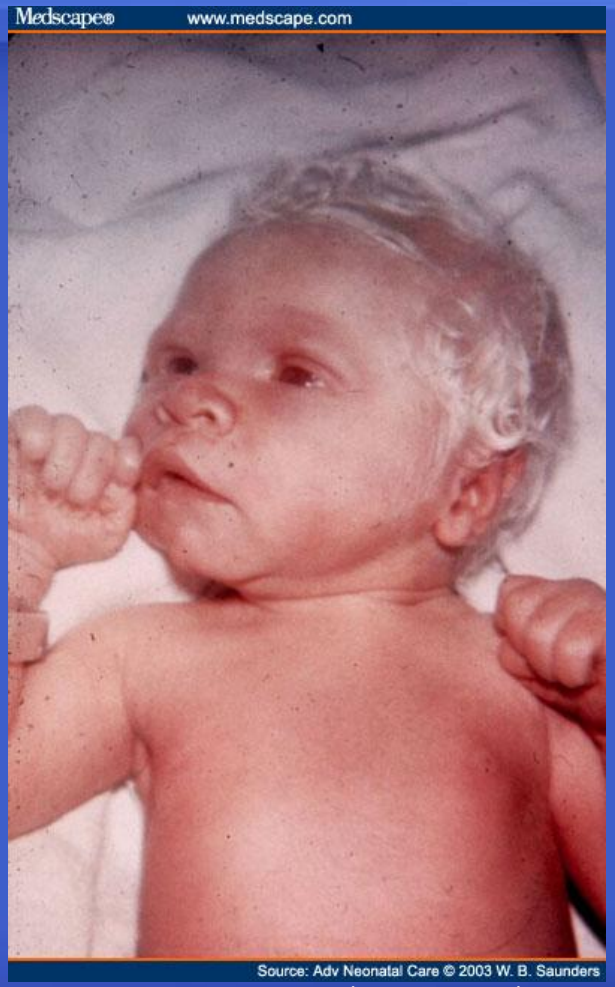


- Autosomal recessive
- Cytopenias
- Platelet and NK-cell dysfunction

www.pathology.ucla.edu/.../case6/image6.gif

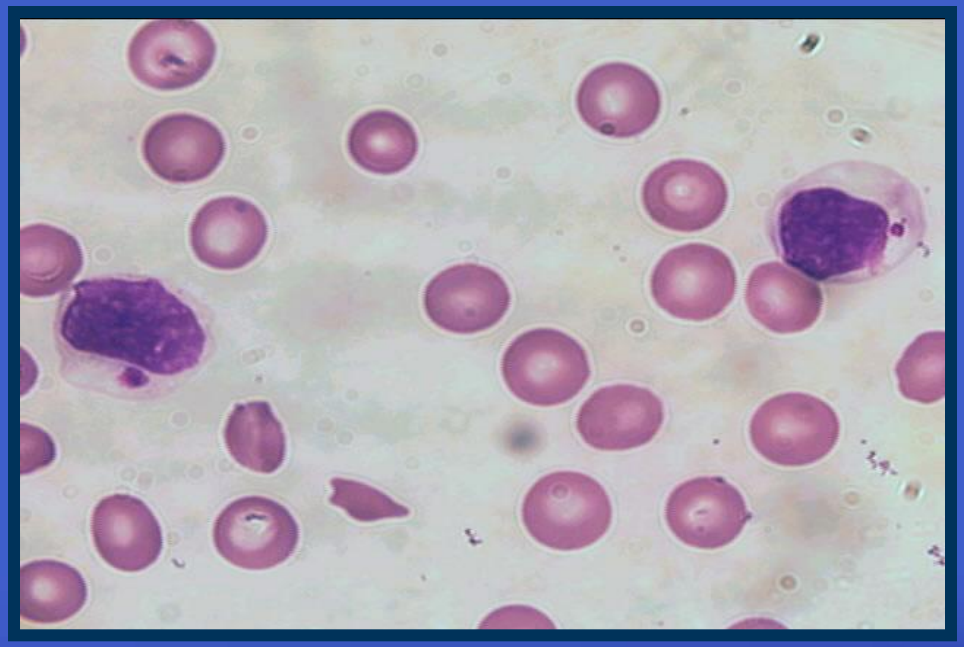
Chédiak-Higashi Syndrome

- Affects many granule-containing cells
  - Melanosomes-partial occulocutaneous albinism
  - Neurons- neurological abnormalities
  - Impaired PMN function-severe, recurrent pyogenic infections (decrease killing and chemotaxis)



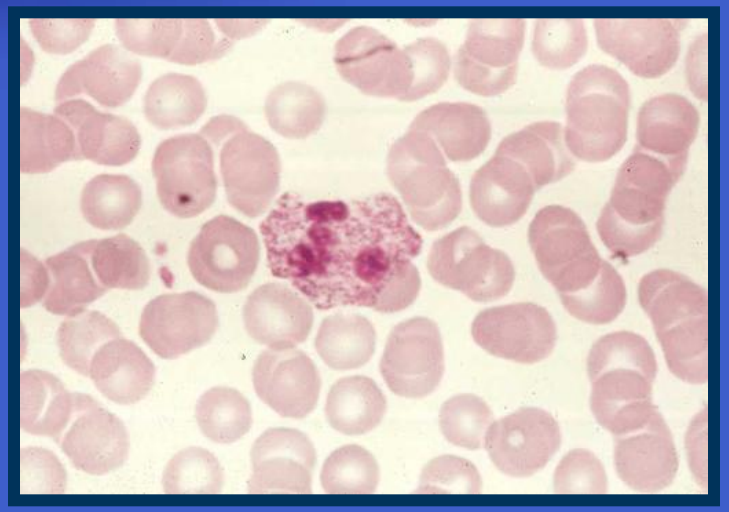
http://www.medscape.com/content/2003/00/46/65/466530/art-adnc466530.fig8.jpg

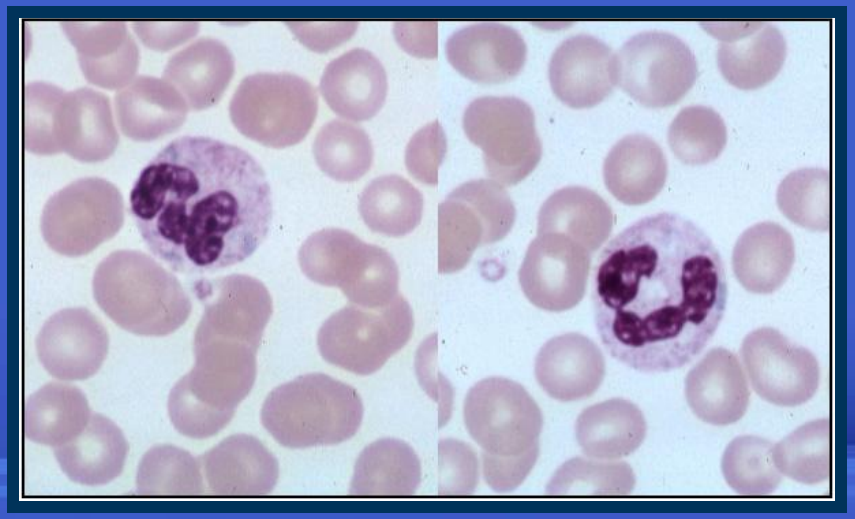
Chédiak-Higashi Syndrome

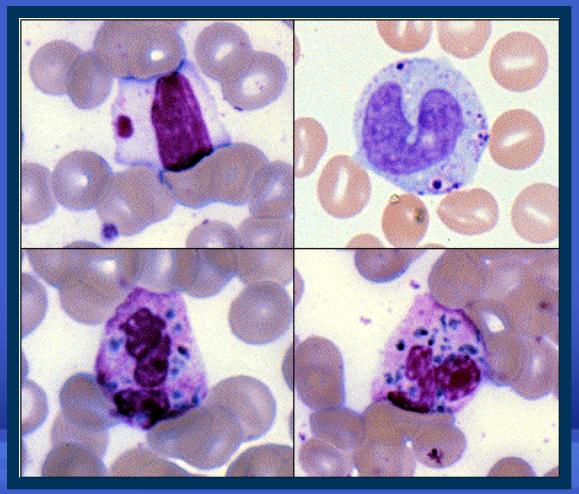


Quiz

- Match the picture:
  - May Hegglin?
  - Chediak-Higashi?
  - Alder Reilley?





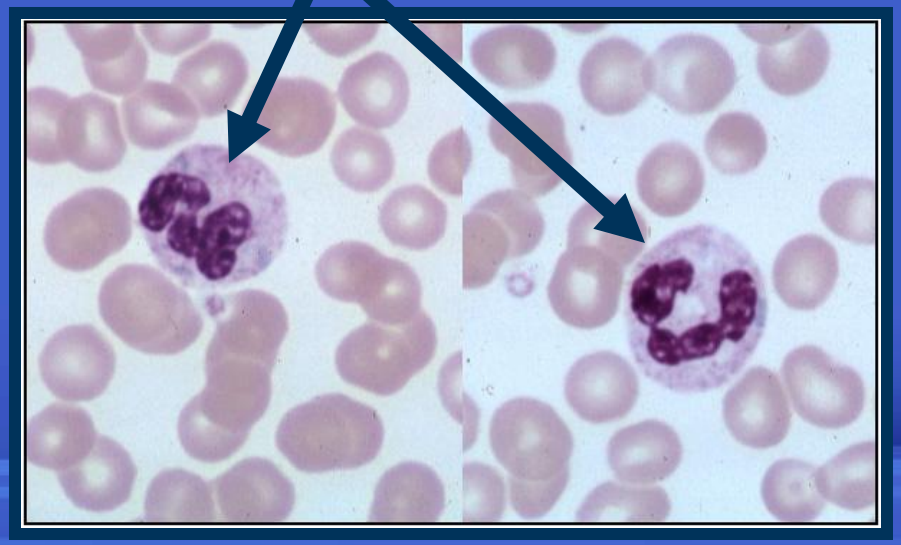


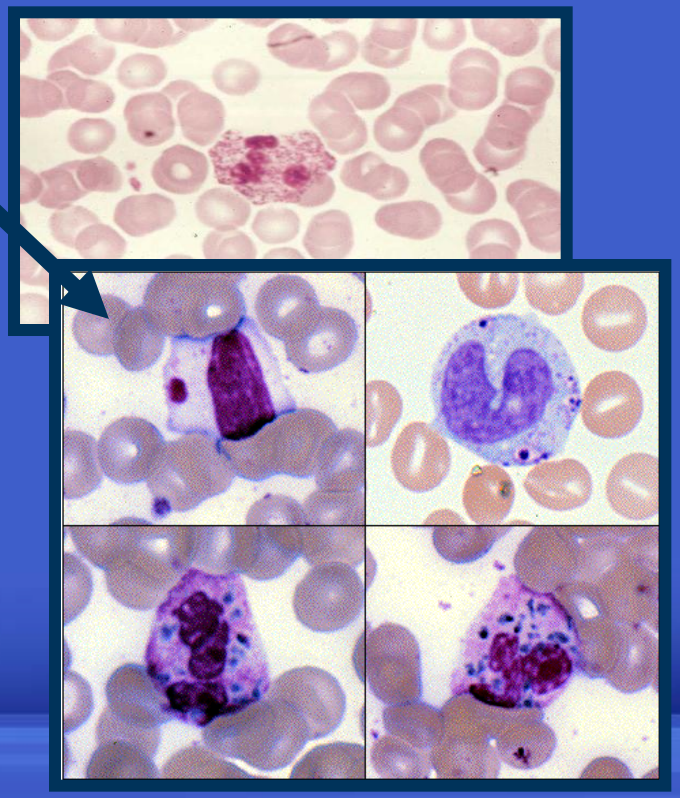
Answer

Alder Reilley

Chediak-Higashi

May Hegglin



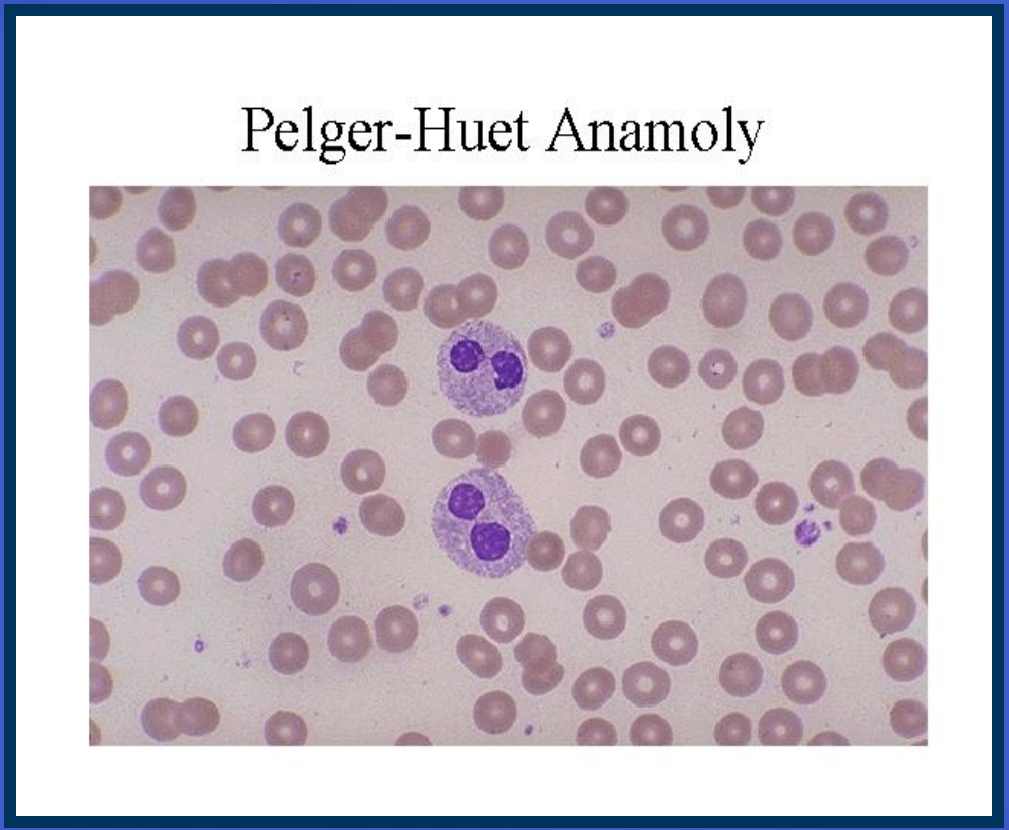


Abnormal Neutrophil Nuclei

- Pelger-Huët anomaly
- Hereditary hypersegmentation of neutrophils

Pelger-Huët Anomaly

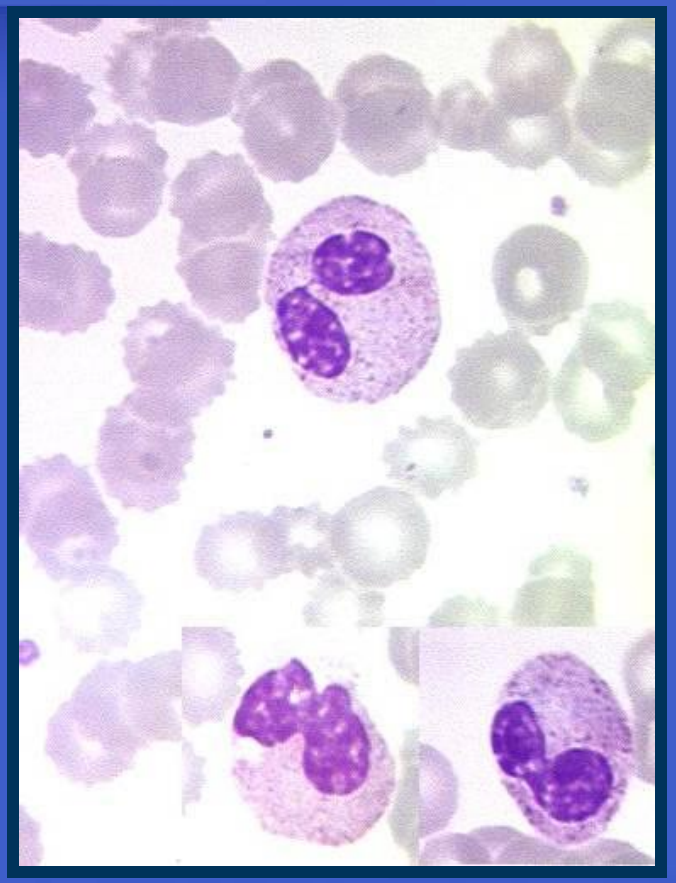
- Bilobed (pince nez) or non-segmented neutrophil nuclei seen in most PMNs
- Coarse clumping of the nuclear chromatin in neutrophils, lymphocytes, and monocytes



http://www.med.unc.edu/medicine/web/Smearreview/img034.jpg

Pelger-Huët Anomaly

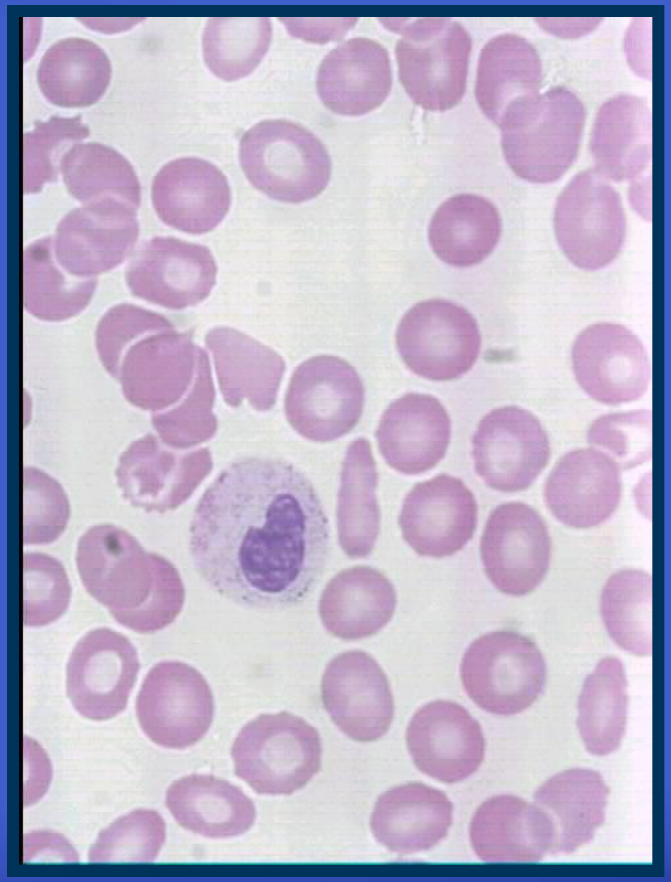
- Autosomal dominant
- No other lineage abnormalities
- No functional abnormalities
- Mutations in lamin ß-receptor (LBR gene on Chrom 1)



http://www.bphealthcare.com/healthcare/galleries/haem/case5-a1.jpg

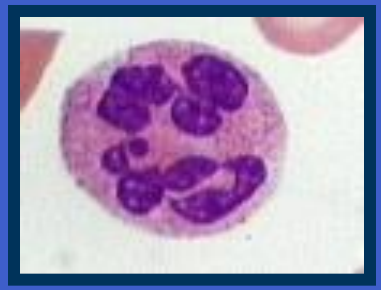
Pelger-Huët Anomaly

- Heterozygous in good health, and their natural resistance to infection is unimpaired
- Homozygous PHA is associated with skeletal anomalies, developmental delay, and seizures



Hereditary Hypersegmentation

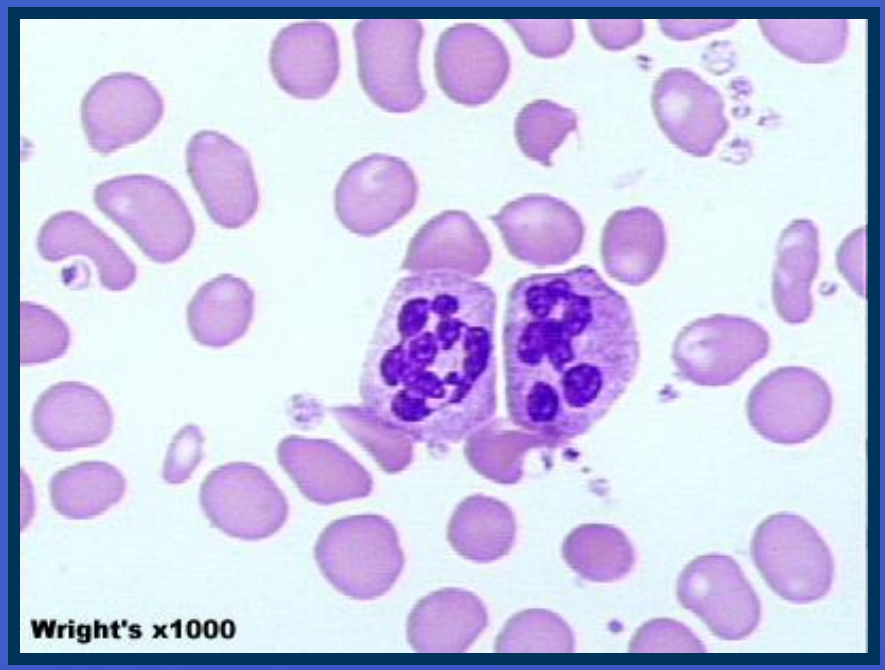
- AKA Undritz anomaly
- More than 3 cells having 5 lobes or a single cell with 6 lobes found in the course of a 100 cell differential (or 5% with 5 lobes)



http://www.med-ed.virginia.edu/courses/path/innes/images/wcdjpeg/wcd%20hyperseg.jpeg

Hereditary Hypersegmentation

- Autosomal dominant
- No other abnormalities
- No associated findings

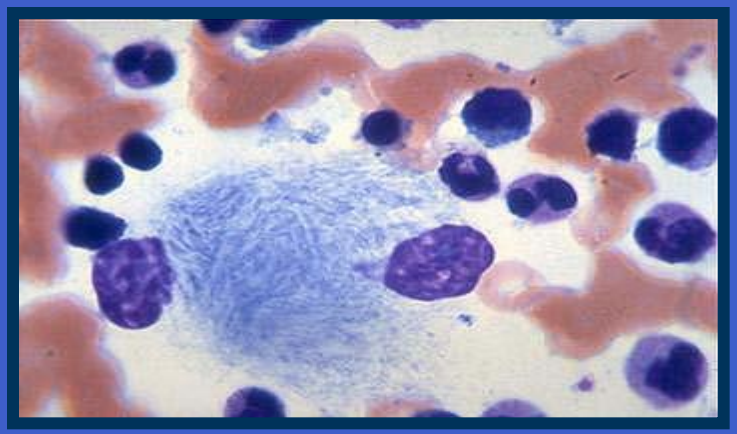


Histiocyte/Macrophage Abnormalities (Bone Marrow Cells)

- Inherited abnormalities
  - Gaucher cell
  - Niemann Pick cell

Gaucher Cell

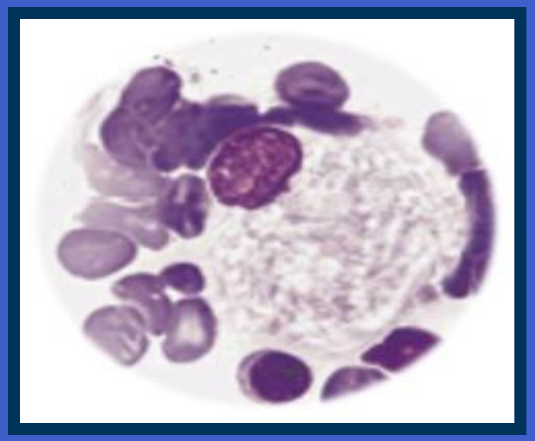
- Crumpled tissue-paper cytoplasm
- Caused by enlarged, elongated lysosomes filled with glucocerebroside
- Accumulation in BM, liver, spleen, and lungs leads to pancytopenia, hepatosplenomegaly, and pulmonary disease
- Infiltration in BM causes thinning of the cortex, pathologic fractures, bone pain, bony infarcts, and osteopenia



http://pathcuric1.swmed.edu/PathDemo/gen1/gen130.jpg

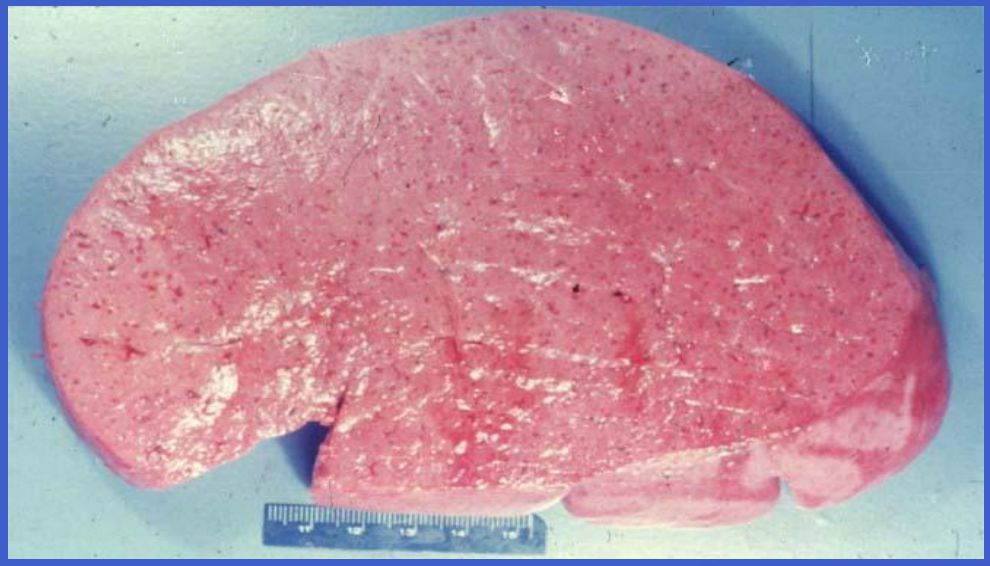
Gaucher Cell

- Seen in
  - Gaucher's disease
  - Myeloproliferative syndromes (CML)= "pseudo-Gaucher" cells



http://www.sfu.ca/biology/faculty/kermode/laboratory/gaucher-cells.jpg

Gaucher Disease

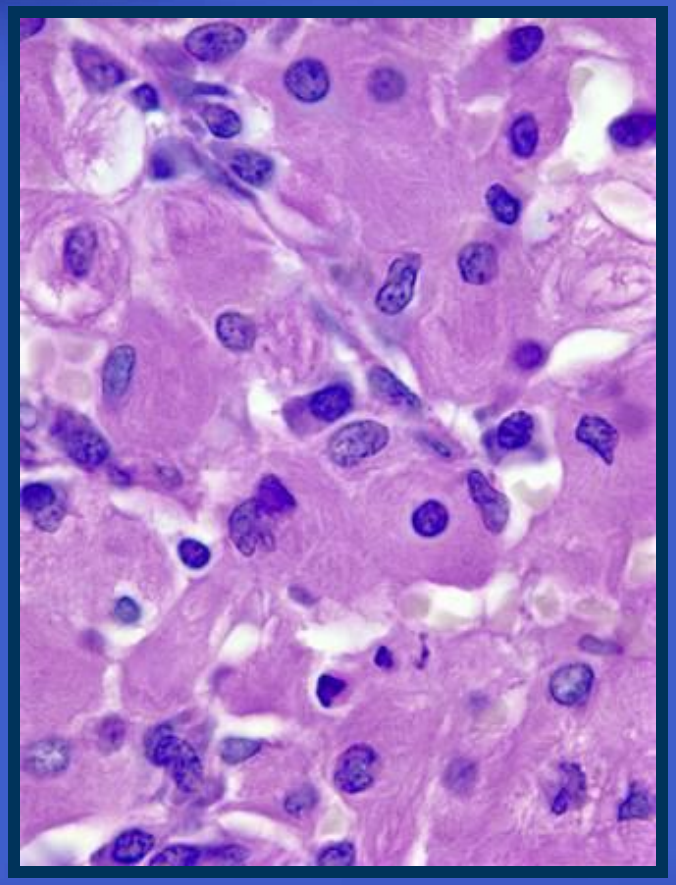


The enlarged spleen shows effacement of white pulp and massive expansion of pale red pulp.

pathology.catholic.ac.kr/.../genetic/ge13.jpg

Gaucher Cell

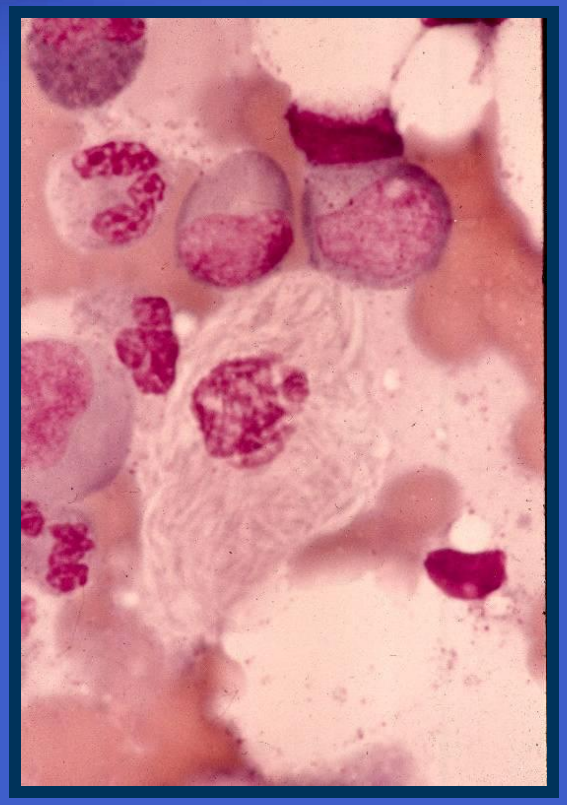
- Acid phosphatase +
- PAS+
- Labs
  - ↓ in WBC acid ß-glucosidase
  - ↑ in serum acid phosphaatase
  - ↑ in serum ACE



http://www.neuropathologyweb.org/chapter10/images10/10-GCl.jpg

Gaucher Cell

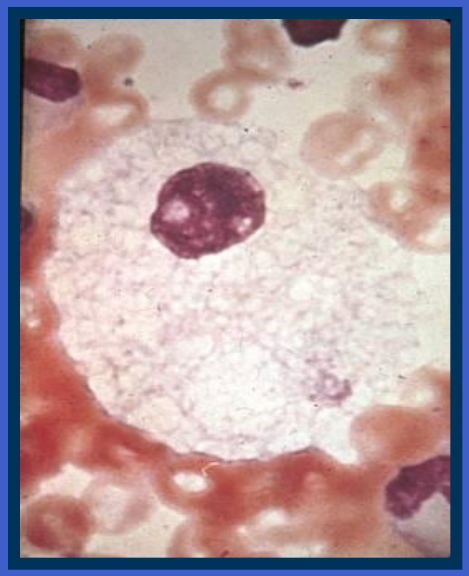
- Enzyme replacement therapy now available (imiglucerase [Cerezyme])



http://www.academic.marist.edu/~jzmz/HematologyI/MicroexamBM25.jpg

Niemann Pick Cell

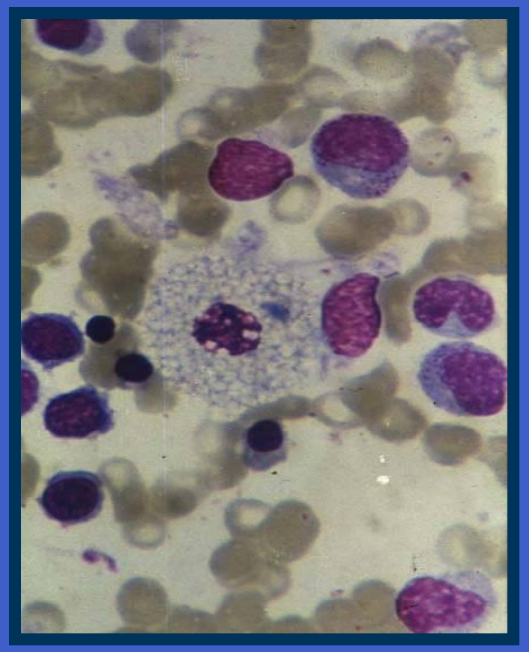
- Foamy, vacuolated cytoplasm
- Accumulation of sphingomyelin
- Seen in Niemann-Pick disease (sphingomyelinase deficiency), Wolman disease, cholesterol ester storage disease, lipoprotein lipase deficiency, and, GM1 gangliosidosis type 2



http://www.thecrookstoncollection.com/Collection/medslides/Slides/Niemann-pick-cell.jpg

Niemann Pick Cell

- Weakly PAS+
- Birefringence on polarized light
- Yellow-green on UV



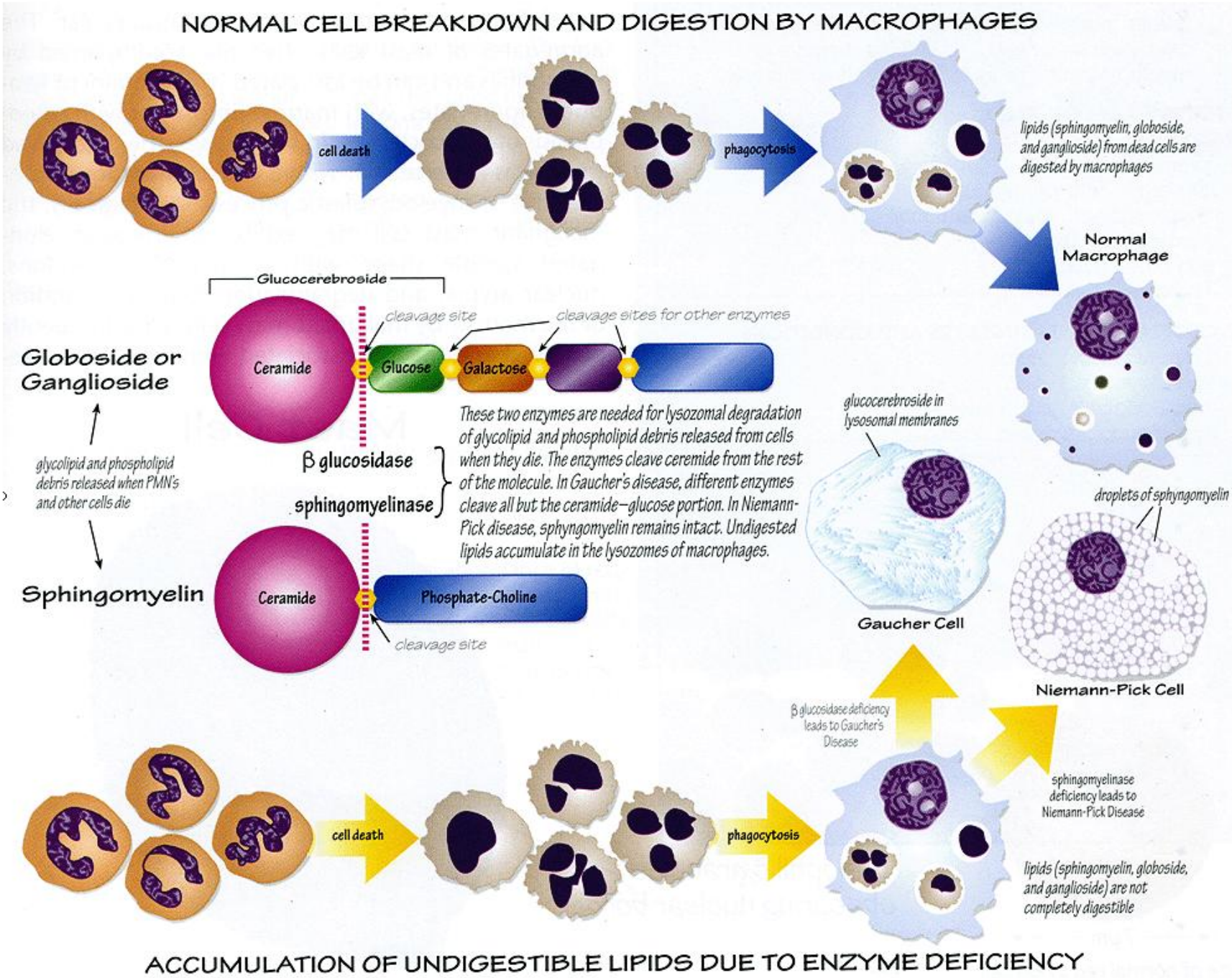
http://pathology.catholic.ac.kr/pathology/specimen/genetic/ge13.jpg

Niemann Pick Disease

- Systemic involvement
  - Progressive lung disease
  - Hepatosplenomegaly
  - Short stature
  - Pancytopenia
- Lab findings
  - ↓ WBC sphingomyelinase
  - May see vacuolation of PB lymphocytes and monocytes

Niemann Pick Disease

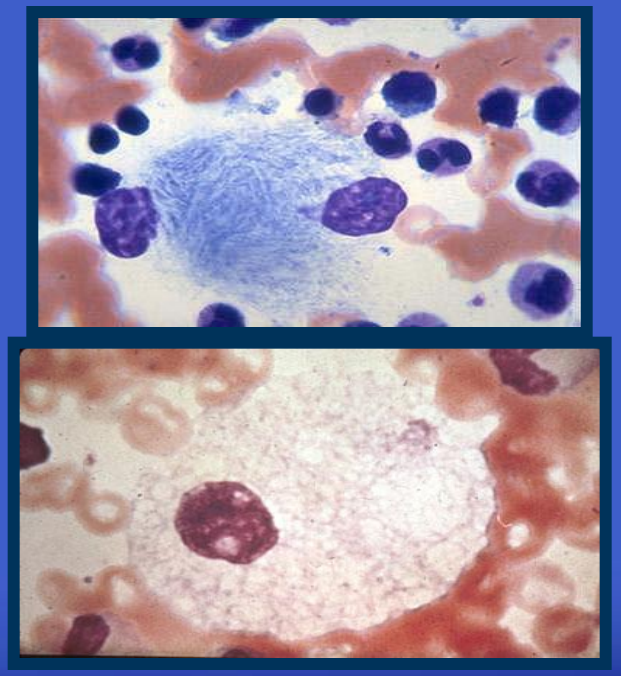
- No specific treatment available
- Generally more rapid clinical course than Gaucher's disease



Color Atlas of Hematology 1998: 327.

Quiz

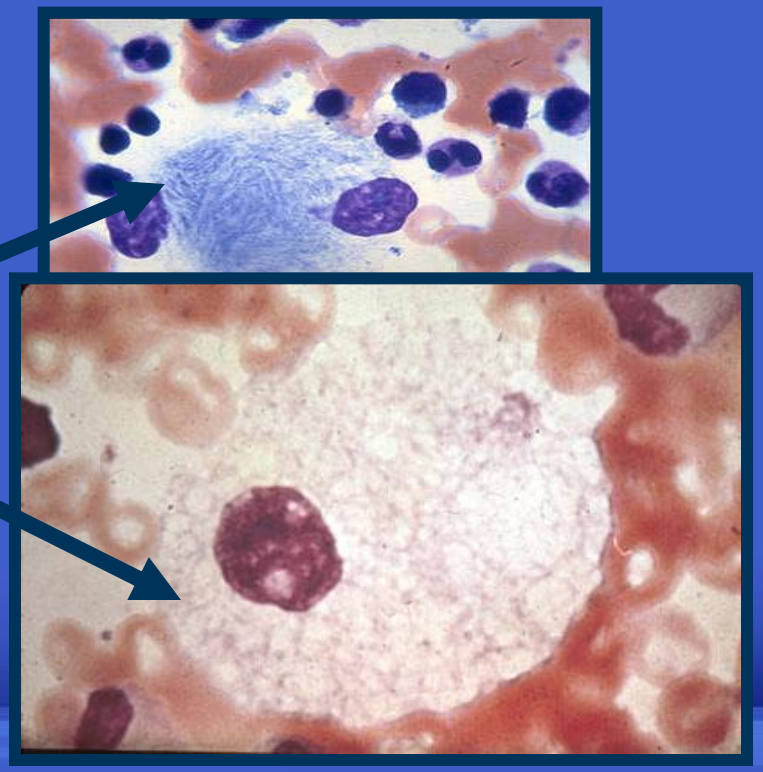
- Name that cell
  - Gaucher cell?
  - Niemann Pick cell?



Answer:

Gaucher cell

Niemann Pick cell



References

- Kjeldsberg, Practical Diagnosis of Hematologic Disorders
- Osler Notes
- Internet