Overview of Anemia and Diagnostic Hematology

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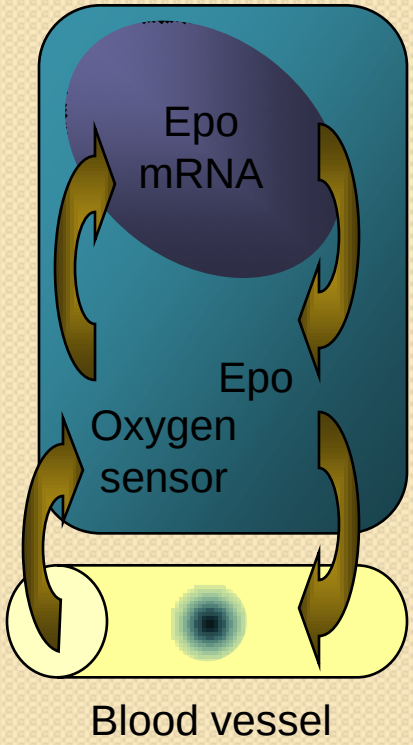
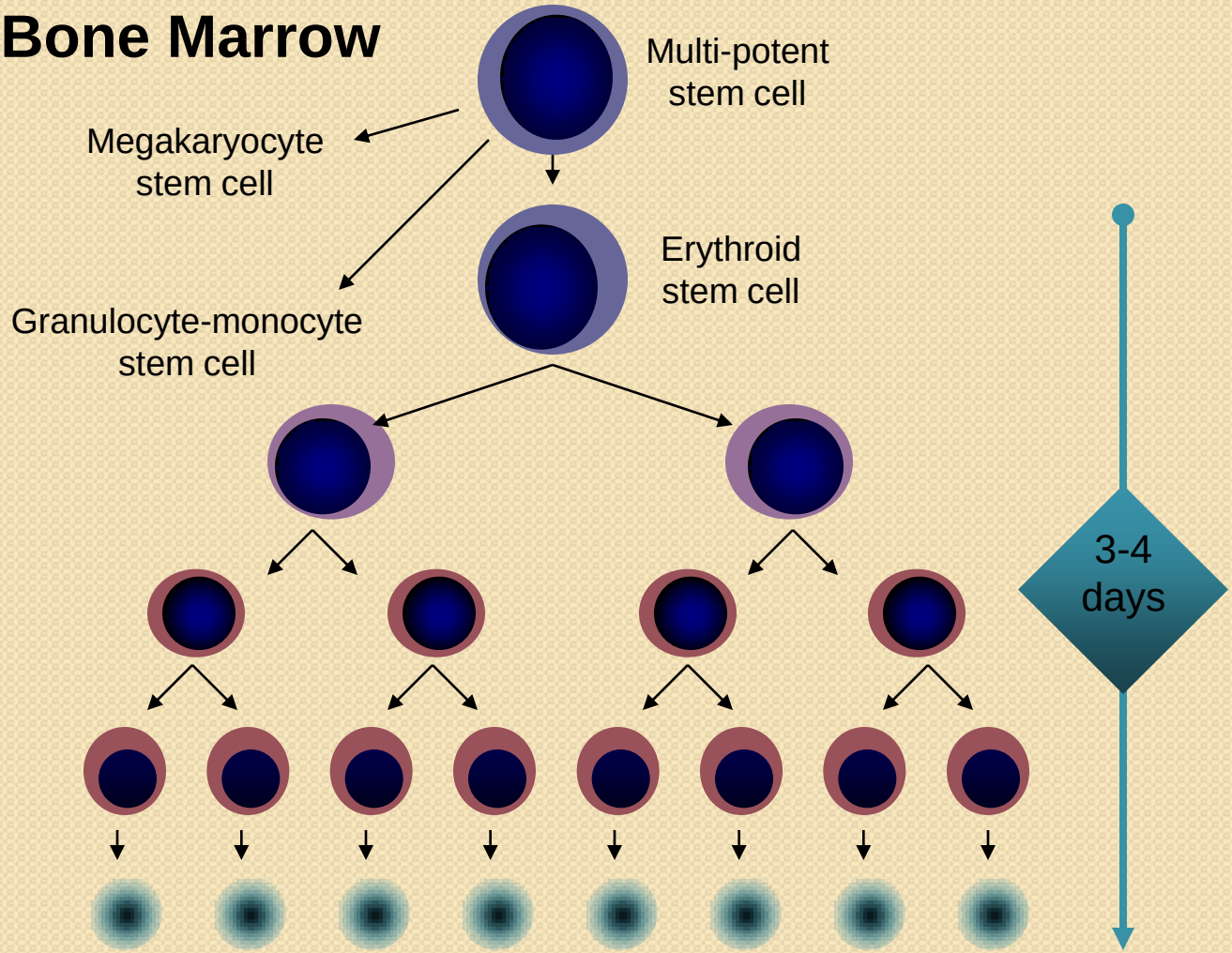
Regulation of Erythropoiesis

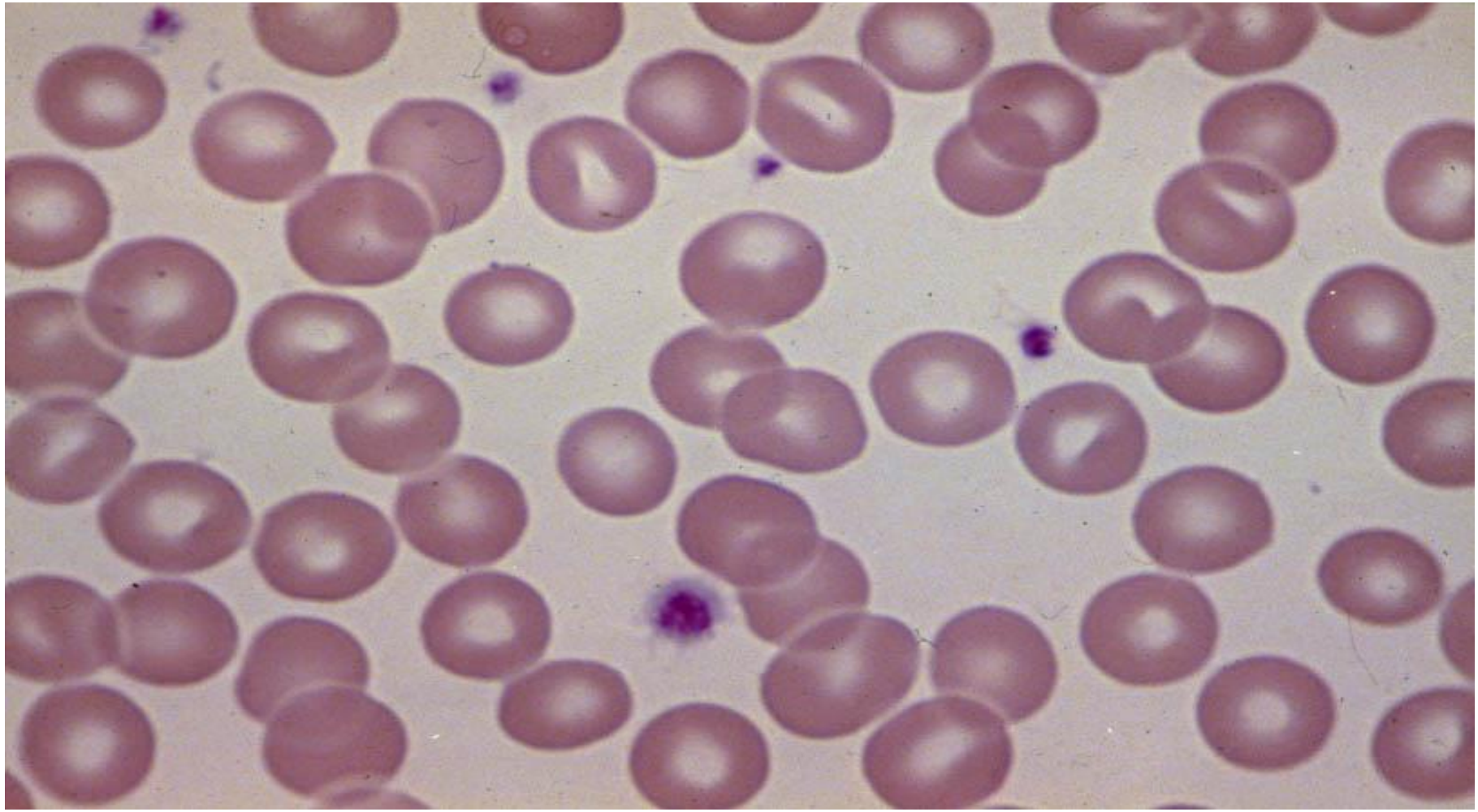
Kidney



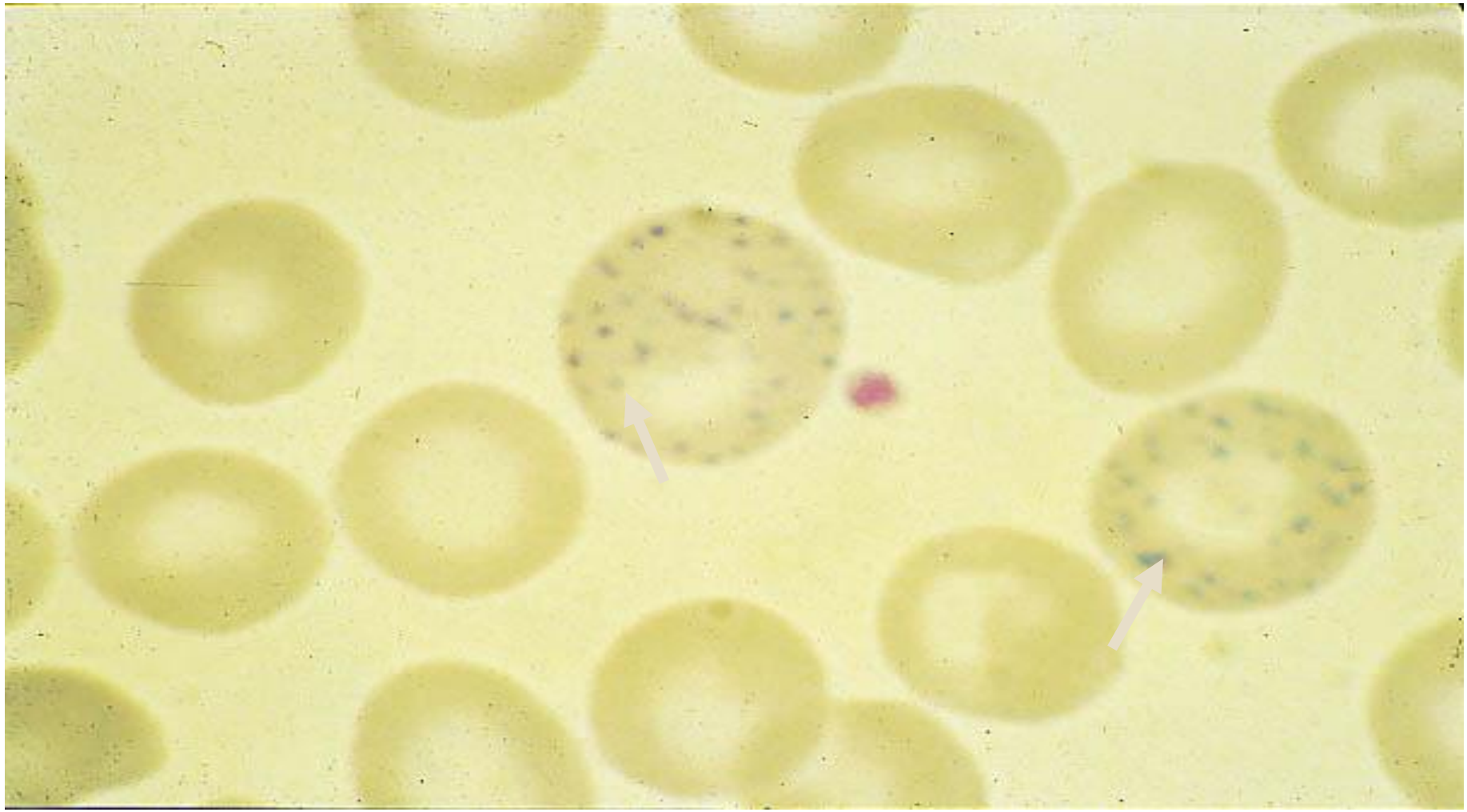
Bone Marrow



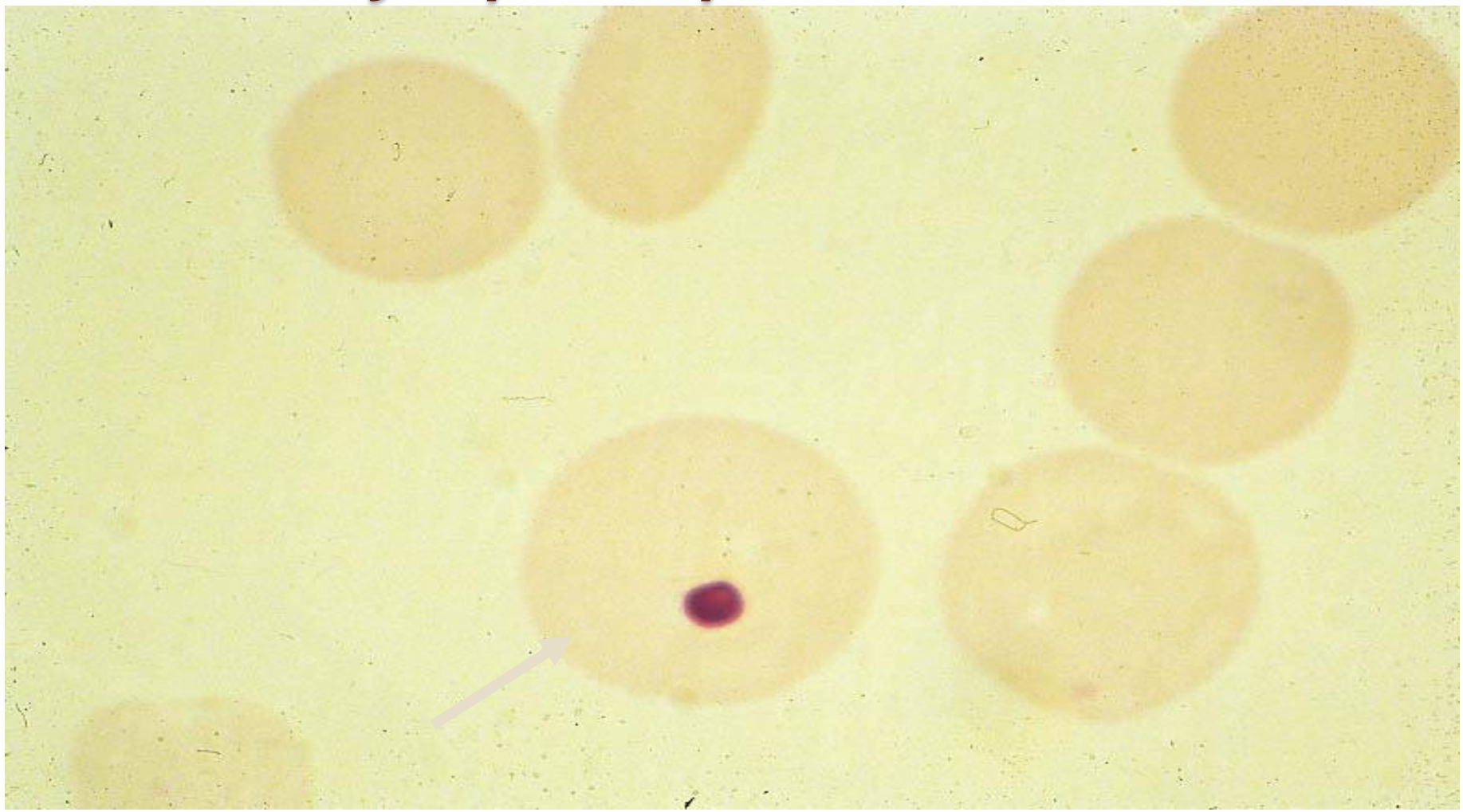
Normal Peripheral Smear



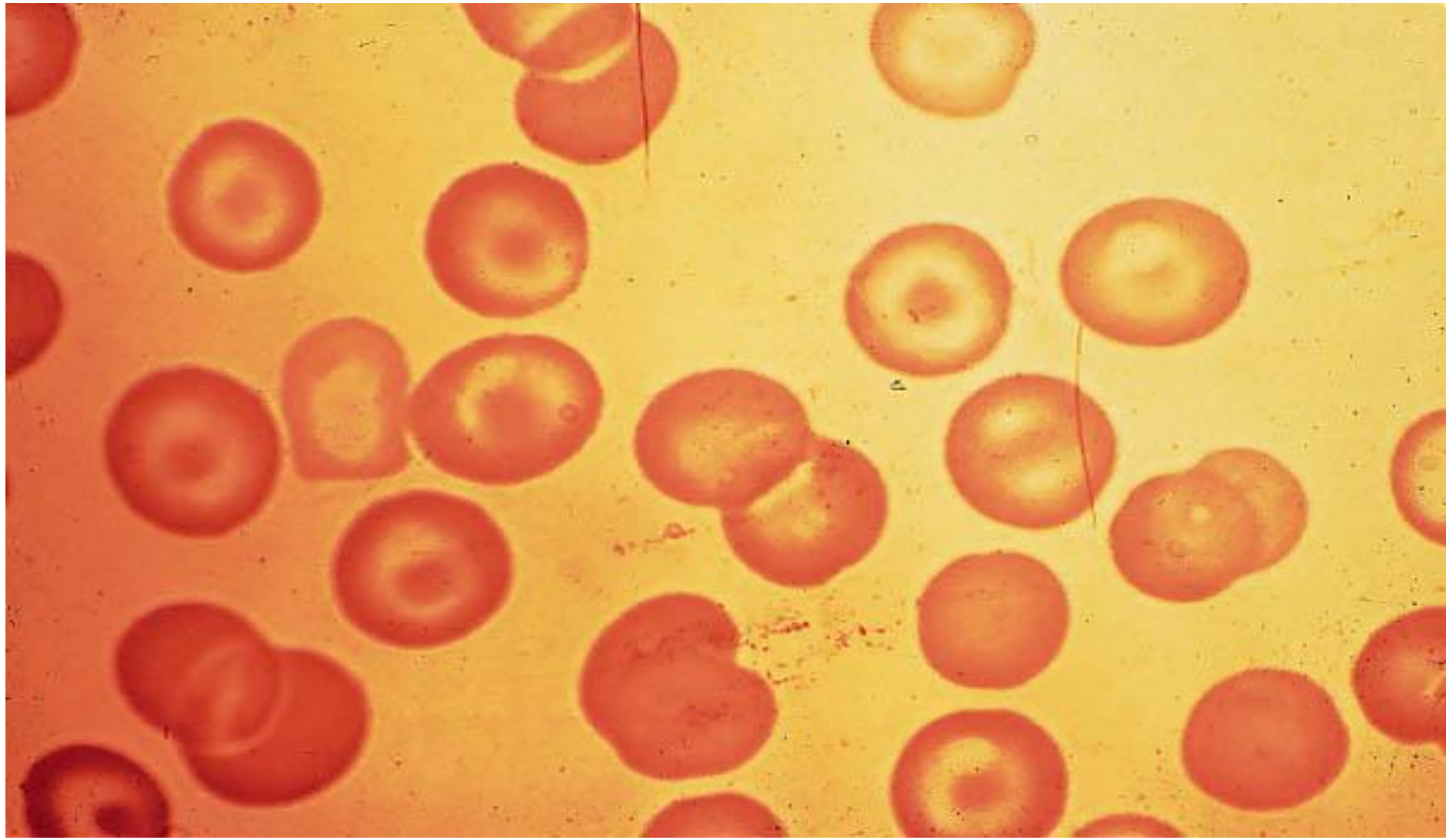
Basophilic Stippling



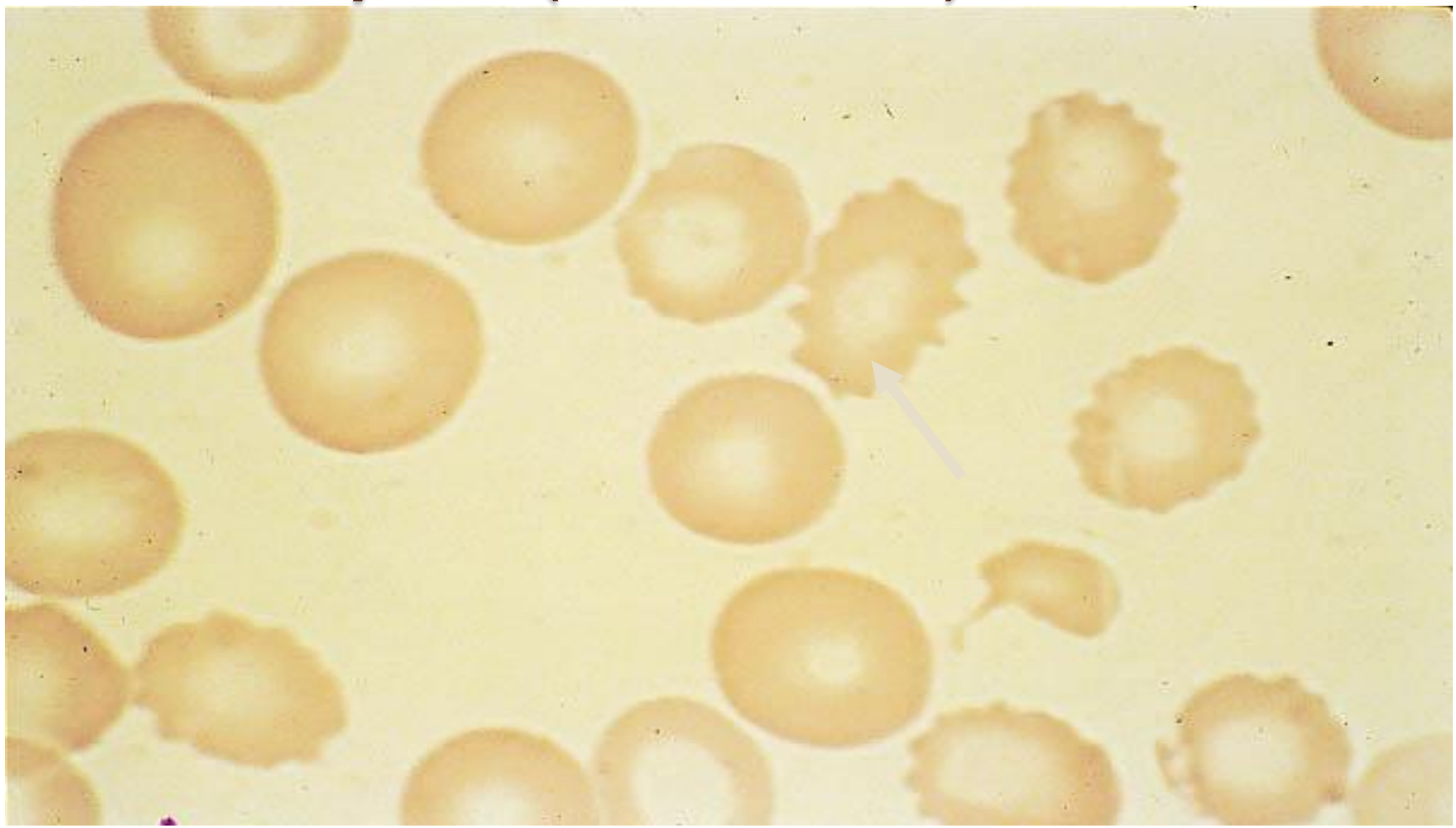
Howell-Jolly Body



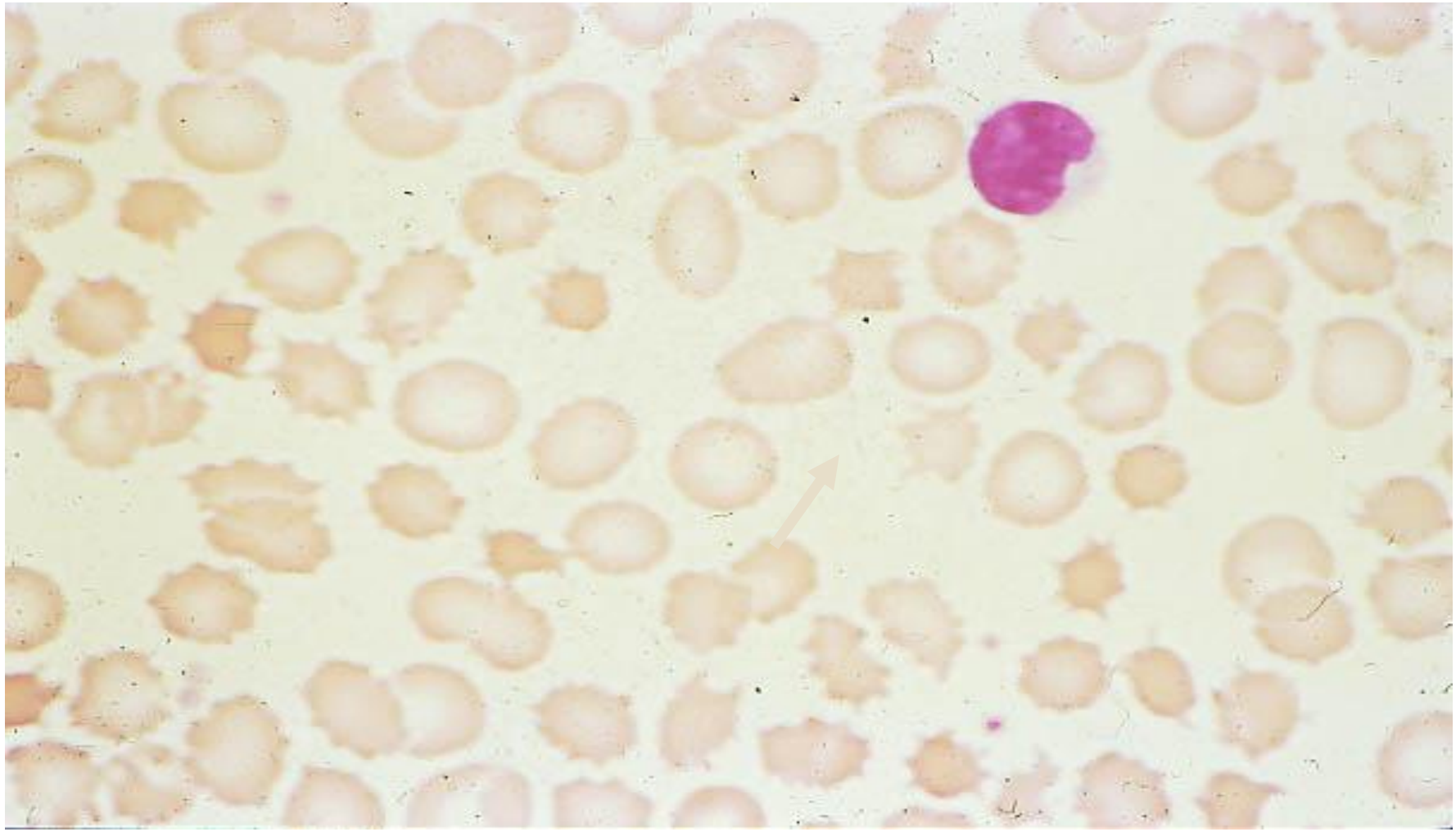
Target Cells



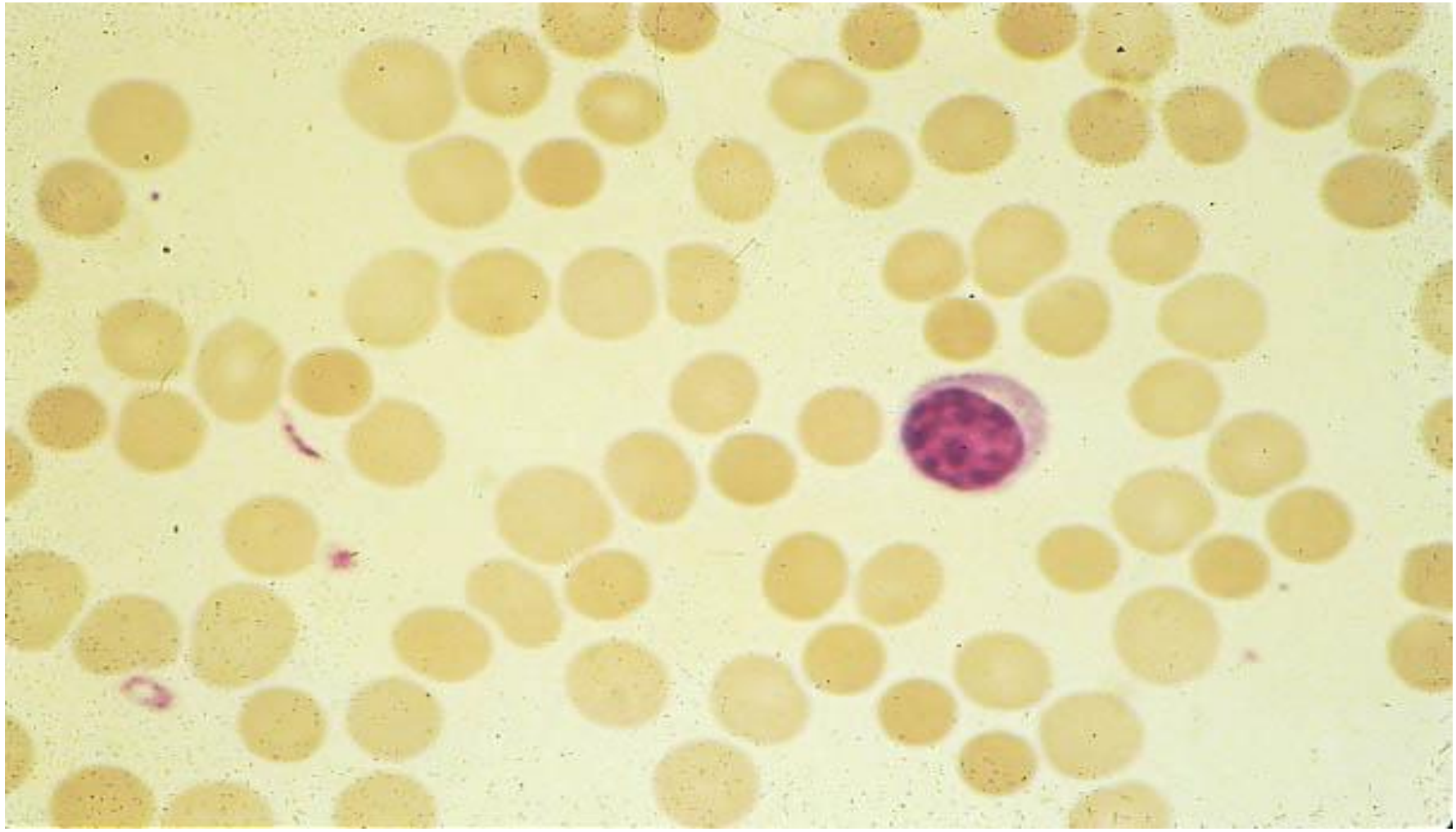
Echinocytes (Burr Cells)



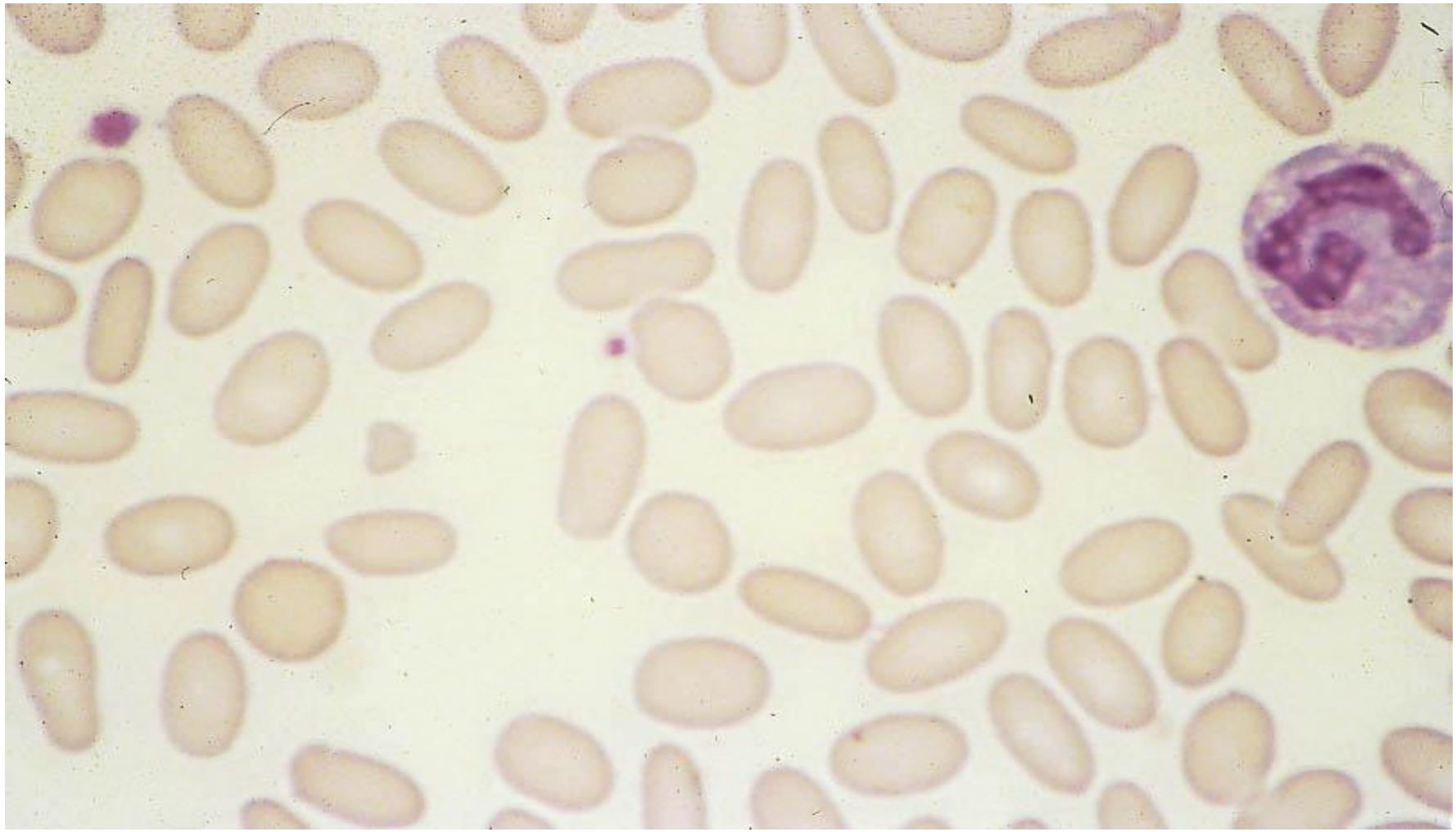
Acanthocytes (Spur Cells)



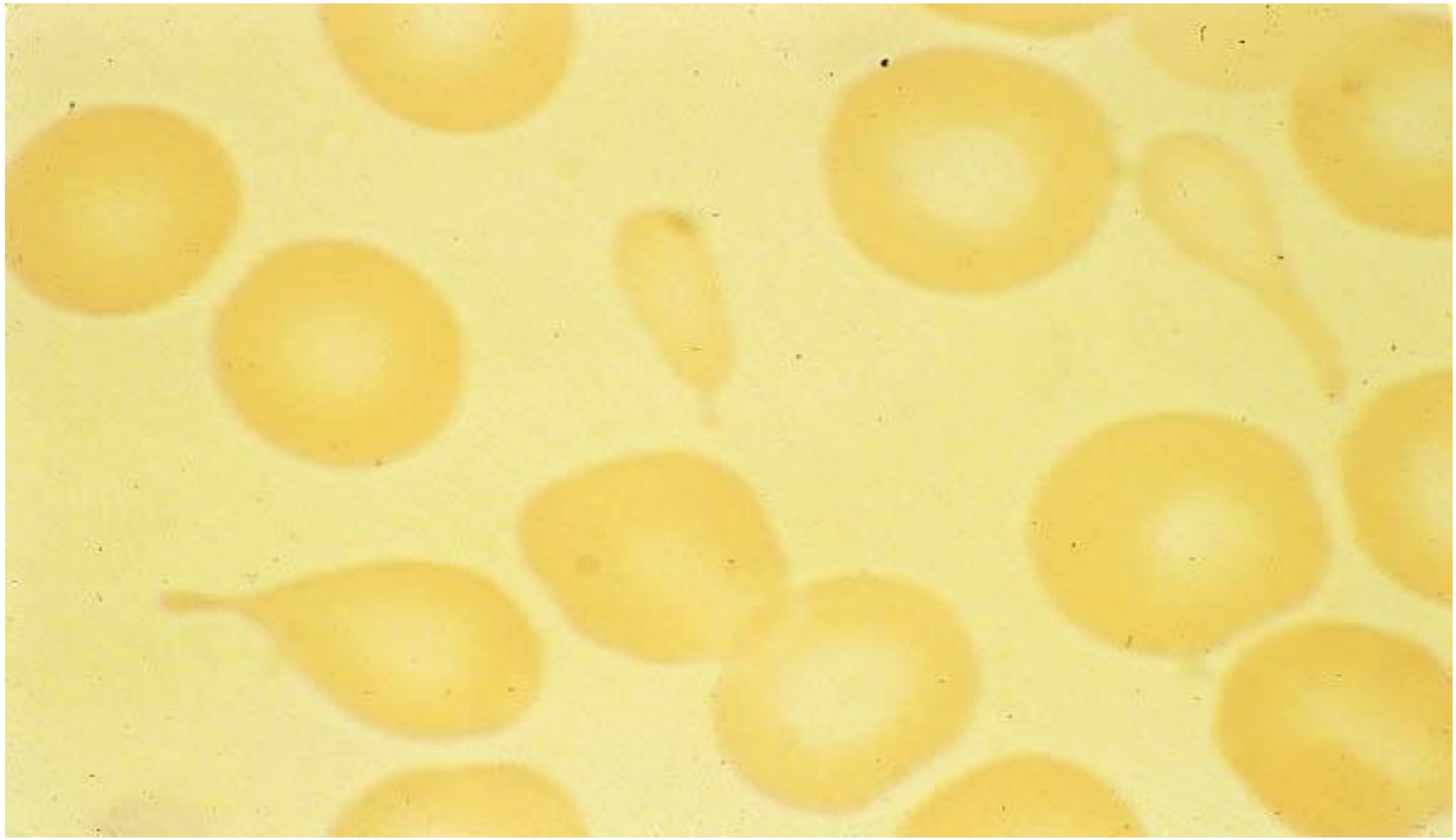
Spherocytes: Hereditary Spherocytosis



Elliptocytes: Hereditary Elliptocytosis



Teardrop Cells



What is Anemia ?

Important to remember

- Anemia is a clinical sign of disease
- It is not a single disease by itself
- Need to look for the underlying cause !
- Will we ignore a fever with out investigation ?
- Its diagnosis is not that simple !! We'll make it
- Its very common and imp. in our practice
- Drug Rx. depends on the cause

Definition of Anemia

- Decrease in the quantum of circulating red blood cell mass and there by ↓ O_2 carrying capacity
- Most common hematological disorder by far
- Almost always a secondary disorder
- As such, critical for all practitioners to know how to evaluate / determine its cause / treat

Classification of Anemia

- Acute vs. chronic
  - Signs and symptoms
- Red cell kinetics
  - Determined by reticulocyte count
- Red cell size
  - Determined by MCV

Laboratory Evaluation of Anemia

- Complete blood count
- Reticulocyte count
- Peripheral smear

RBC Abnormalities

- Number
  - RBC Count
  - Hgb - Hemoglobin
  - Hct - Hematocrit
- Size
  - MCV – Mean corpuscular volume
- Shape
  - RDW – RBC distribution width
  - Peripheral Smear
- Hgb Content
  - MCH – Mean corpuscular Hgb
  - MCHC – Mean corpuscular Hgb concentration

Hemoglobin (Hgb) & Hematocrit (Hct)

- Hgb
  - The iron-containing oxygen transport protein in RBCs
  - "Normal" varies with age, gender, race, altitude, degree of sexual maturation, heredity
- Hct
  - The proportion of blood volume made up of RBCs
  - Usually an estimation, apprx. 3x Hgb level

Mean Corpuscular Volume (MCV)

- Reflective of size for RBC
- Normal □80 – 100 femtoliter
  - Low □microcytic anemia
  - Normal □normocytic anemia
  - High □macrocytic anemia

RBC Distribution Width (RDW)

- Essentially measures the variation in the size of the RBC
- Helpful in the diagnosis of microcytic anemias
- Normal □ 12 – 14%
  - Iron Def. Anemia □ 14 – 25%

Mean Corpuscular Hgb (MCH) & Mean Corpuscular Hgb Concentration (MCHC)

- Reflect the hemoglobin content of RBC
- MCH (normal 27 – 31 picograms/cell)
  - Low hypochromic anemia
  - Normal normochromic anemia
  - High hyperchromic anemia
- MCHC (normal 32 – 36 g/deciliter)

Classification of Anemia Based on RBC Kinetics and Size

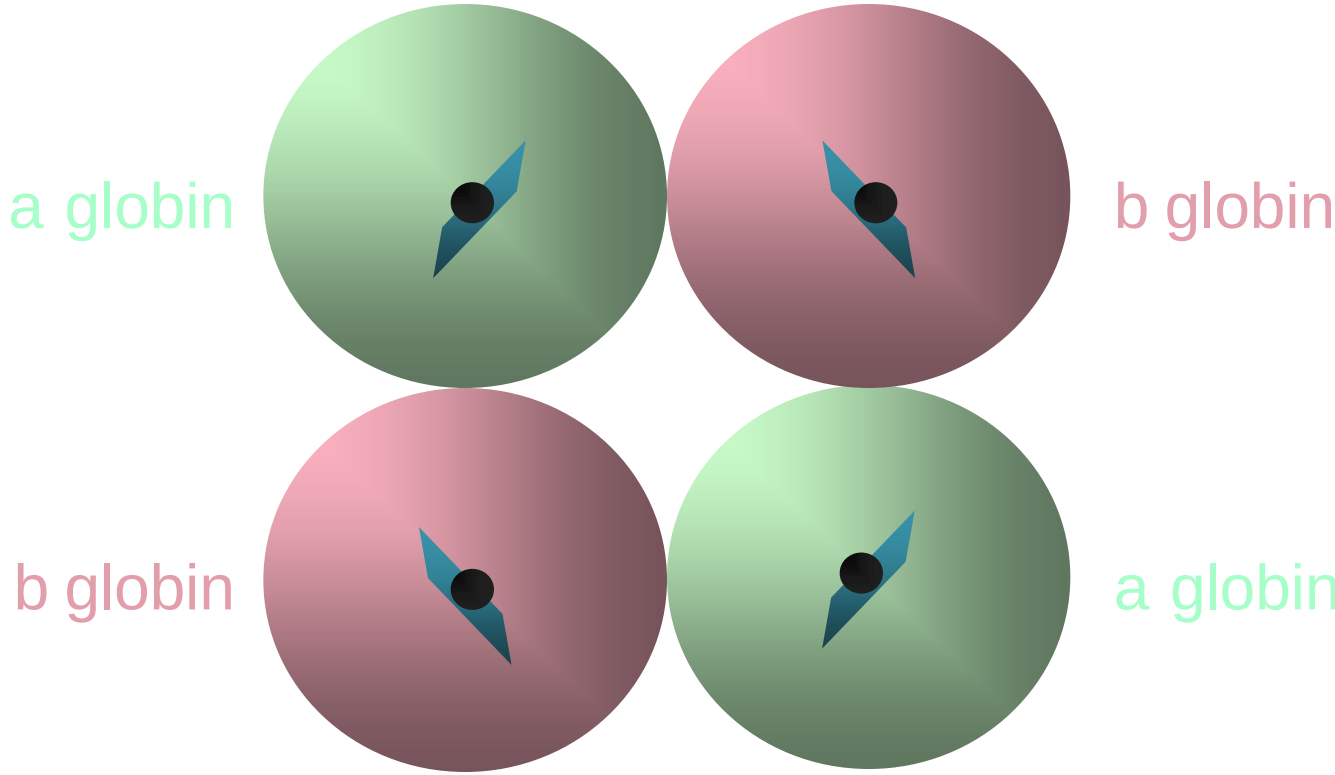
Retic count	MCV		
	Microcytic	**Normocytic**	**Macrocytic**
Low	**Common**	**Common**	**Common**
High	**Uncommon**	**Common**	**Uncommon**

Microcytic Hypochromic Anemia: Diagnosis

- Mild (MCV > 70 fl)
  - Iron deficiency
  - Thalassemia
  - Lead toxicity
  - Sideroblastic anemia
  - Anemia of chronic disease
- Severe (MCV < 70 fl)
  - Iron deficiency
  - Thalassemia

Thalassemia: Impaired globin gene prouction

Hgb A tetramer



Thalassemia

- Decreased production of normal globin chains:

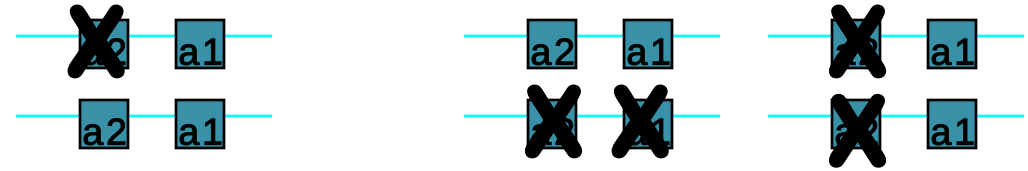
- a thalassemia deficiency of a gene(s)
- b thalassemia deficiency of b gene(s)

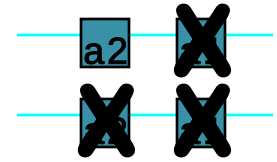
Alpha Thalassemia: Laboratory Findings

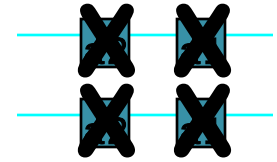
α chains	Hgb (g/dl)	MCV (fl)	RDW	Hemoglobin Analysis
αα/αα	Normal	Normal	Normal	Normal
αα/-α	12-14	75-85	Normal	Normal
α-/α- or -/αα	11-13	70-75	↑	Normal with Hgb Barts
--/- α	7-10	50-60	↑ ↑	Normal with Hgb Barts
- -/- -	-	-	-	Not viable

Alpha Thalassemia: Clinical features

- Absence of 1-2 alpha chains
  - Common
  - Asymptomatic
  - Does not require therapy
- Absence of 3 alpha chains
  - Microcytic anemia (Hgb 7-10)
  - Splenomegaly
- Absence of 4 alpha chains
  - Hydrops fetalis (non-viable)







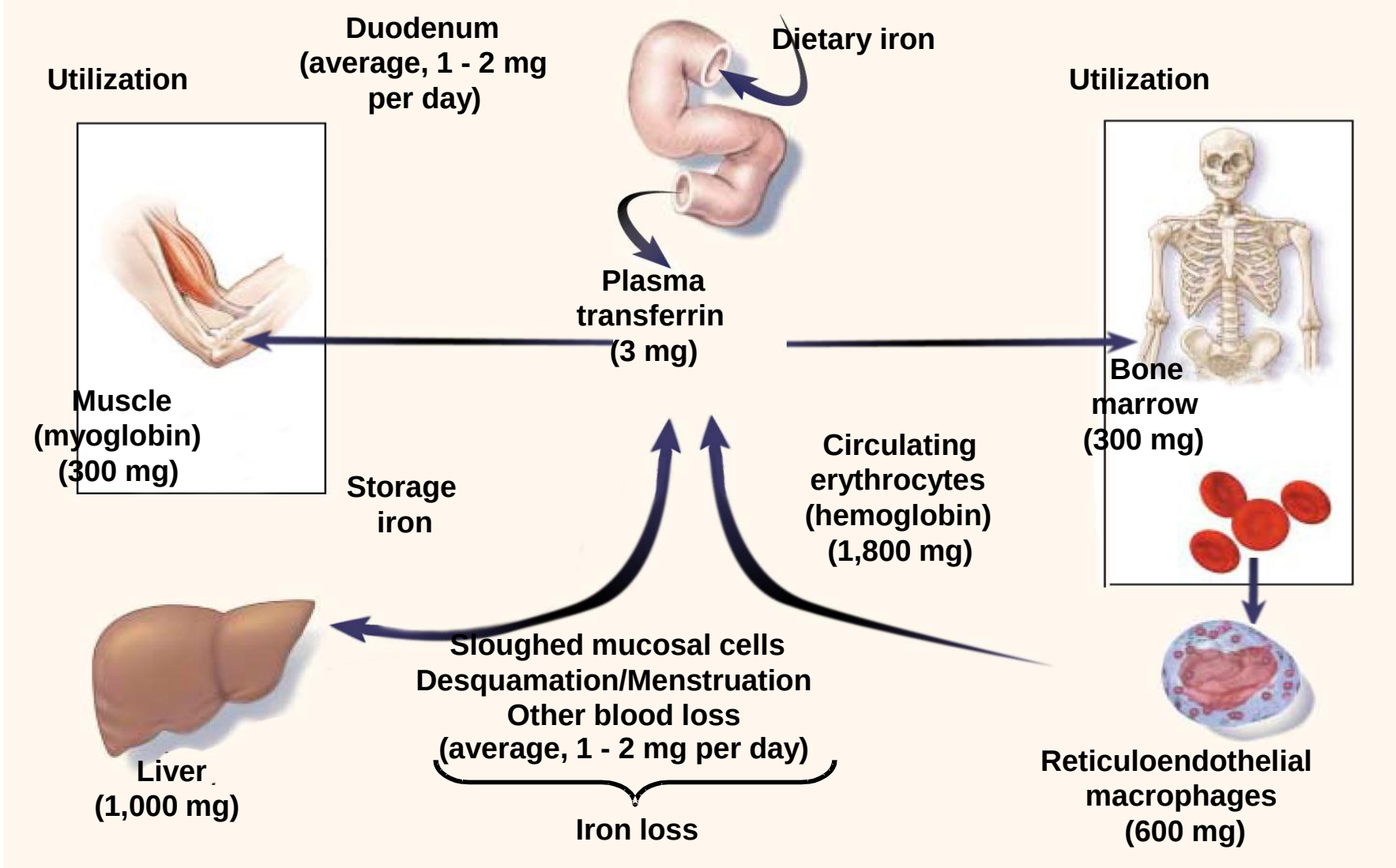
Beta Thalassemia

Clinical Syndrome	Genotype	Hgb (g/dl)	Hgb analysis
Minor (Trait)	/β^+ or β/β°	10-13	↑ Hgb A2, ↑ Hgb F
Intermedia	β^+/β^+	7-10	↑ Hgb A2, ↑↑ Hgb F
Major (Cooleys)	β^+/β° or β°/β°	< 7	↑ Hgb A2, ↑↑↑ Hgb F

Iron Deficiency Anemia

- A world-wide problem
  - 3% of toddlers age 1-2 years
  - 2-5% of women of child bearing age
- Iron metabolism
- Iron stores
- Laboratory findings of iron deficiency
- Causes of iron deficiency
- Treatment

Body Iron Distribution and Storage



Major Iron Compartments

Metabolic	
Hemoglobin	1800-2500 mg
Myoglobin	300-500 mg
Storage	
Iron storage	0-1000 mg
Transit	
Serum iron	3 mg
Total	3000-4000 mg

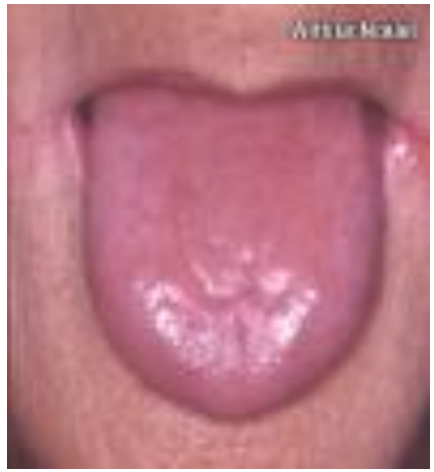
Development of Iron Deficiency

- Depletion of iron stores
  Ferritin low
  BM iron absent
- Compromised iron delivery
  Serum iron low
  TIBC high
  sTfR high
- Iron deficient anemia
  Hgb low
  MCV low

Systemic Manifestations of Iron Deficiency

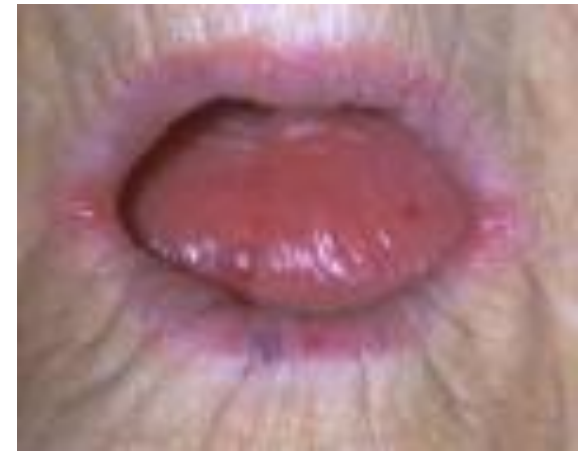
- Behavioral and neuropsychiatric manifestations
- Pica (pagophagia)
- Angular stomatitis
- Glossitis
- Esophageal webs and strictures
- Koilonychia

Systemic Manifestations of Iron Deficiency



Glossitis

Koilonychia



Angular chelitis

Causes of Iron Deficiency

- Increased iron requirements
  - Blood loss
    - Gastrointestinal tract
    - Genitourinary tract
    - Blood donation
  - Pregnancy and lactation
- Inadequate iron supply
  - Insufficient dietary iron
  - Impaired iron absorption
    - Gastric surgery
    - Intestinal malabsorption
    - Celiac disease

Unexplained iron deficiency: "Gastrointestinal sideropenia"

- Consider in patients with relapsed/refractory iron deficiency:
  - Celiac disease
  - Atrophic body gastritis
  - *H. pylori* infection
  - Gastric bypass surgery

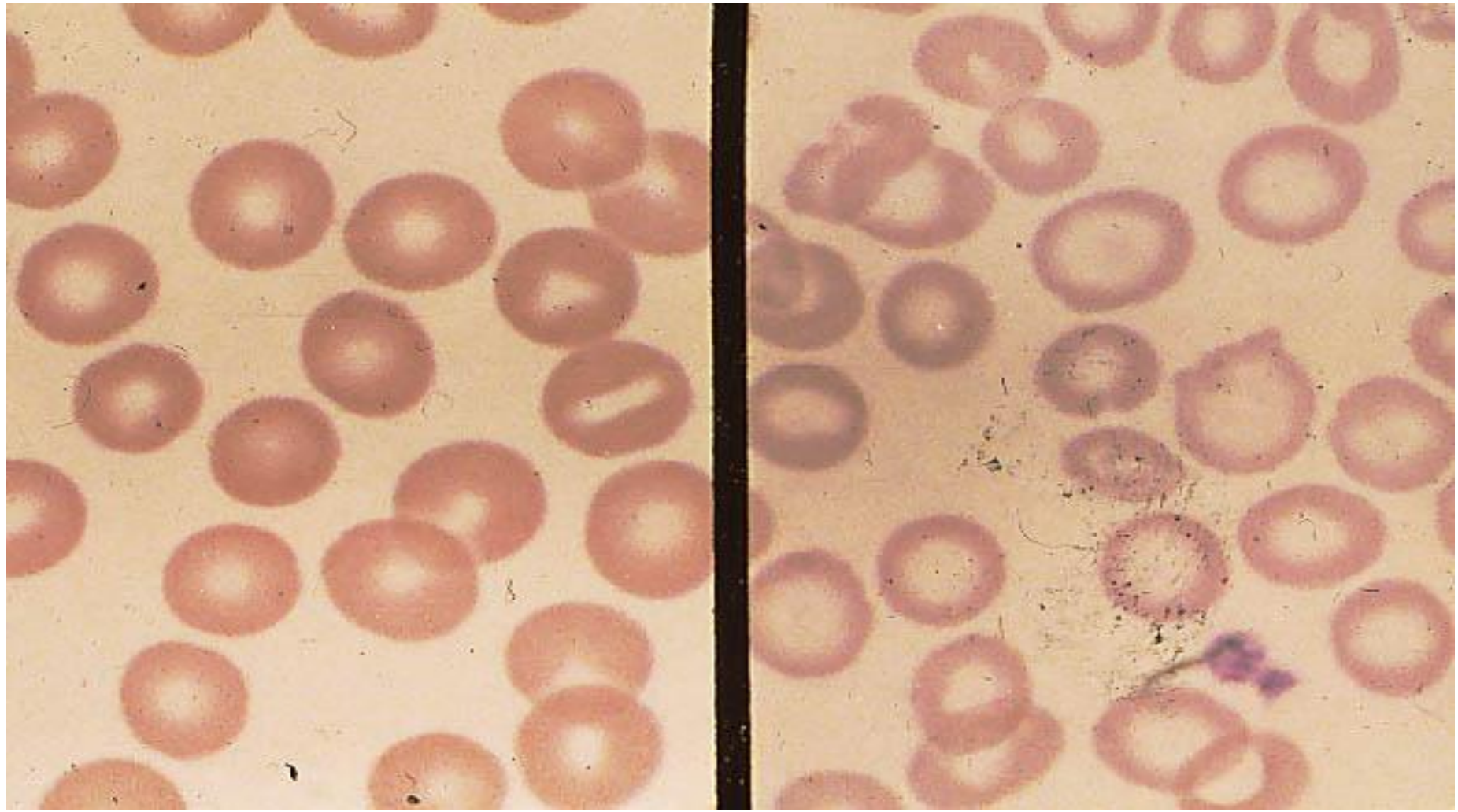
Treatment with Oral Iron: General Principles

- Ferrous salts are absorbed better than ferric
- All ferrous salts are absorbed to the same extent
- Ascorbic acid increases absorption and toxicity
- Iron is absorbed best on an empty stomach
- Iron should not be given with antacids

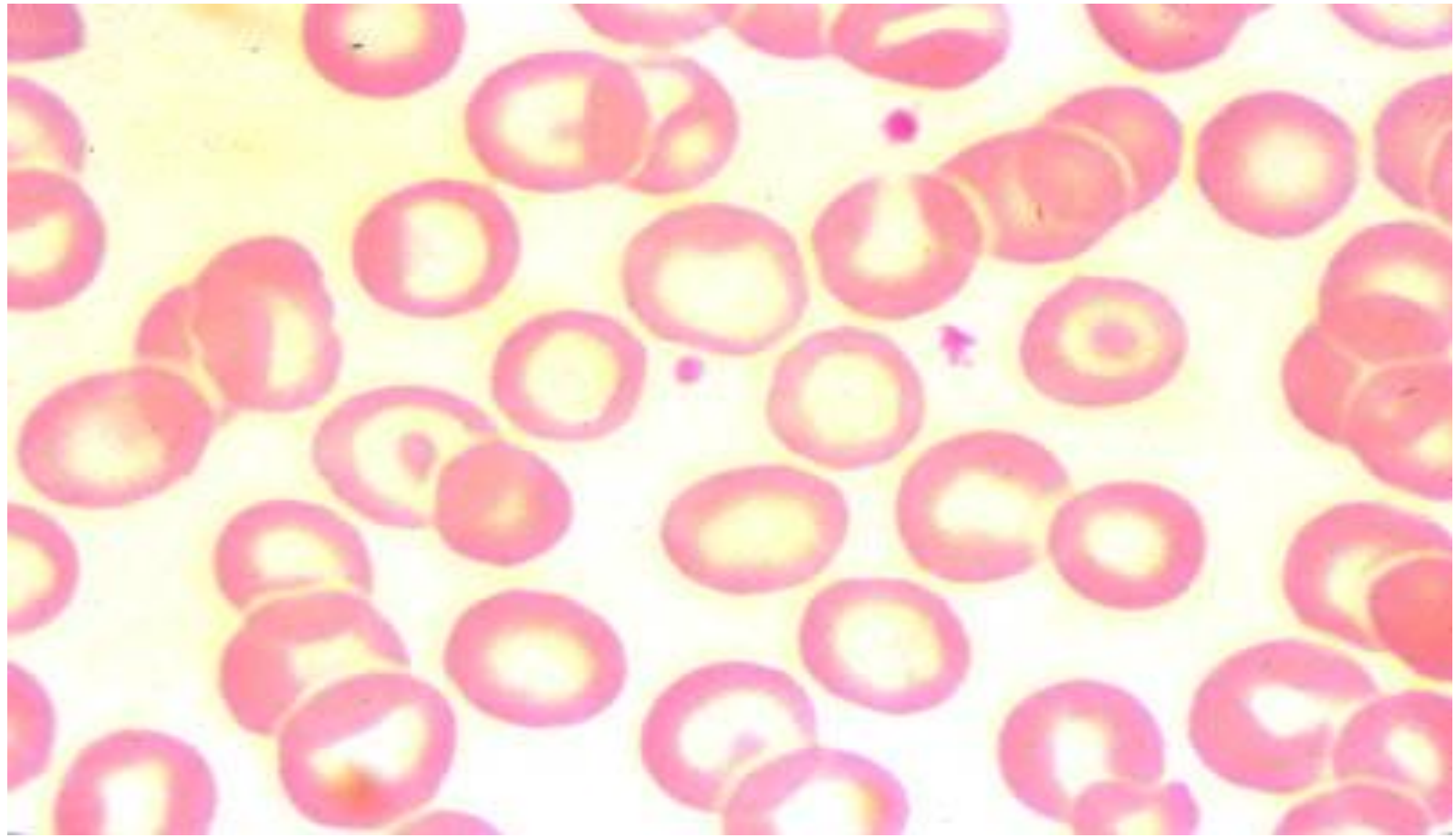
Use of Parenteral Iron

- Agents available
  - Iron dextran (total dose replacement; 1/300 anaphylaxis)
  - Iron polysaccharide (125 mg/d maximum; ~1/1000 anaphylaxis)
- Indications
  - Malabsorption
  - Iron-limited response to erythropoietin
  - Toxicity/noncompliance with oral iron
- Response
  - Maximal increase in hemoglobin synthesis
  - Rapid increase in iron stores

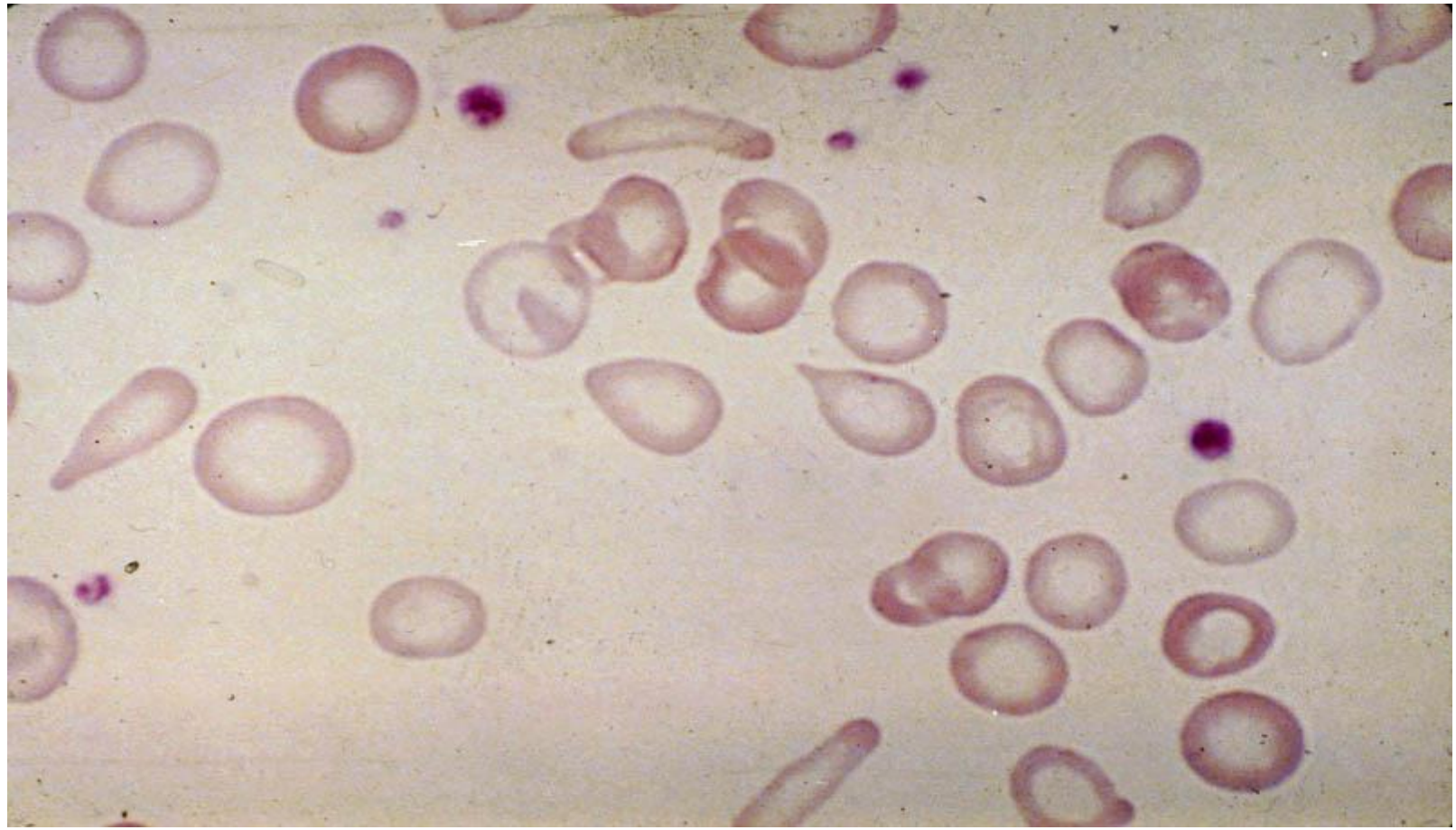
Normal Hypochromic Microcytic



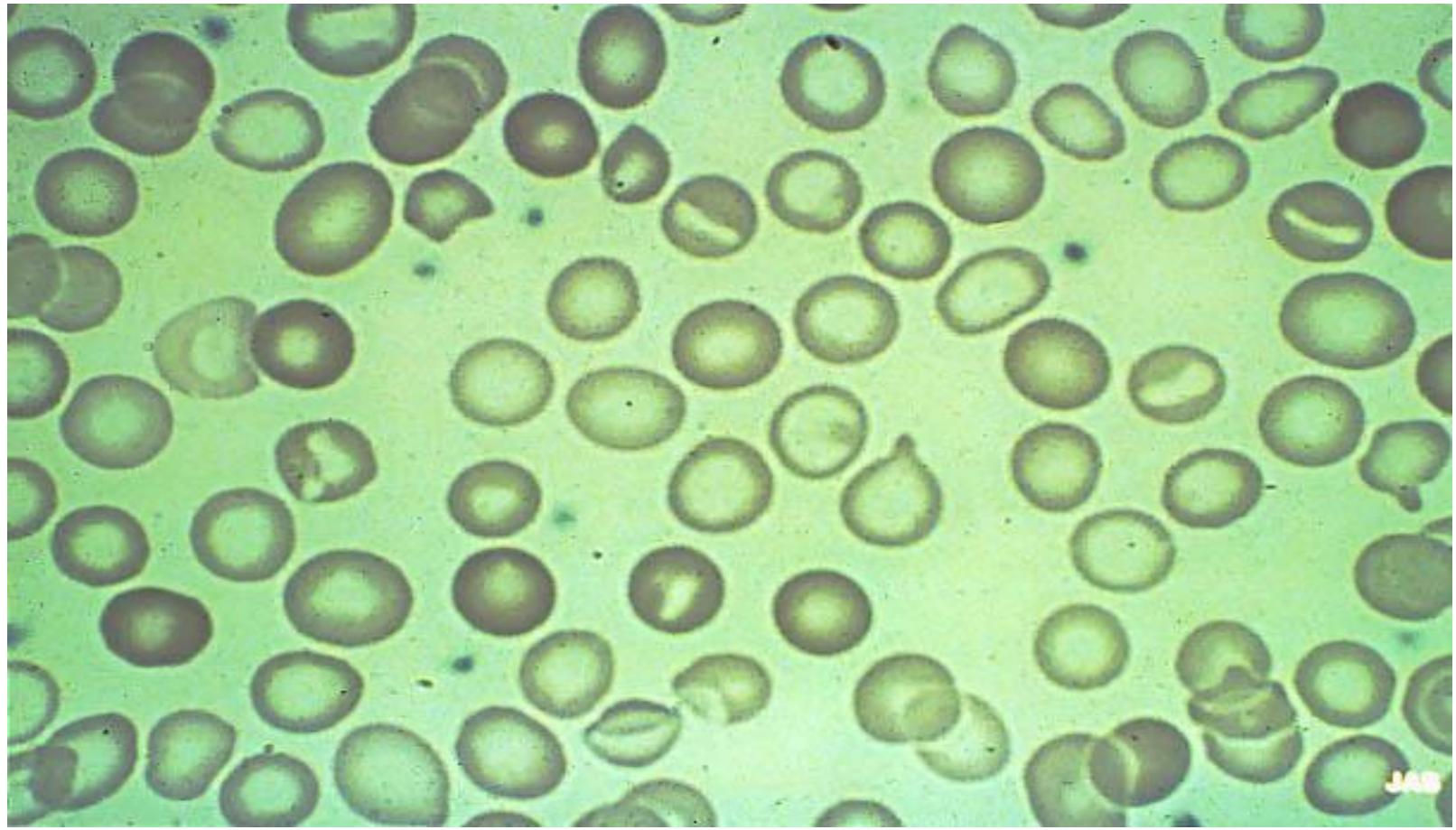
Hypochromia without Anisocytosis: Thalassemia Trait



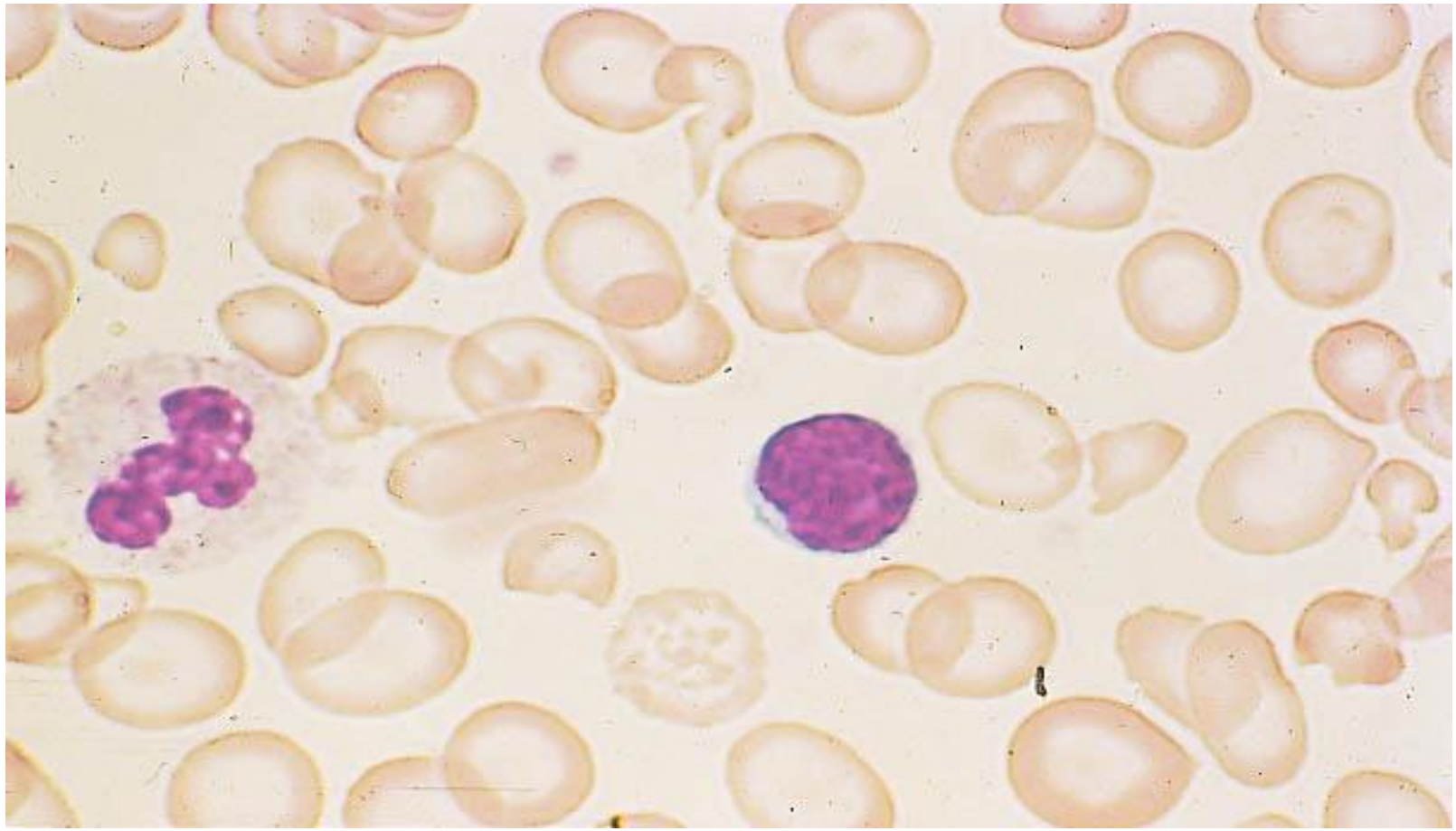
Severe Hypochromia: Iron Deficiency Anemia



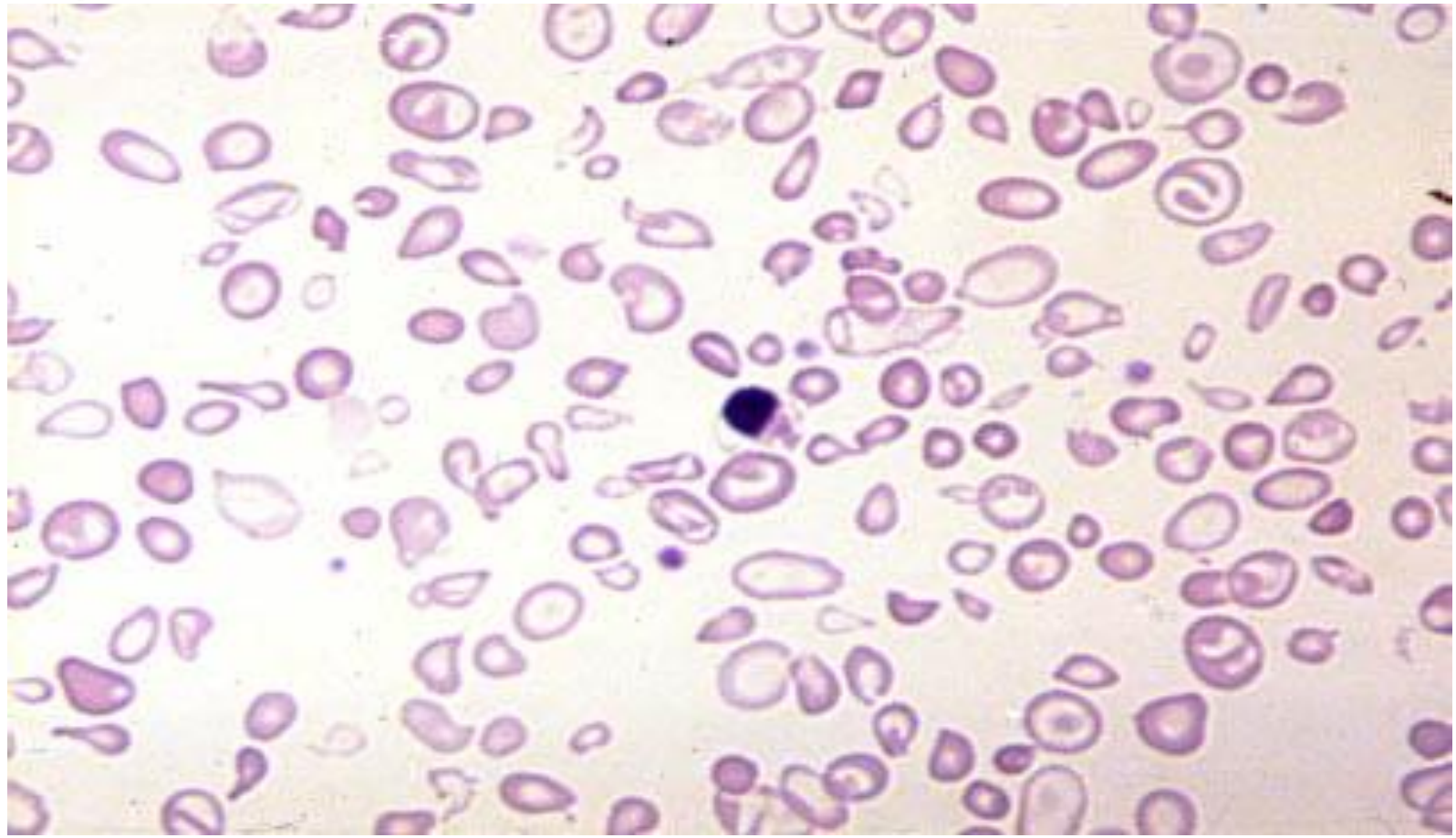
Mixed Population: Treated Iron Deficiency Anemia



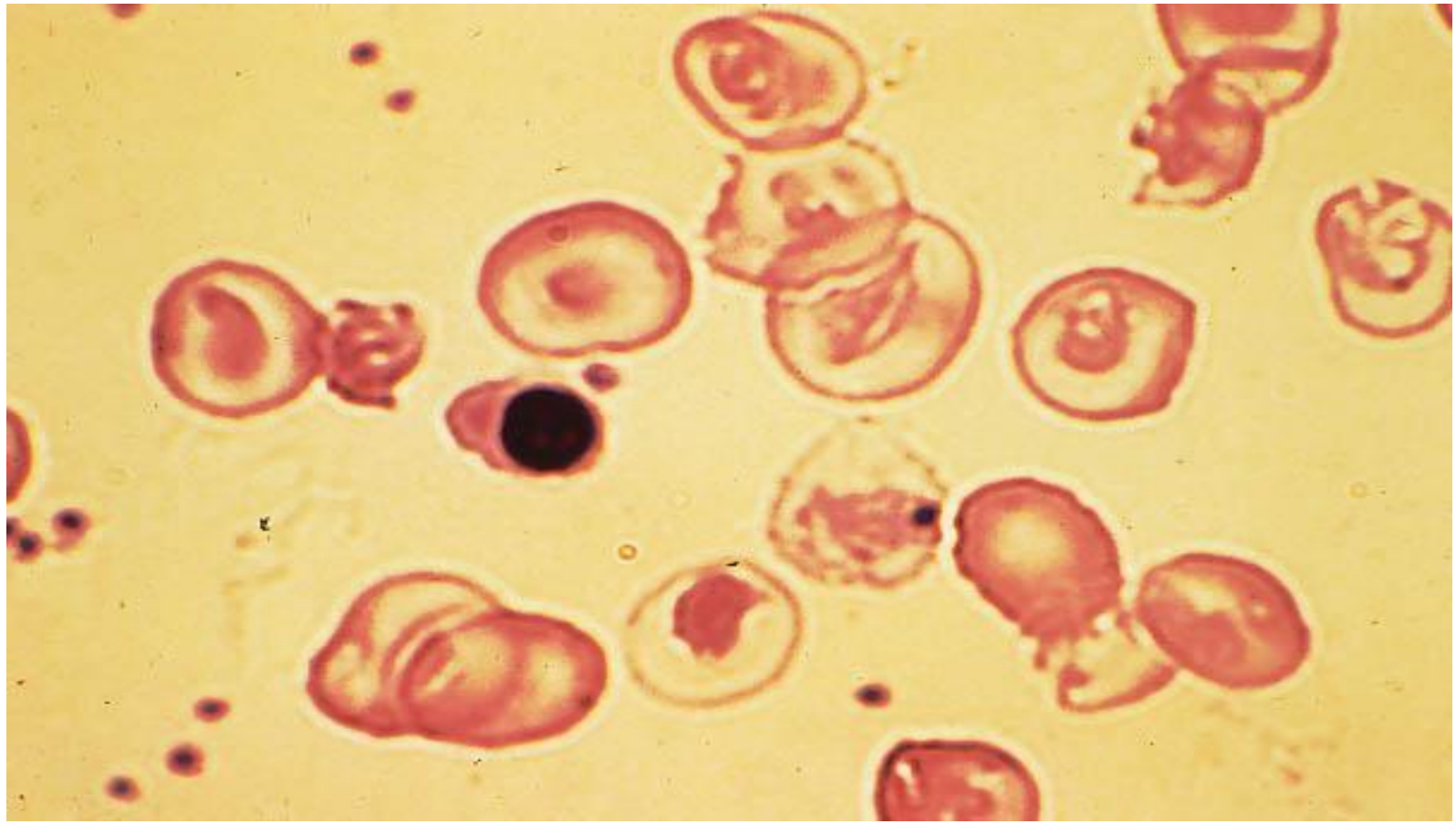
Microcytic Hypochromia: Alpha Thalassemia (α-/--)



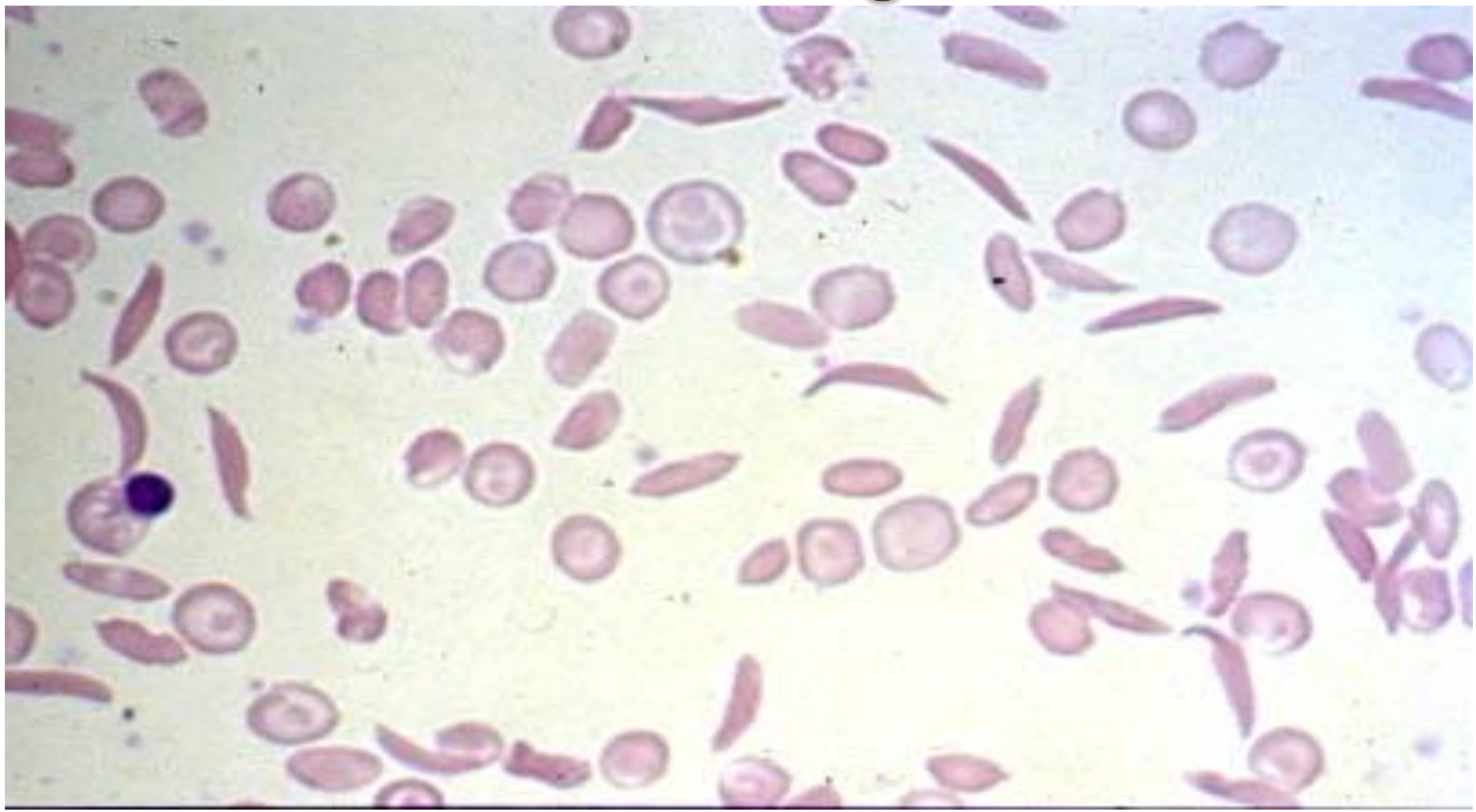
Microcytic Hypochromia: Beta Thalassemia Major



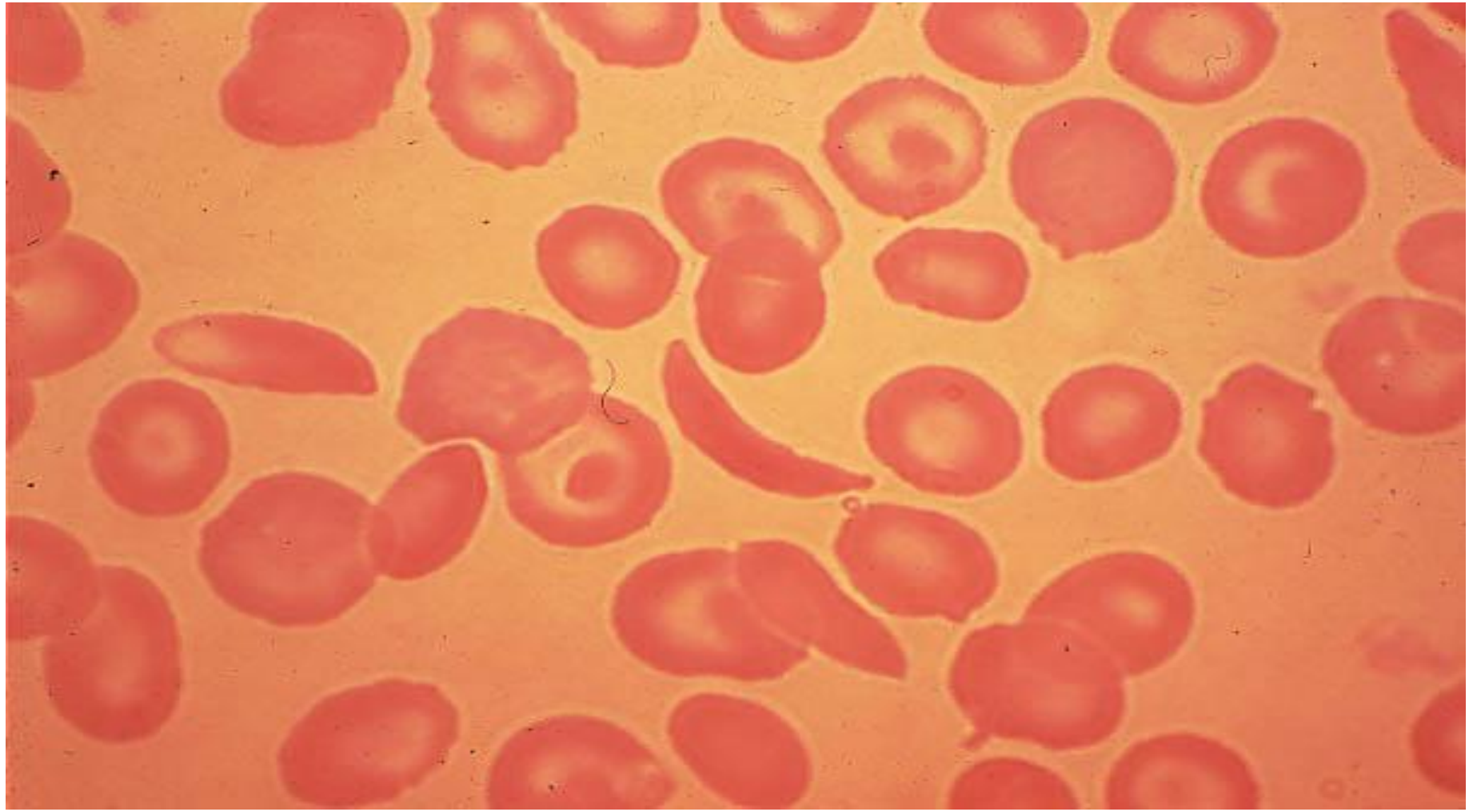
Microcytic Hypochromia: Beta Thalassemia Major



Sickle Cell Anemia: Hgb SS



Hemoglobin S-Beta Thalassemia



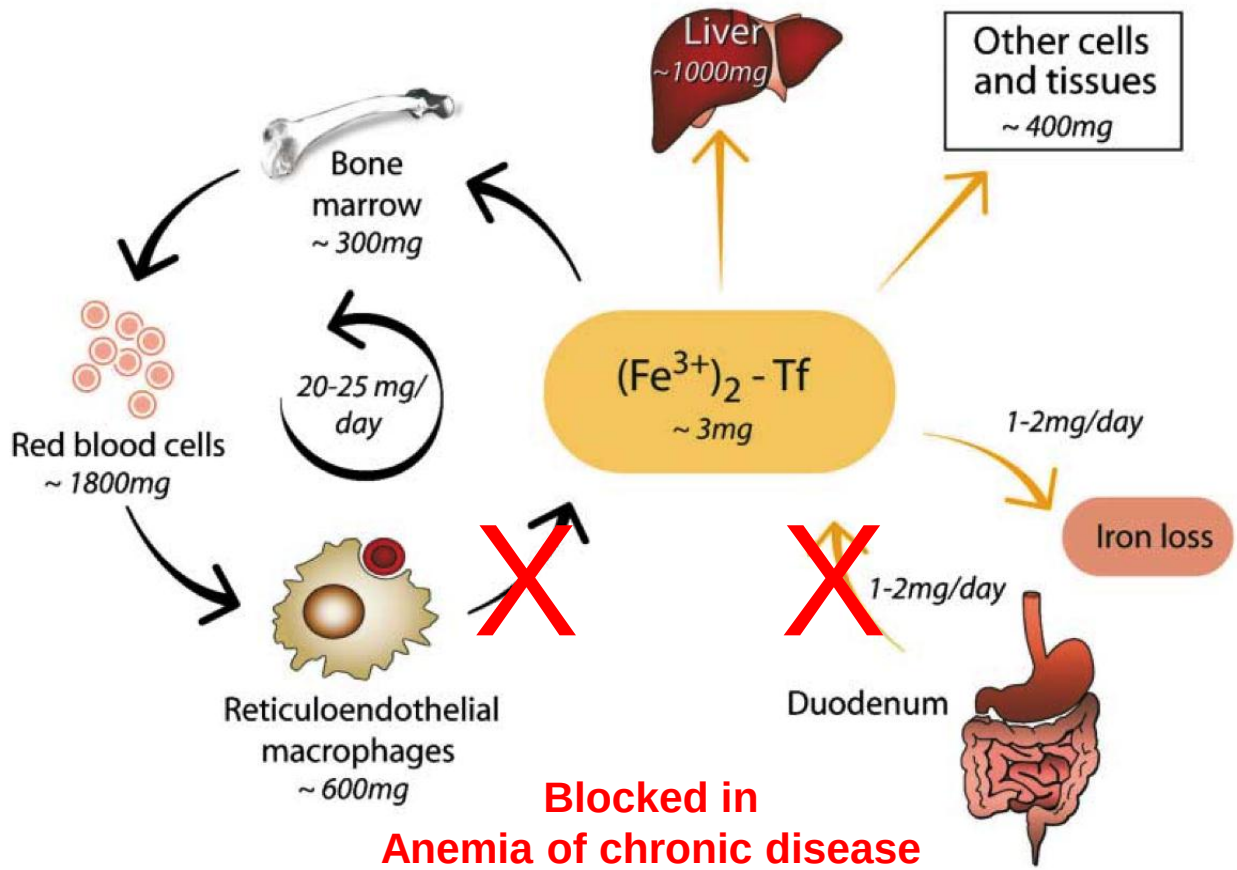
Normocytic Anemia with Low Reticulocyte Count

- Decreased stimulation of bone marrow
  - Anemia of chronic disease
  - Chronic renal insufficiency
  - Metabolic disorders
- Isolated decrease in RBC precursors
- Bone marrow damage
  - Fibrosis
  - Stem cell damage
  - Infiltration with tumor/infection
- Intrinsic bone marrow disease
  - Myelodysplasia/sideroblastic anemia

Anemia of Chronic Disease

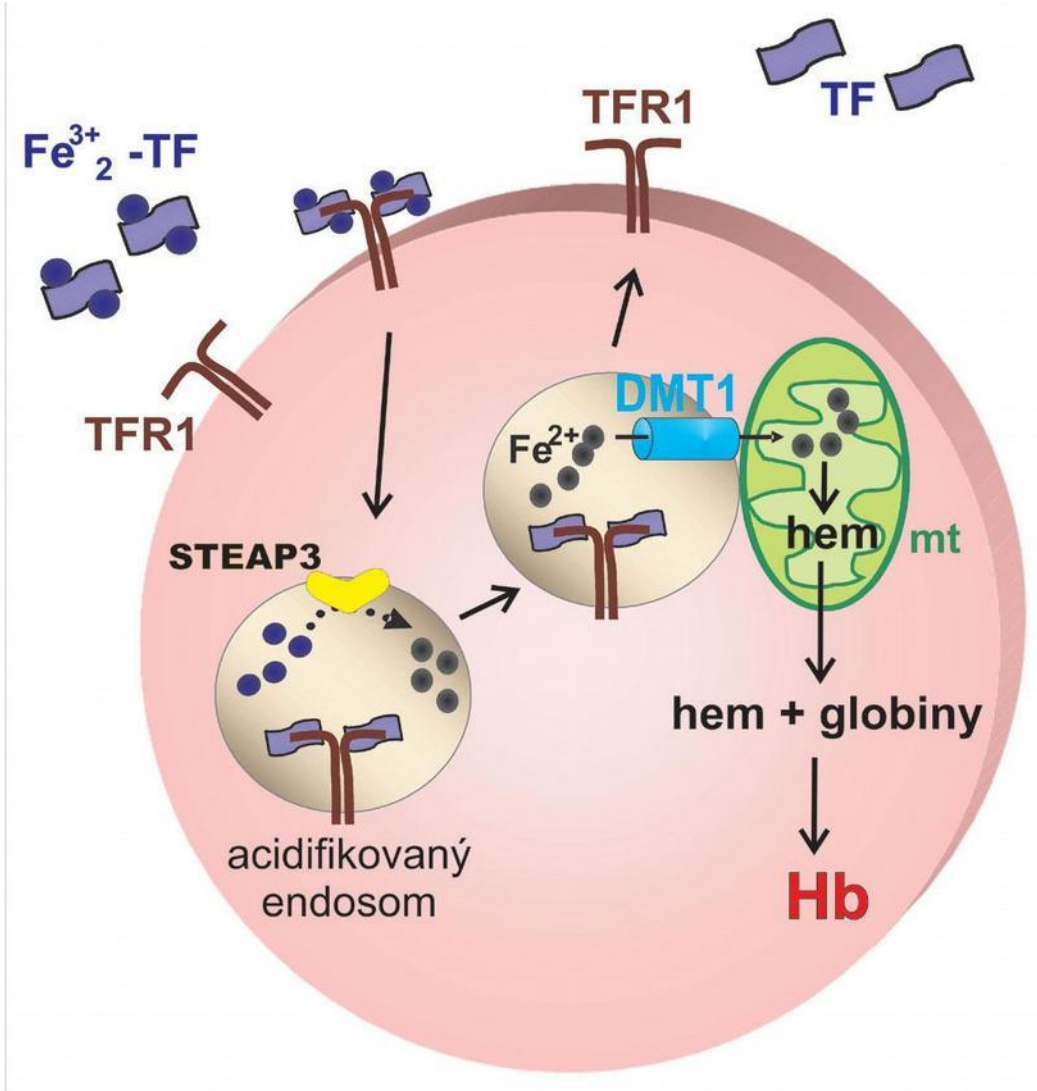
- Associated conditions — Prevalence
  - Infection — 20-95%
    - Viral, bacterial, TB, parasitic, fungal
  - Autoimmune disease — 8-17%
    - RA, SLE, Sarcoidosis, IBD, Vasculitis
  - Cancer — 30-77%
  - Chronic solid organ rejection — 8-70%
- Characteristics
  - Anemia of variable severity (mild-severe)
  - Low erythropoietin level
  - Low reticulocyte count
  - WBC and platelet counts are normal

Iron Transfer Between Cells and Tissues: Impaired in Anemia of Chronic Disease



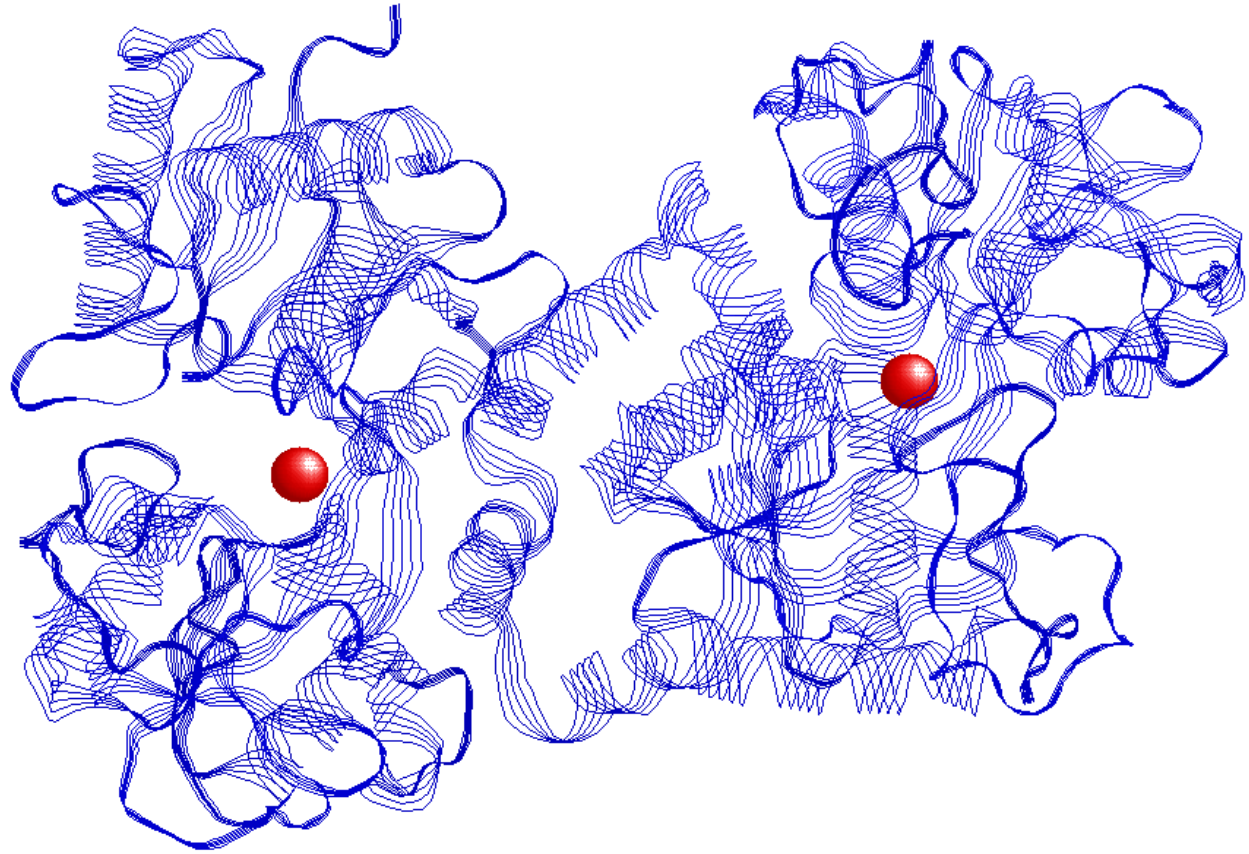
Hentze, et.al, Cell 117: 285 (2004)

Feroportin – DMT



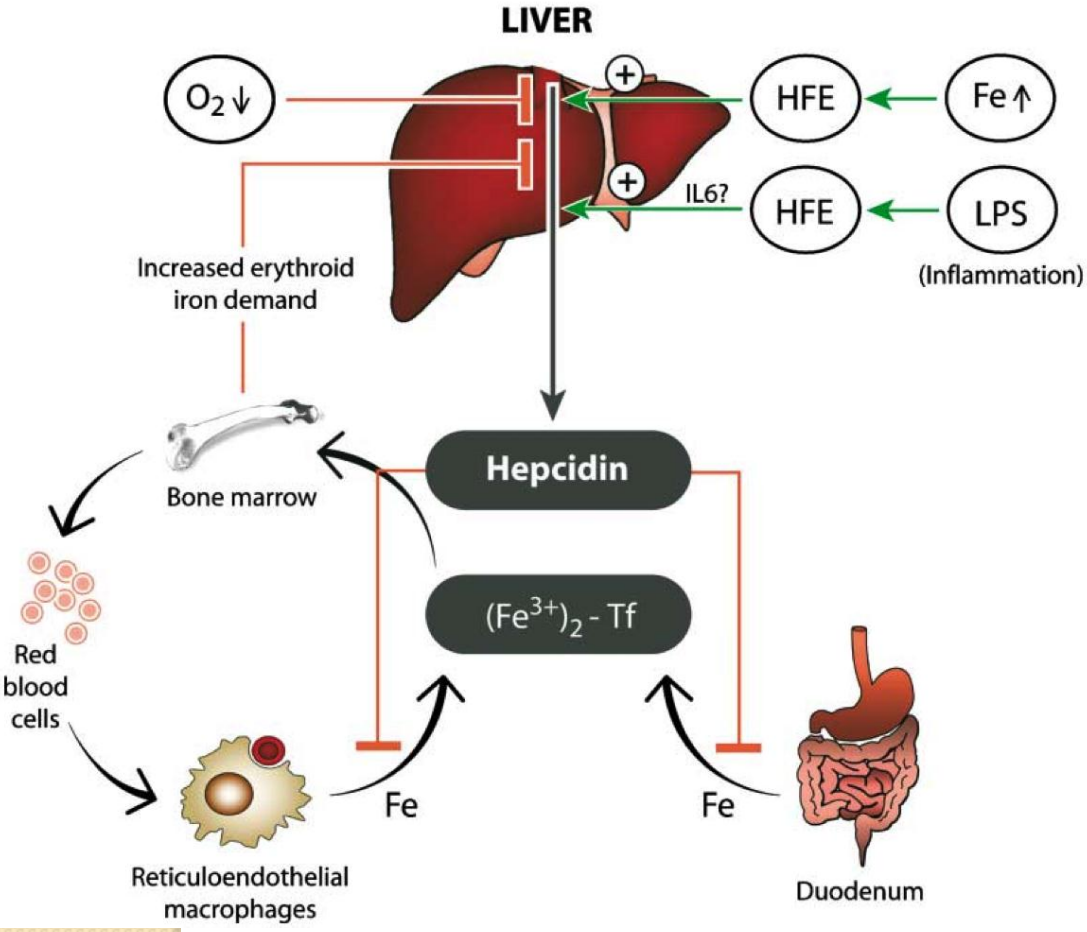
- **Εξαγωγή του Fe από το κύτταρο**
- **Μεταφορά Fe εντός του κυττάρου**

Transferrin



- **Κύρια πρωτείνη μεταφοράς**
- **Δεσμέυει το δισθενή Fe**

Iron Transfer Between Cells and Tissues: Mediated by Hepcidin



Hepcidin

Iron overload ↑

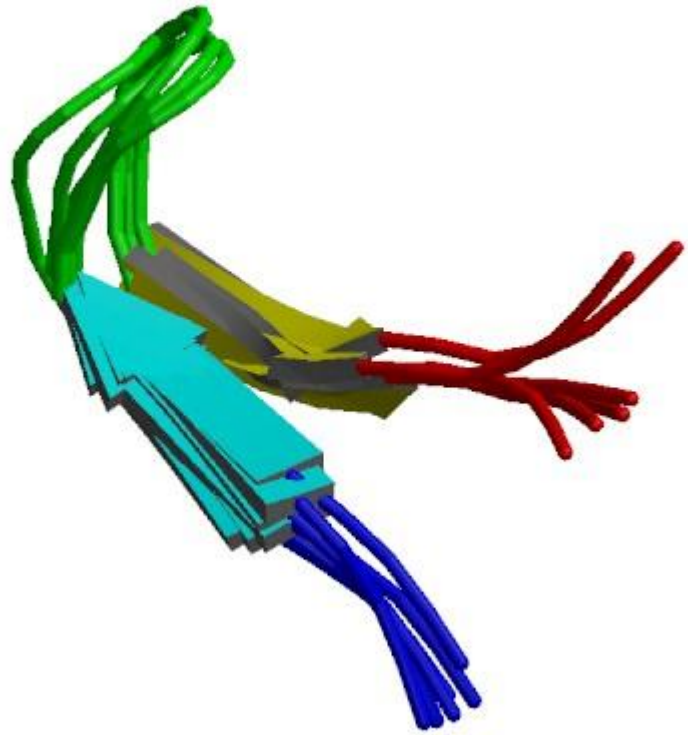
Anemia of chronic disease ↑

Iron deficiency ↓

Increased iron demand (hemolysis) ↓

Hentze, et.al, Cell 117: 285 (2004)

Hepsidin



- **Πεπτιδική ορμόνη που εκκρίνεται από το ήπαρ.**
- **Υπερπαραγωγή εψιδίνης οδηγεί σε μείωση της απορρόφησης από το εντεροκύτταρο και σε μειωμένη απελευθέρωση από τις ενδοκυττάριες αποθήκες.**

Summary

- Hepcidin plays a pivotal role in control of iron
- Increased hepcidin: Anemia of chronic disease
- Decreased hepcidin: Hemochromatosis
  - Decreased hepcidin transcription in liver (HFE, Hemojuvelin orTfR2)

Anemia of Chronic Disease

- Treatment options
  - Underlying condition
  - RBC transfusion
  - Erythropoietic agent
  - Iron supplement not usually indicated
- Hepcidin inhibitors (?)

Anemia of Chronic Renal Disease

- Characteristics
  - Widespread - 8% of US population has increased creatinine
  - 23% of patients with chronic renal disease have HCT ≤ 30%
  - Long-term anemia is a risk for LVH
  - Risk factor for mortality
- Etiology
  - Insufficient production of erythropoietin

Anemia in the Elderly

- General principles:
  - Anemia in elderly defined as Hgb <13 g/dl for men; Hgb < 12 g/dl for women
  - ~3 million individuals in the US age >65 are anemic
  - Anemia more common in females <75 years; more common in males >75 years

Potential Mechanisms of Anemia in the Elderly

- Dysregulation of the inflammatory response
- Blunting of hypoxia/erythropoietin sensing mechanism
- Sarcopenia
- Alterations in the stem cells
- Decrease in sex steroids (testosterone)
- Frequent co-morbid medical conditions
- Polypharmacy

Pure Red Cell Aplasia

- Normocytic anemia with reticulocyte count < 0.5%
- Absent erythroid precursors in marrow
- Caused by Parvovirus B19
- Clinical setting
  - *Immunocompetent* patients with chronic hemolysis
  - *Immunodeficient* patients with persistent viremia

Macrocytic Anemia with Low Reticulocyte Count

- Megaloblastic anemia
  - Vitamin B12 deficiency
  - Folate deficiency
- Non-megaloblastic macrocytic anemia
  - Liver disease
  - Hypothyroidism
  - Drug-induced (DNA synthesis block)
  - Myelodysplastic syndrome

Folate and Cobalamin Daily Requirements

	Diet	
	Vitamin B12 (Cobalamin)	**Folate**
Source	Animal products	Widespread
Body stores	5 mg	5 mg
Daily requirement	2-5 μg	50-200 μg
Daily intake	10-20 μg	400-800 μg
Dietary deficiency	Rare	Common

Vitamin B12 Deficiency: Common Mechanisms

- Intragastric events
  - Inadequate dissociation of cobalamin from food protein
  - Total or partial gastrectomy
  - Absent intrinsic factor secretion
- Proximal small intestine
  - Impaired transfer of cobalamin from R protein to intrinsic factor
  - Usurpation of luminal cobalamin
    - Bacterial overgrowth
    - Diphylobothrium latum (fish tapeworm)
- Distal small intestine
  - Disease of the terminal ileum

Pernicious Anemia

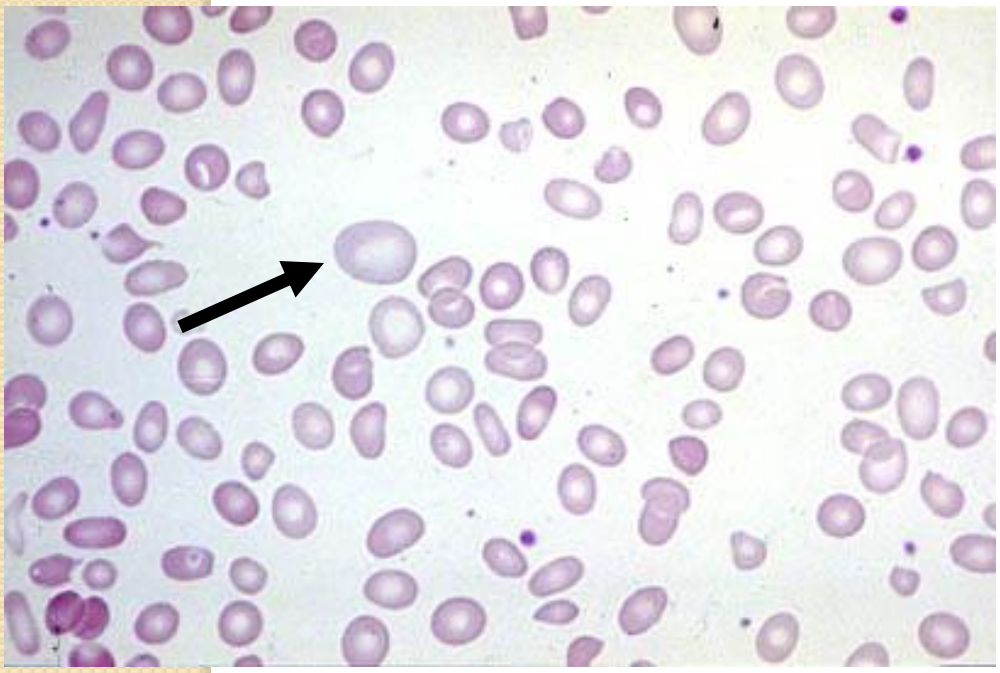
- Most common cause of vitamin B_{12} deficiency
- Occurs in all ages and ethnic backgrounds
- Associated with other autoimmune diseases
  - Screen for thyroid disease every 1-2 years
- Pernicious anemia is a systemic disease
  - Gastrointestinal tract involvement
  - Neurologic involvement

Pernicious Anemia: Laboratory Diagnosis

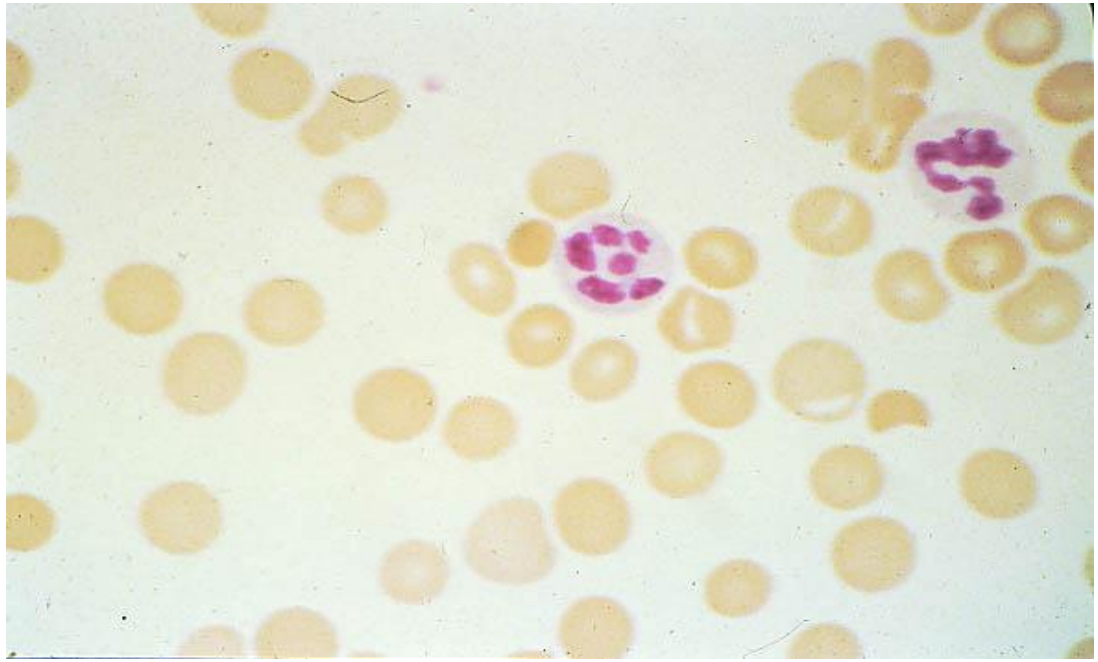
- Anti-intrinsic factor antibodies
  - Specific but not sensitive
- Anti-parietal cell antibodies
  - Sensitive but not specific
- Schilling test
  - Procedure
    - Absorption of radiolabeled cobalamin ± Intrinsic factor
    - Measure urinary excretion of radioactivity
  - Specific but not sensitive

Megaloblastic anemia

Macro-Ovalocytes



Hypersegmented Neutrophils



Treatment of Vitamin B12 Deficiency

- Parenteral cobalamin
  - 1 mg/day x 7 days
  - 1 mg/week x 4 weeks
  - 1 mg/month for life
- Oral cobalamin
  - 1 mg/day for life

Folate Deficiency

- Minimum daily folate requirement is 50 µg
- Usual dietary folate 50-500 µg
- Absorption in small intestine
- Causes of folate deficiency
  - Dietary (90%)
    - Alcohol abuse
    - Pregnancy
  - Malabsorption
  - Drug-induced
- Treatment - oral folic acid supplementation

Normocytic Anemia with High Reticulocyte Count

- Bleeding may have similar laboratory findings as hemolysis
- High reticulocyte count may lead to macrocytosis
- Diagnosis is usually ascertained
- Clinical manifestations of long term hemolysis
  - Cholelithiasis
  - Risk of aplastic crisis (Parvovirus B19)
- Classification
  - Hereditary vs. acquired
  - Extravascular vs. intravascular
  - Immune vs.non-immune

Hemolytic Anemia with Extravascular Hemolysis

- Extravascular (reticuloendothelial system)
  - Hereditary
    - Hemoglobinopathies (sickle cell anemia)
    - Enzymopathies (G6PD deficiency)
    - Membrane defects (hereditary spherocytosis)
  - Acquired
    - Immune mediated
      - Autoimmune hemolytic anemia
    - Non-immune mediated
      - Spur cell hemolytic anemia
      - Paroxysmal nocturnal hemoglobinuria (PNH)

Glucose-6-Phosphate Dehydrogenase Deficiency

- G-6-PD: reduces NADP/oxidizes glucose-6-phosphate
  - Detoxifies free radicals and peroxides
- Sex-linked disorder
- 8% of African-American males
- Hemolytic anemia occurs in the presence of stress (infection or drugs)
  - African form - mild hemolysis
  - Mediterranean form - more severe
    - Unique sensitivity to fava beans

Acquired Hemolytic Diseases

- Immune mediated hemolytic anemia
- Non-immune mediated

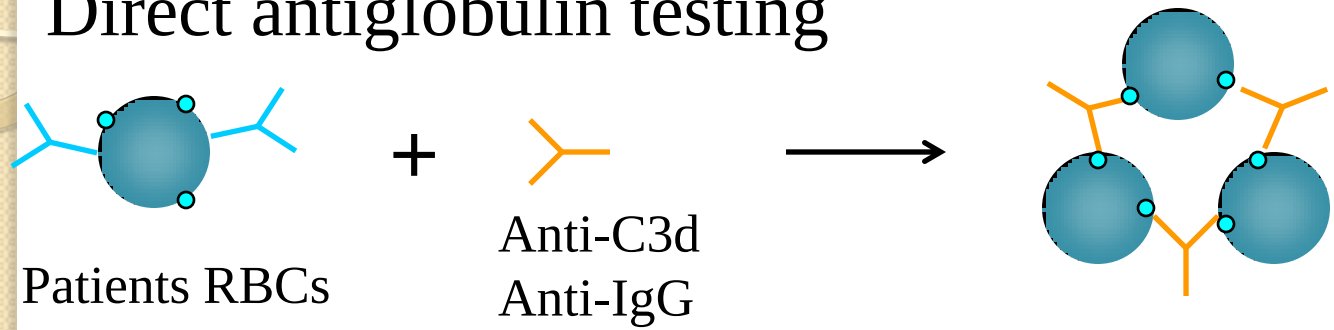
Autoimmune Hemolytic Anemia

- Warm antibodies (IgG-mediated)
  - Primary 45%
  - Secondary 40%
    - Lymphoproliferative disease
    - Connective tissue disease
    - Infectious disease
  - Drug-induced 15%

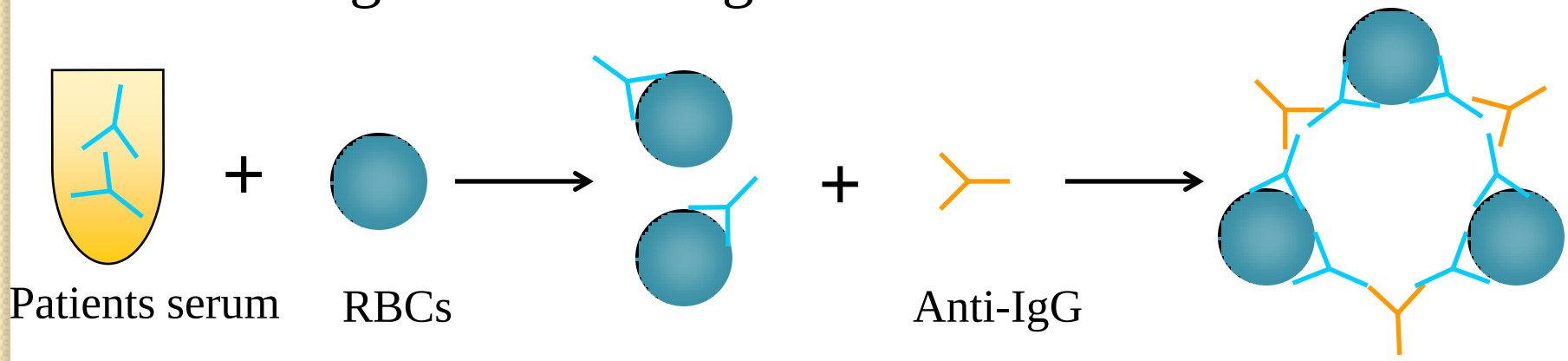
- Laboratory testing
  - Normocytic/macrocytic anemia
  - Peripheral smear: spherocytosis

Anti-Globulin (Coombs) Testing

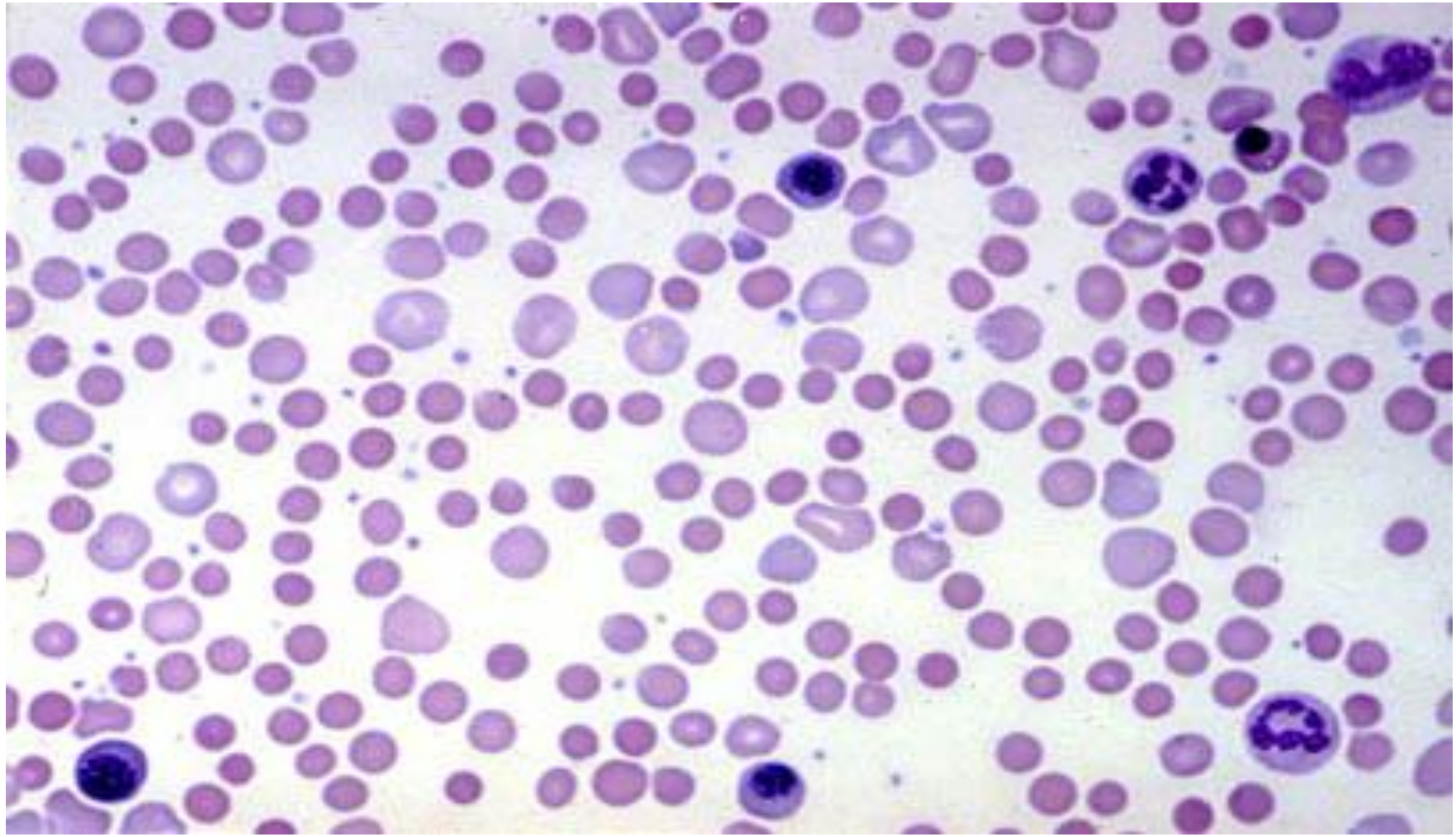
Direct antiglobulin testing



Indirect antiglobulin testing



Spherocytes: Autoimmune Hemolytic Anemia



Treatment of Autoimmune Hemolytic Anemia

- Treat underlying disease if indicated
- Prednisone (1 mg/kg/day for 2 weeks, then taper)
- Splenectomy
- Other
  - Immunosuppressive agents (Rituximab)
  - IVIG
- Similar approach to ITP

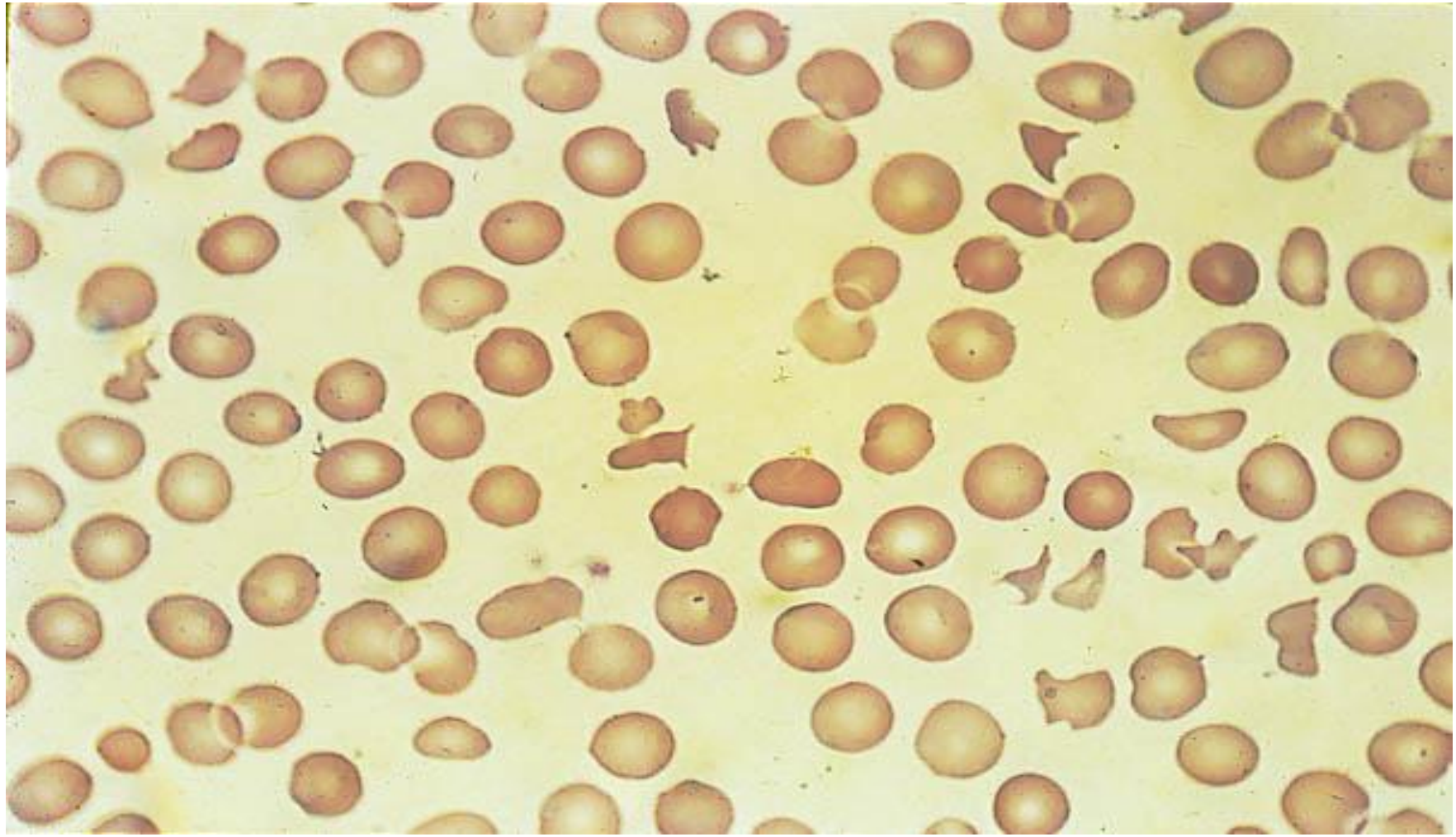
Hemolytic Anemia with Intravascular Hemolysis

- Mechanical damage (microangiopathy)
- Chemical damage
- Infection
- ABO incompatibility

Differential Diagnosis of Microangiopathic Hemolytic Anemia

- Thrombotic thrombocytopenic purpura (TTP)
- Hemolytic uremic syndrome (HUS)
- Disseminated intravascular coagulation (DIC)
- Vasculitis
- Malignant hypertension
- Metastatic neoplasm with vascular invasion
- Preeclampsia/HELLP syndrome of pregnancy

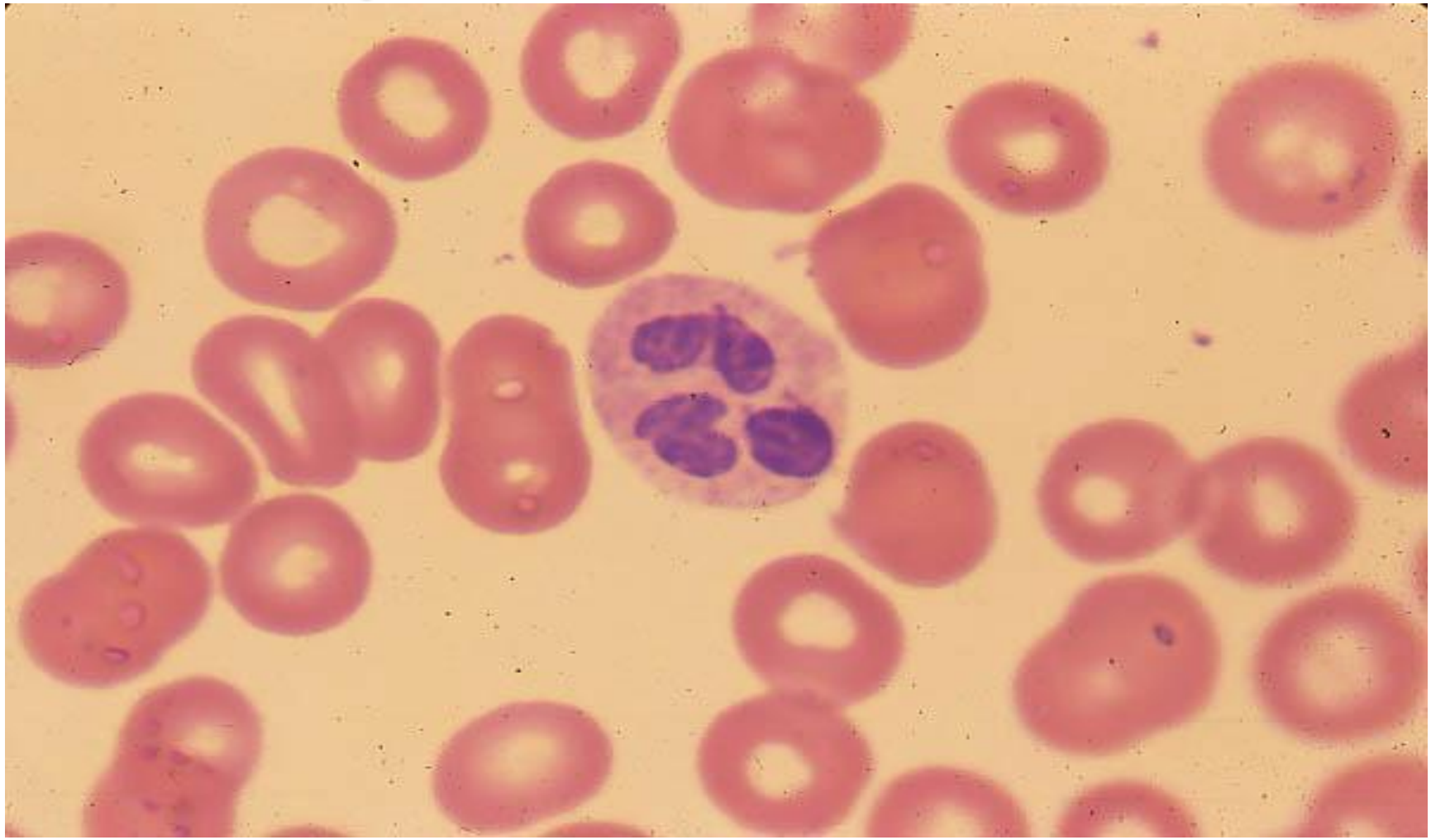
Schistocytes: Microangiopathic Hemolytic Anemia



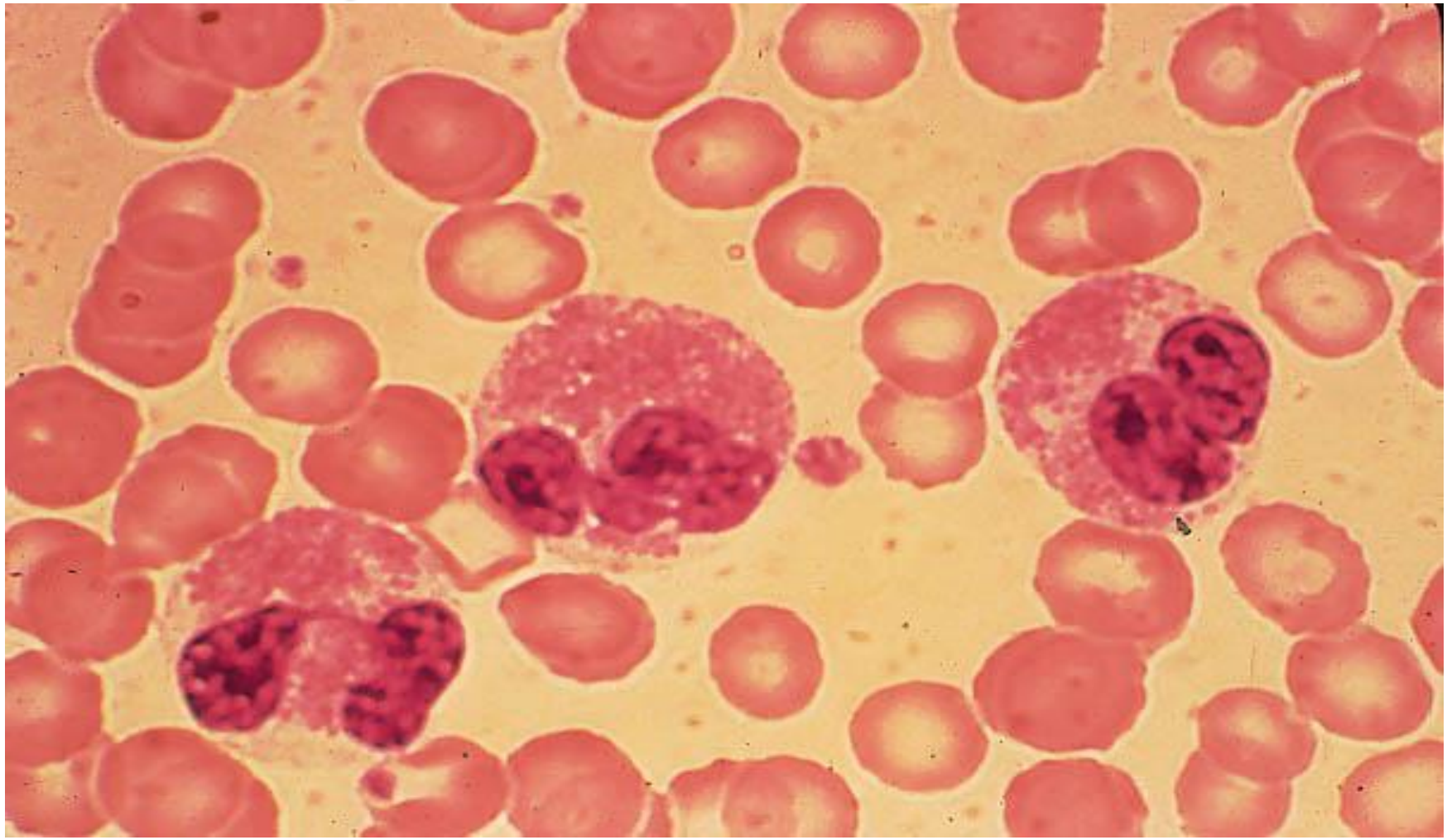
Morphology of Leukocytes

- Normal WBC populations
  - Neutrophils (granulocytes)
  - Lymphocytes
  - Monocytes
  - Eosinophils
  - Basophils

Neutrophil

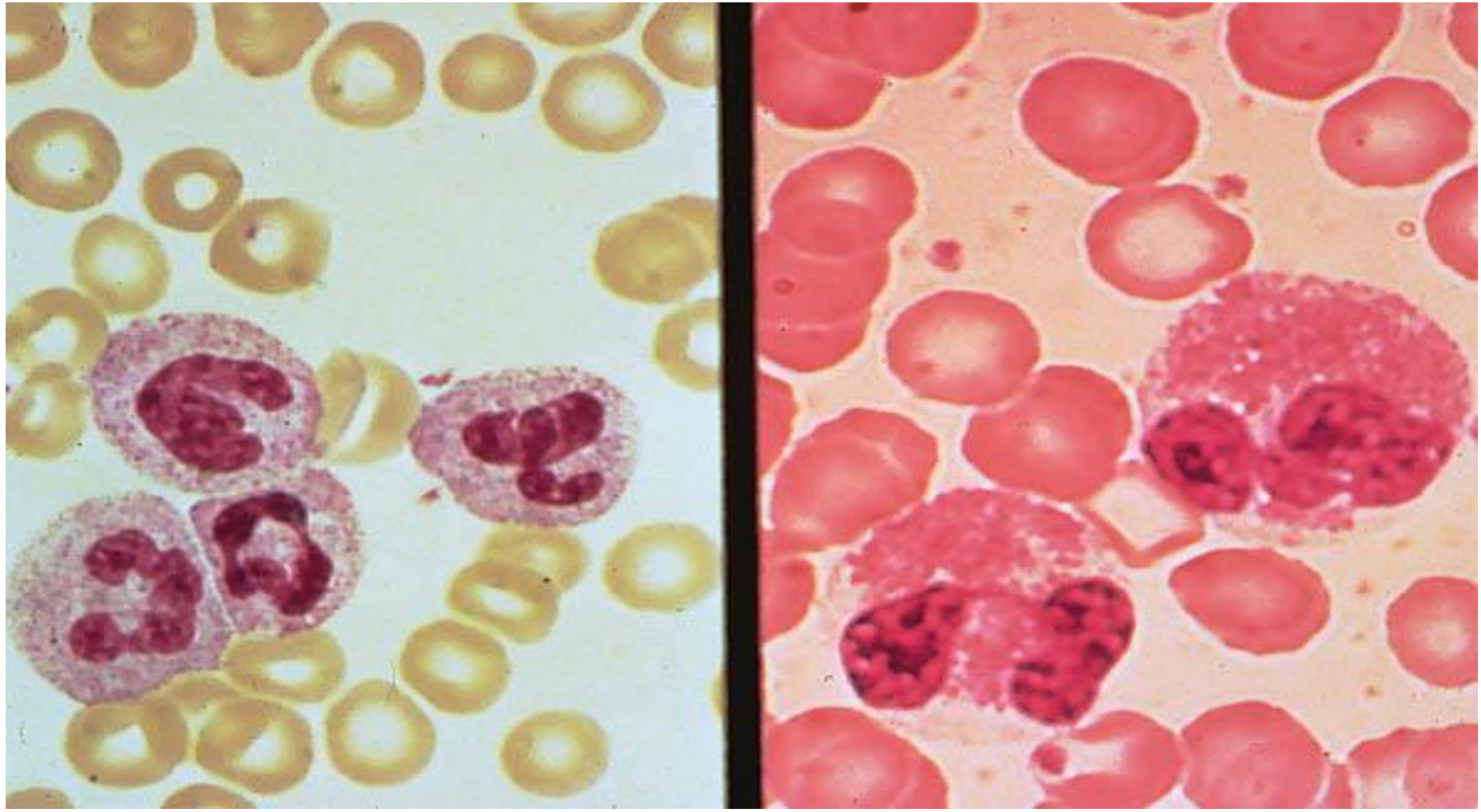


Eosinophil

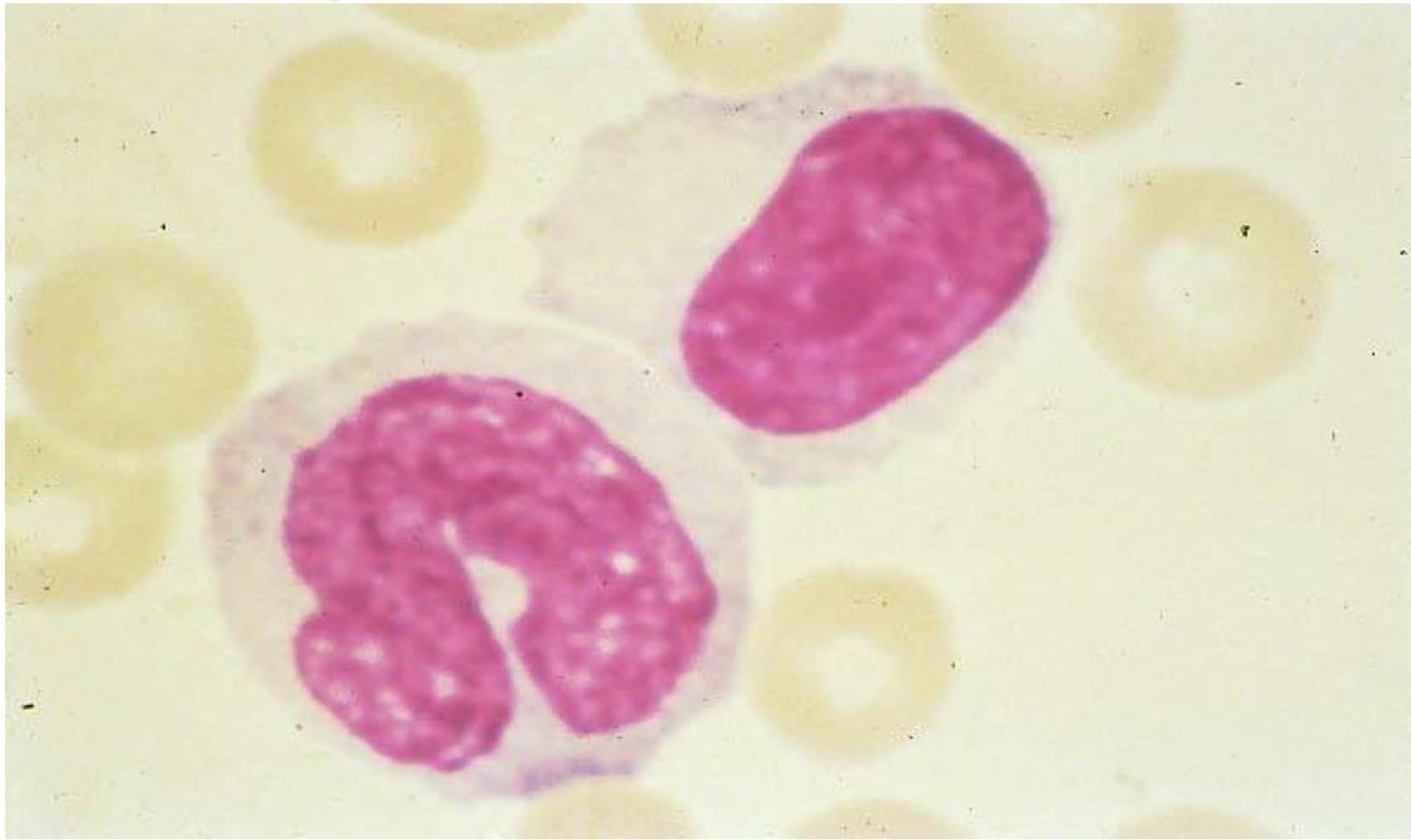


Neutrophil

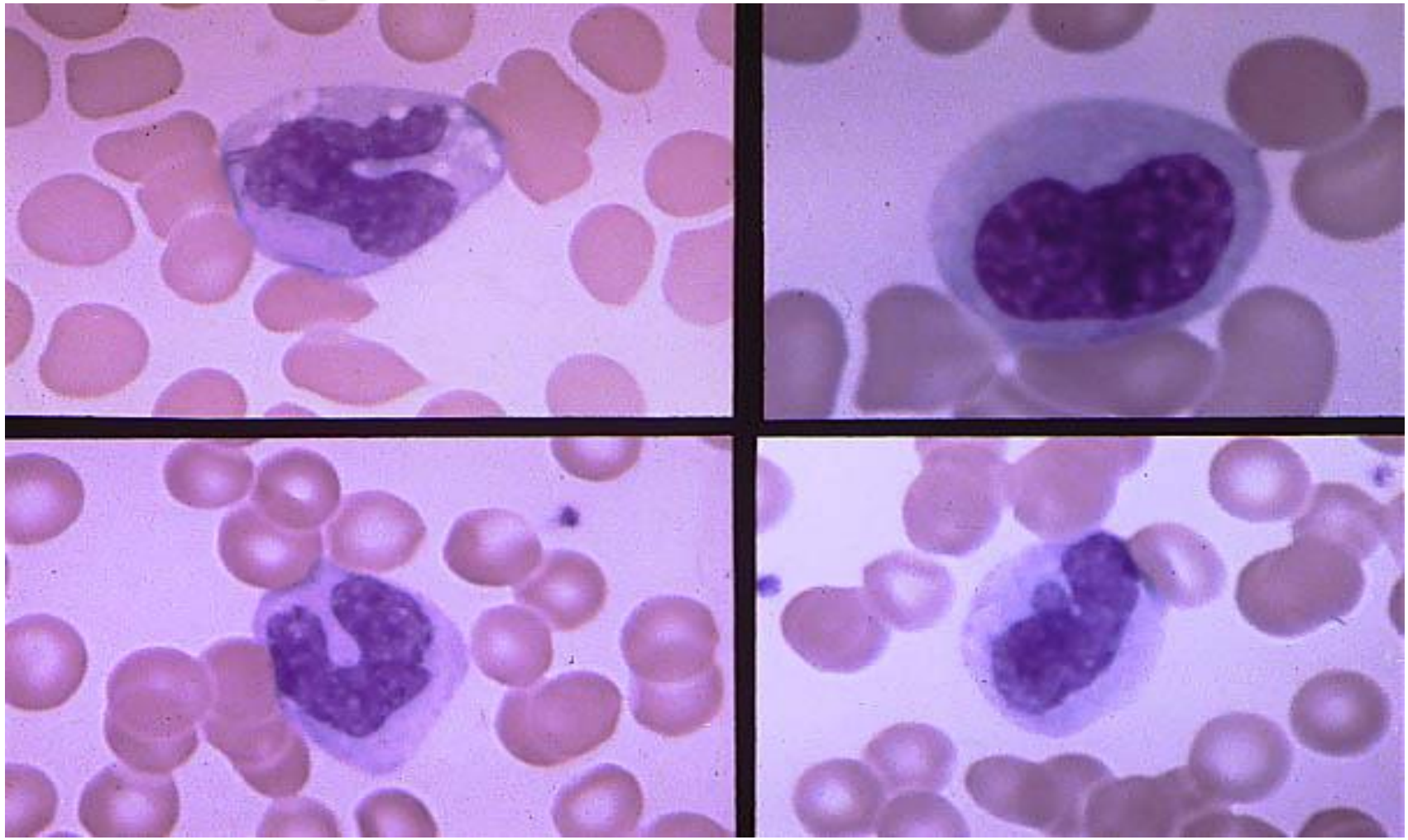
Eosinophil



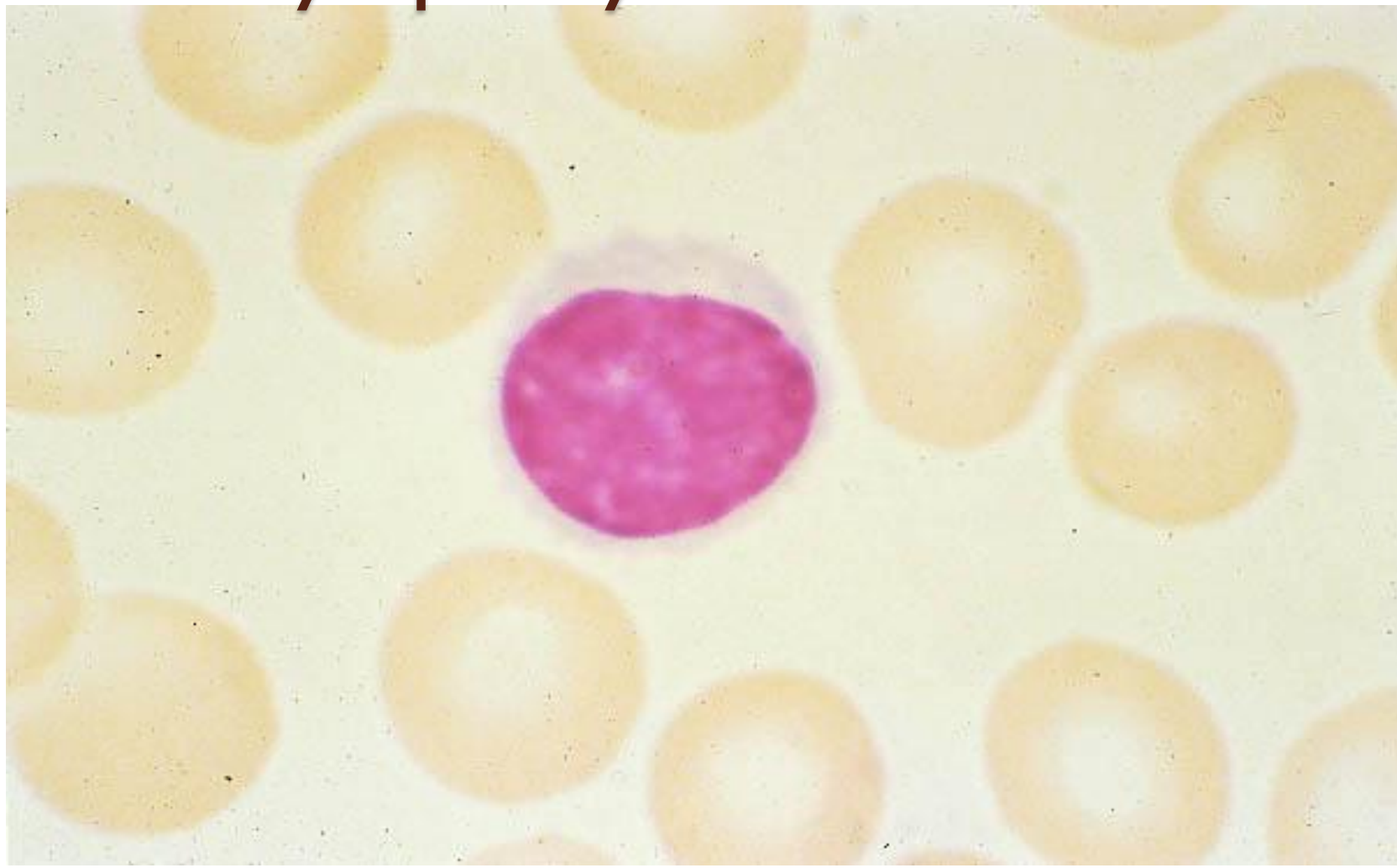
Monocytes



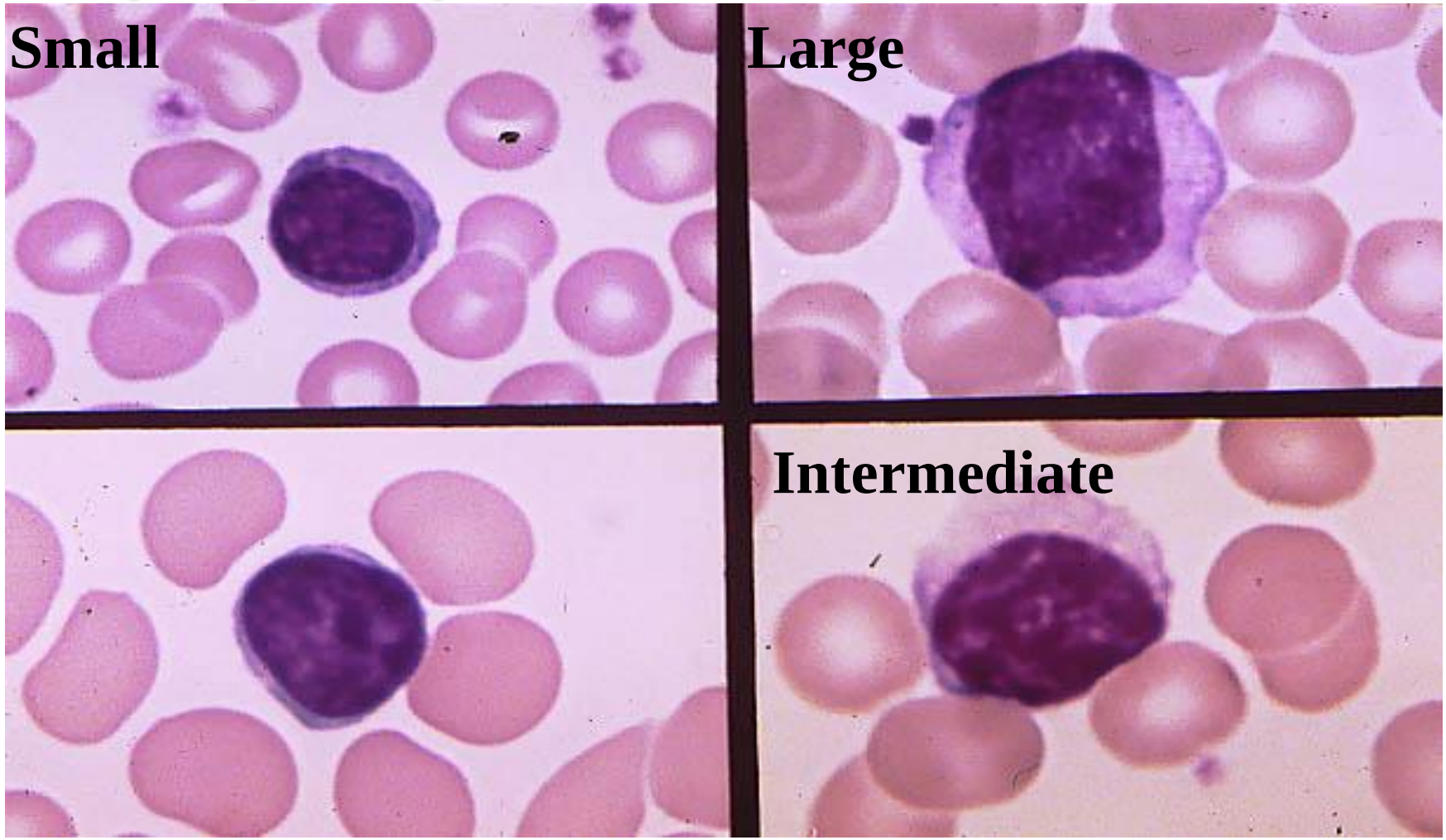
Monocytes



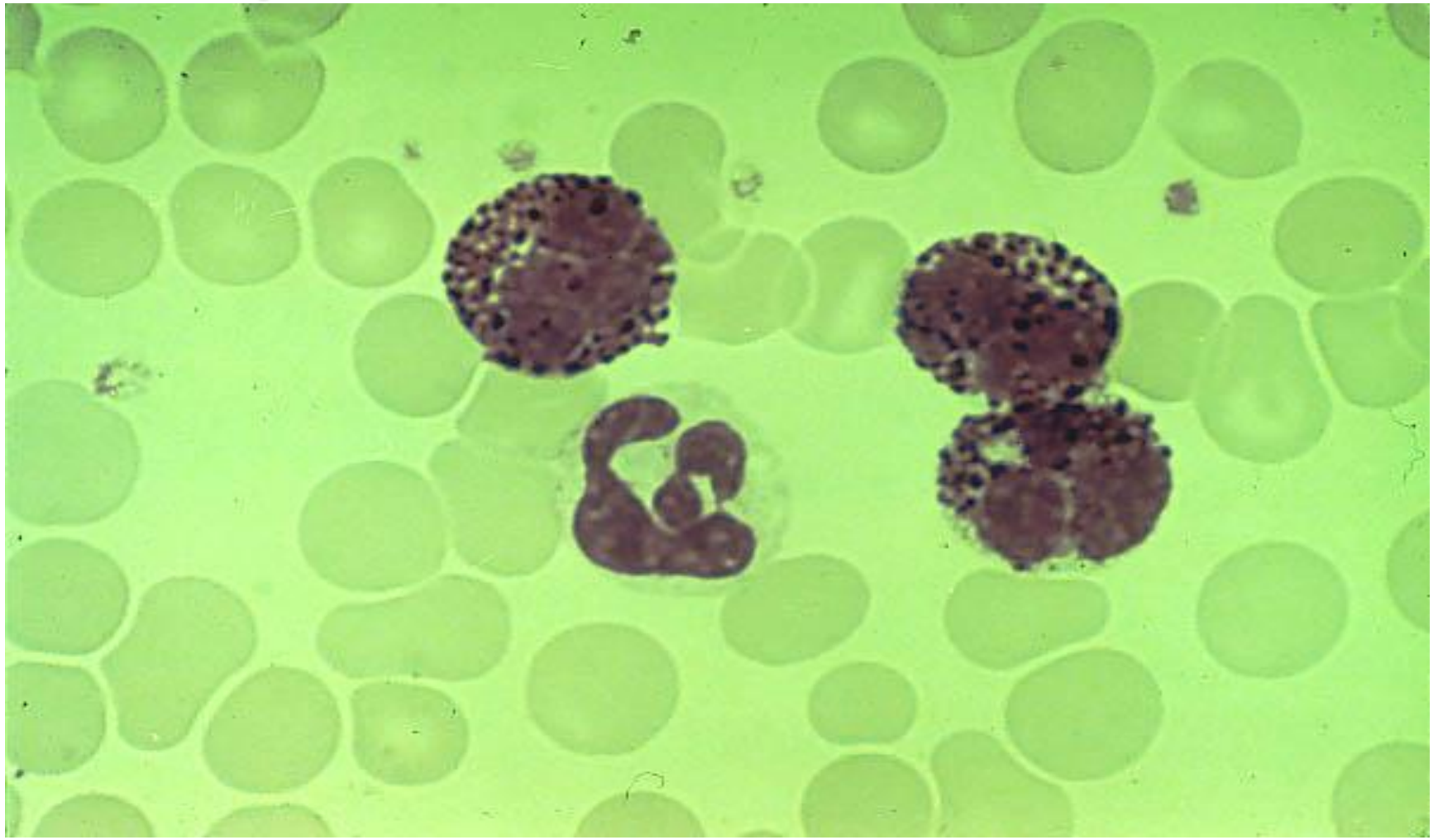
Small Lymphocyte



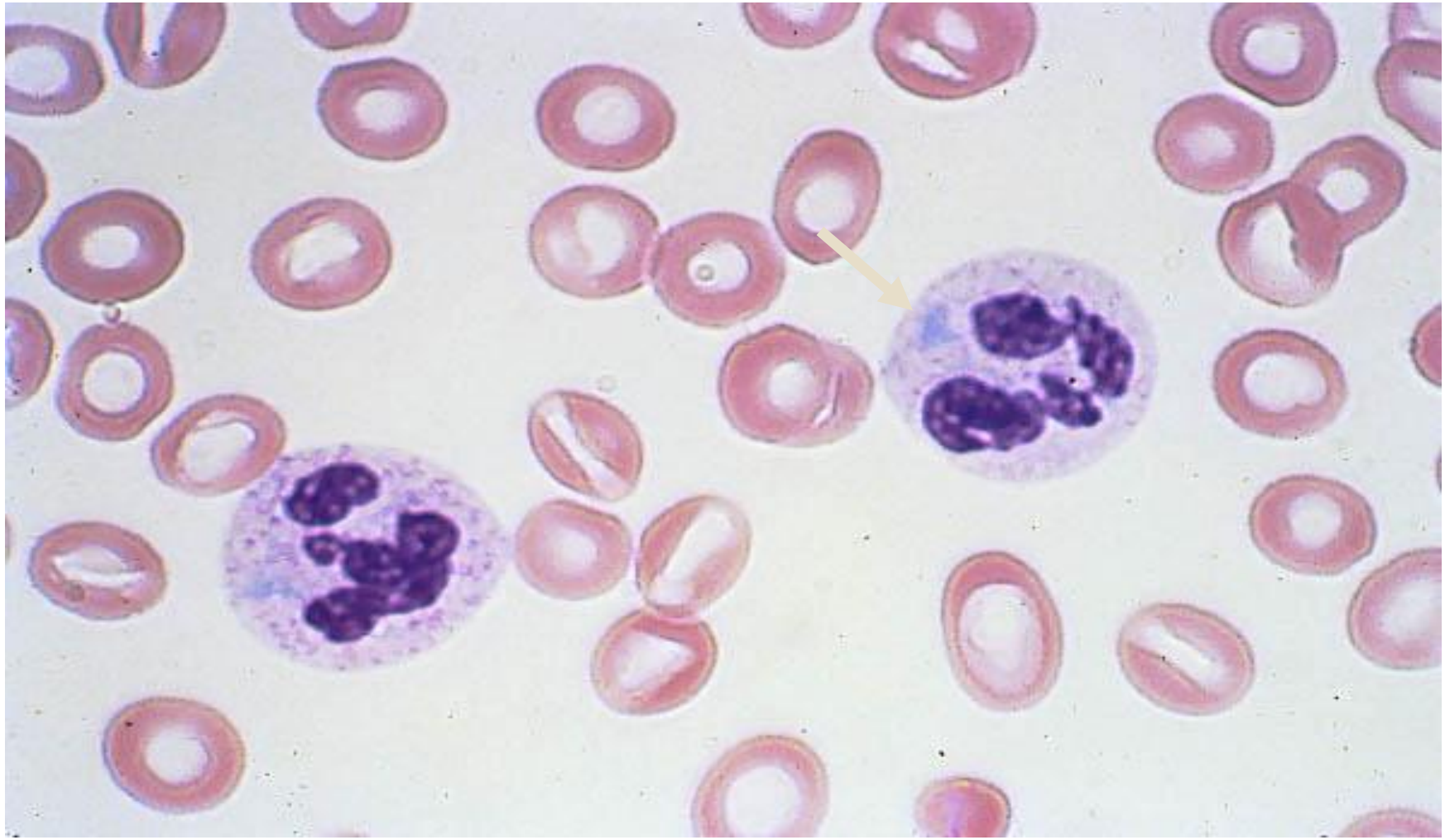
Lymphocytes



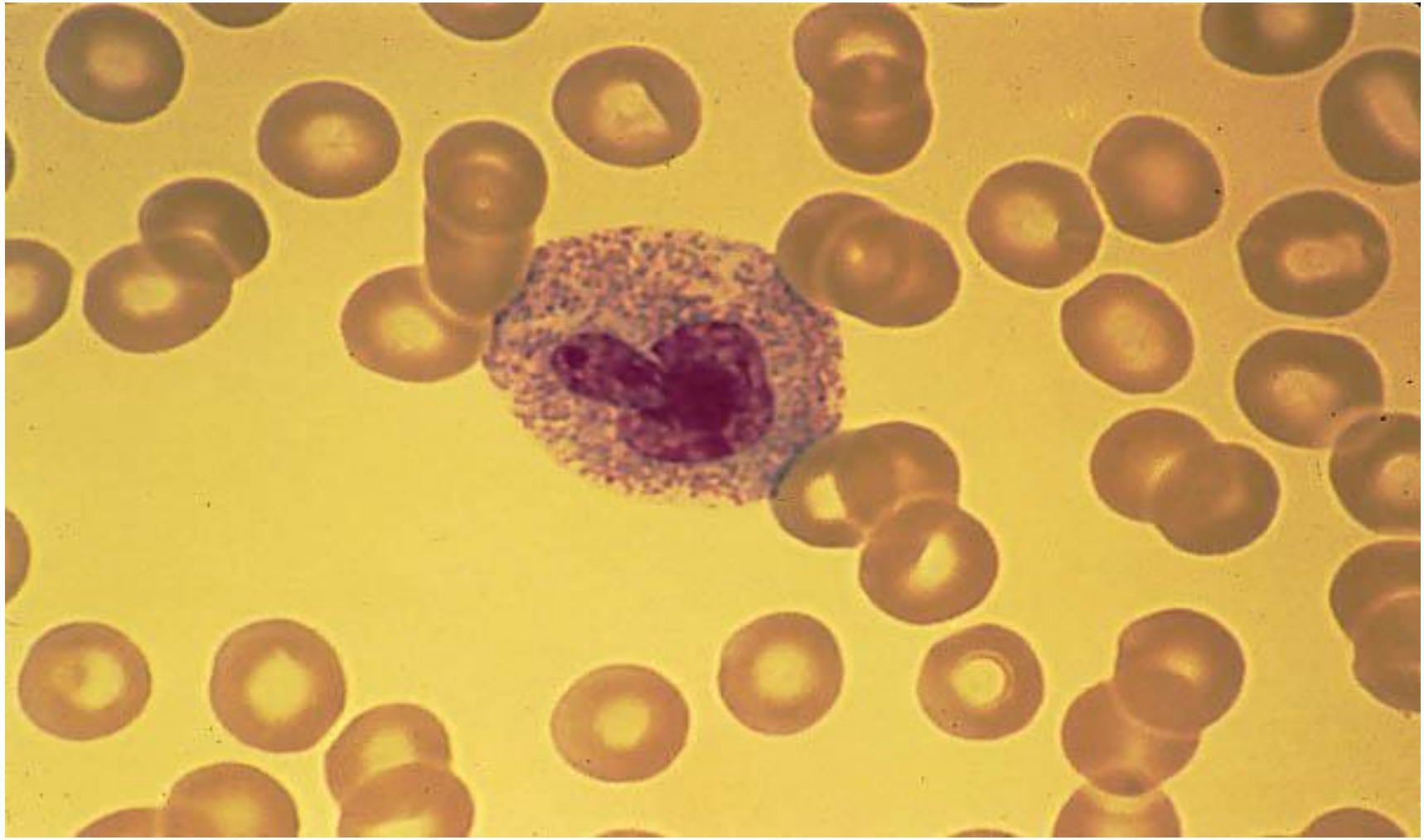
Basophils



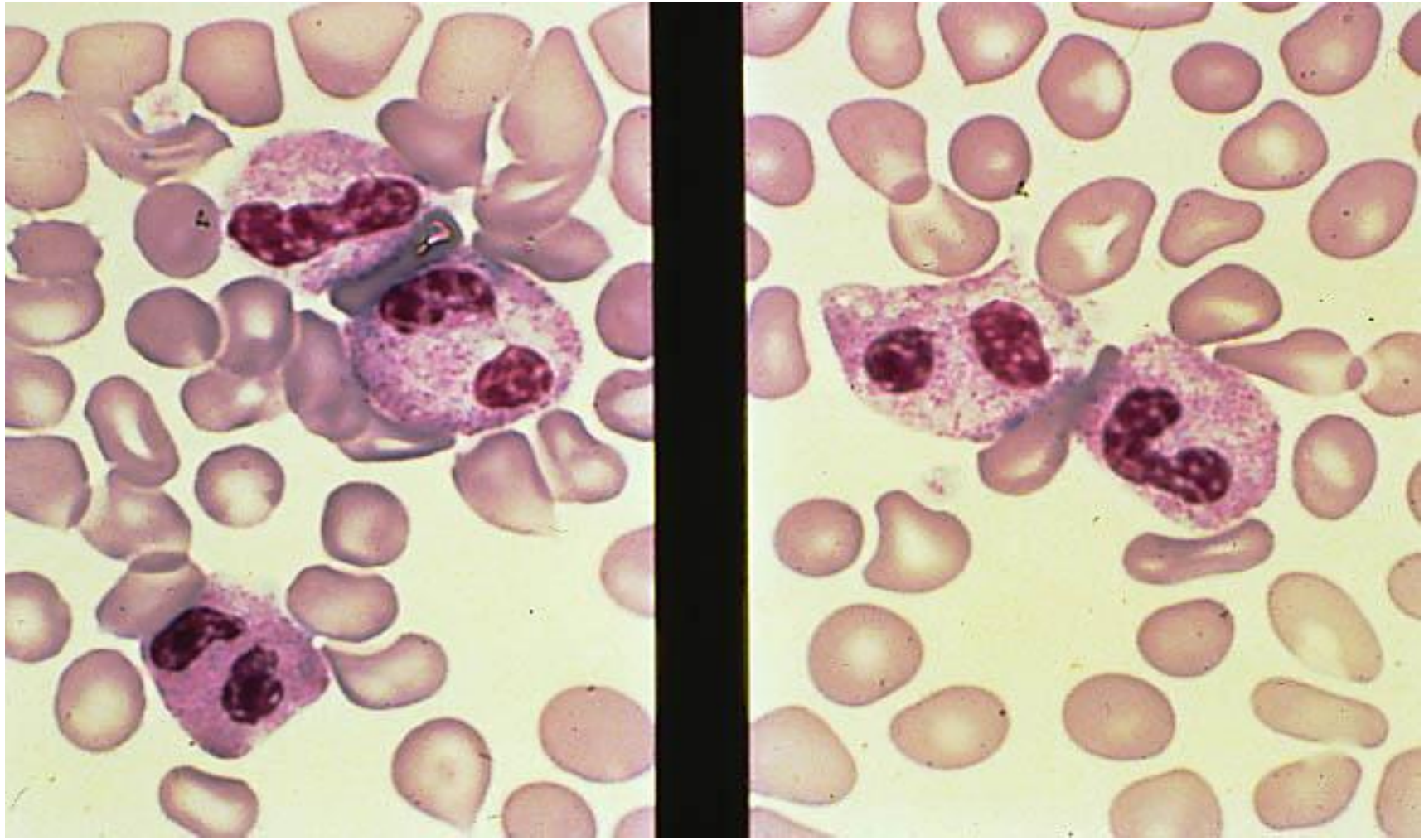
Dohle Bodies



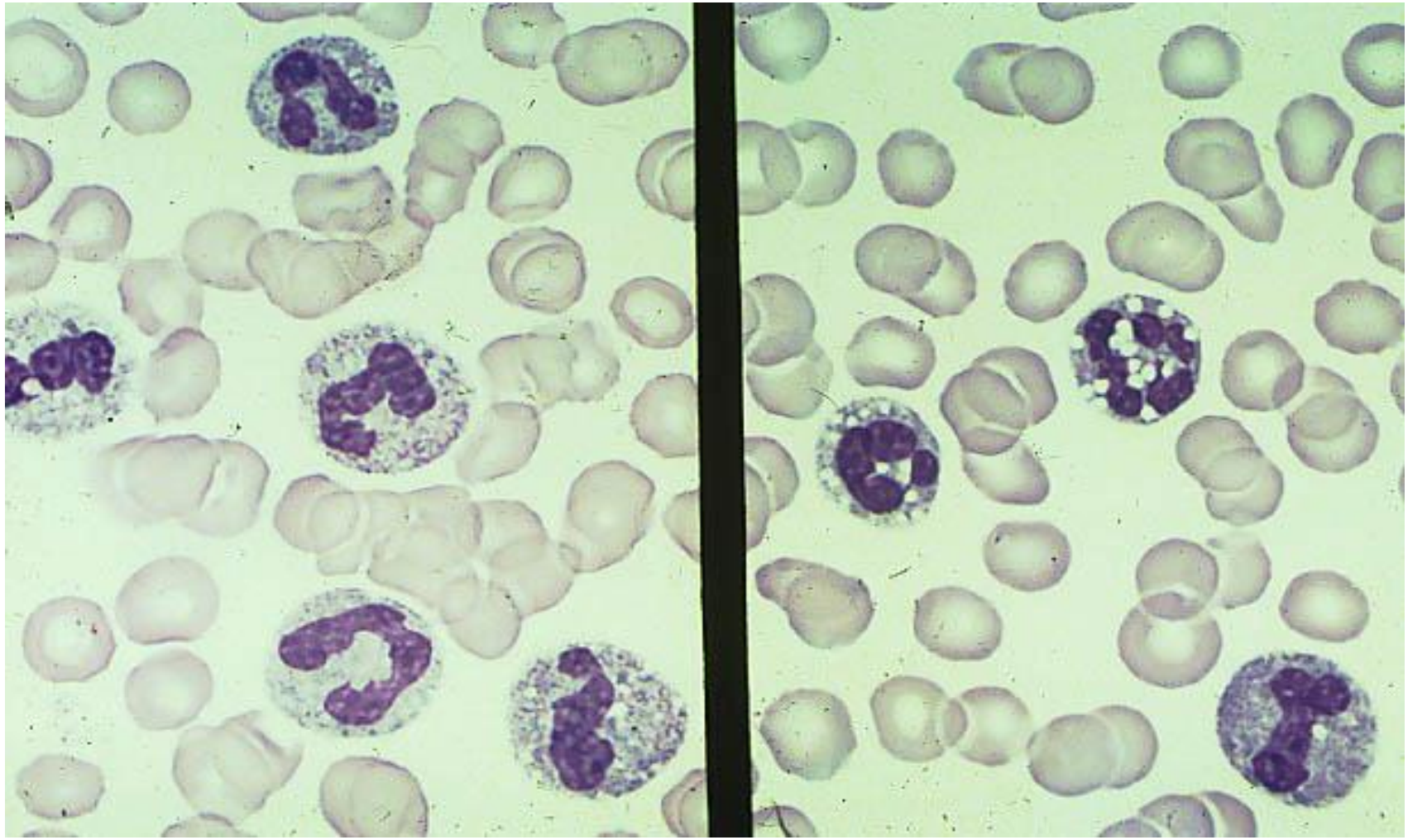
Toxic Granulation



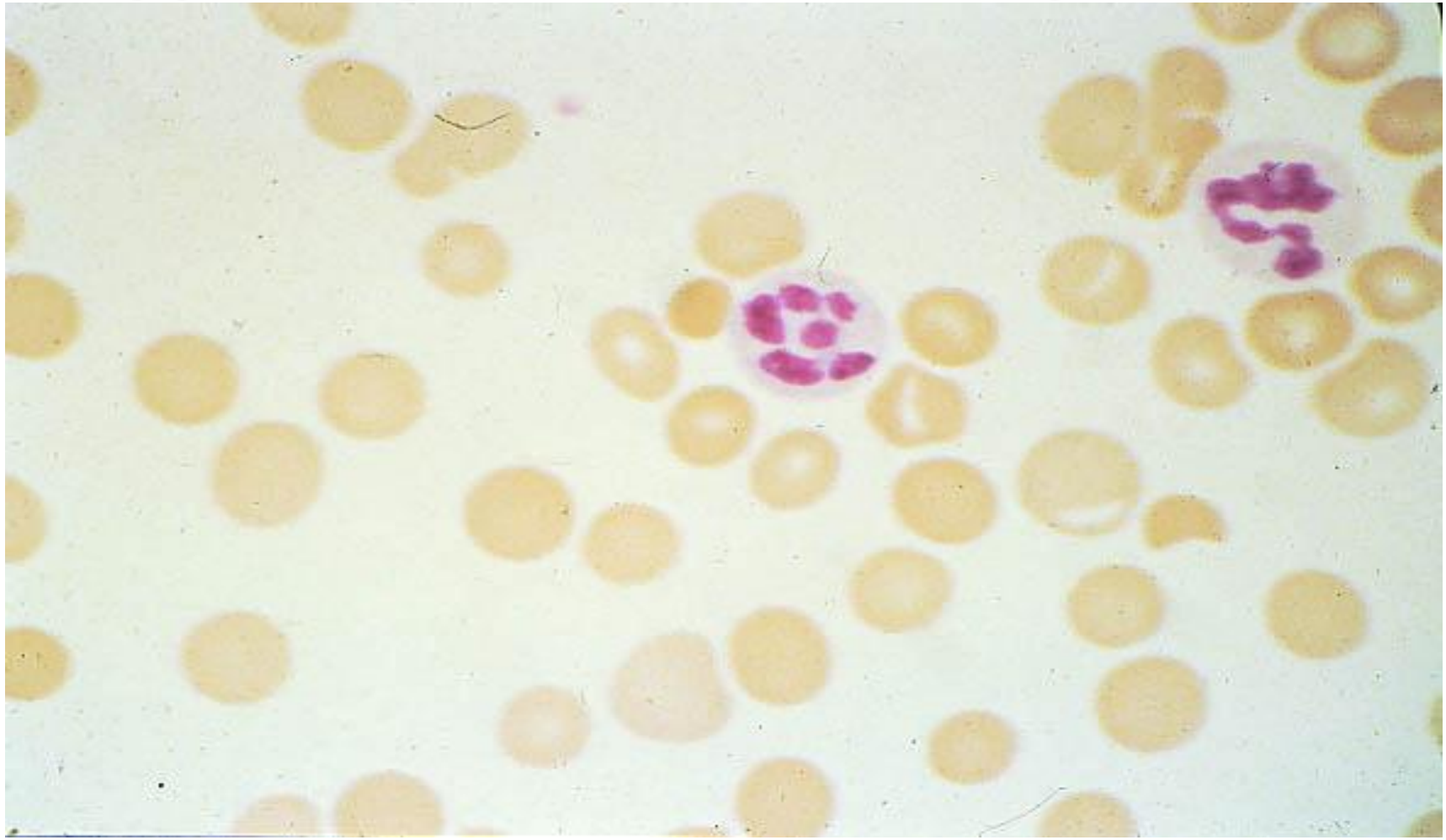
Toxic Granulation



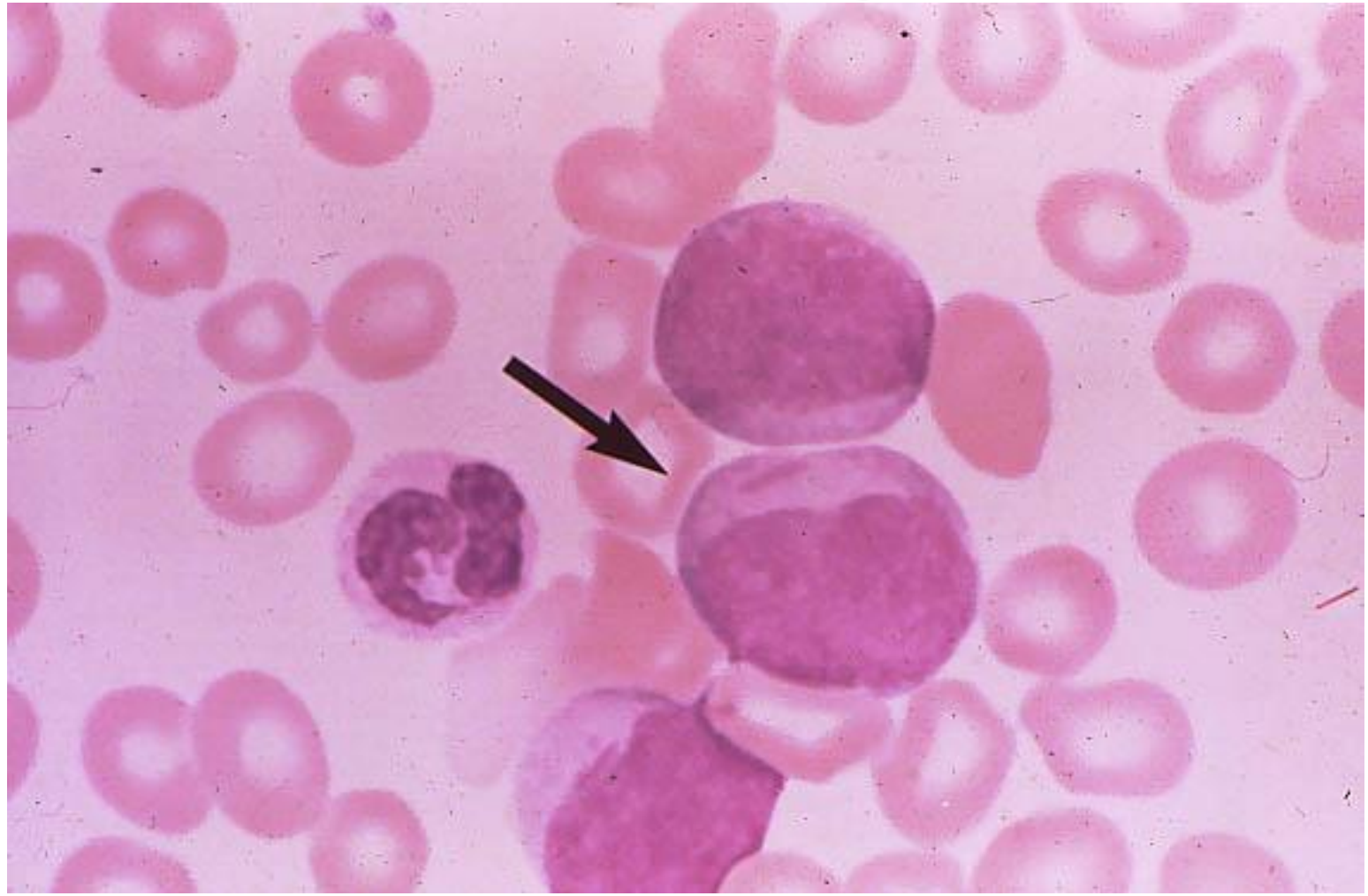
Toxic Granulation and Vacuole Formation



Hypersegmented Neutrophils



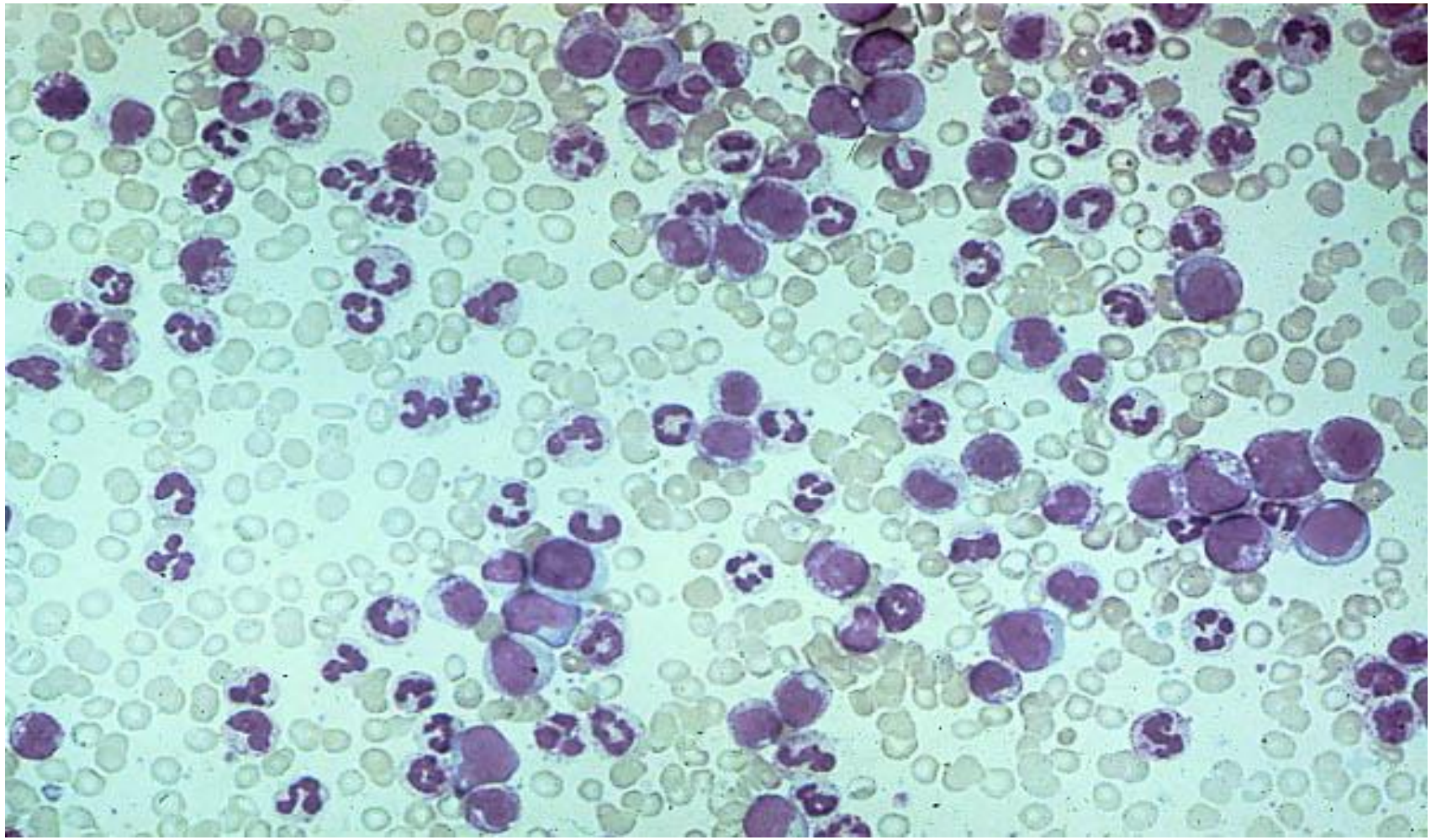
Auer Rod: Acute Myeloid Leukemia



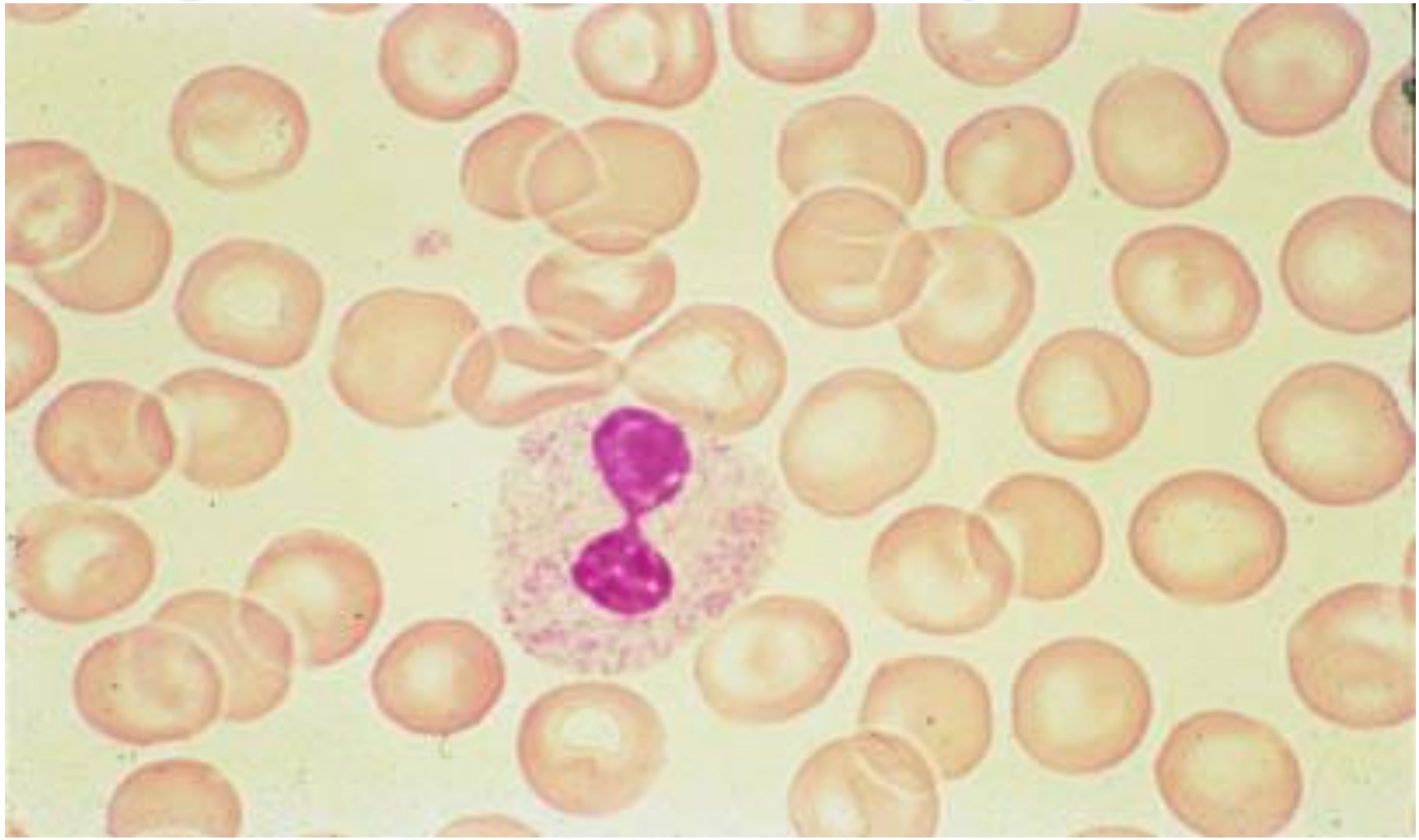
Myeloid Leukemias and Leukemoid Reaction

- Bone marrow exam is almost always indicated
  - Cytogenetic analysis
  - Flow cytometry analysis

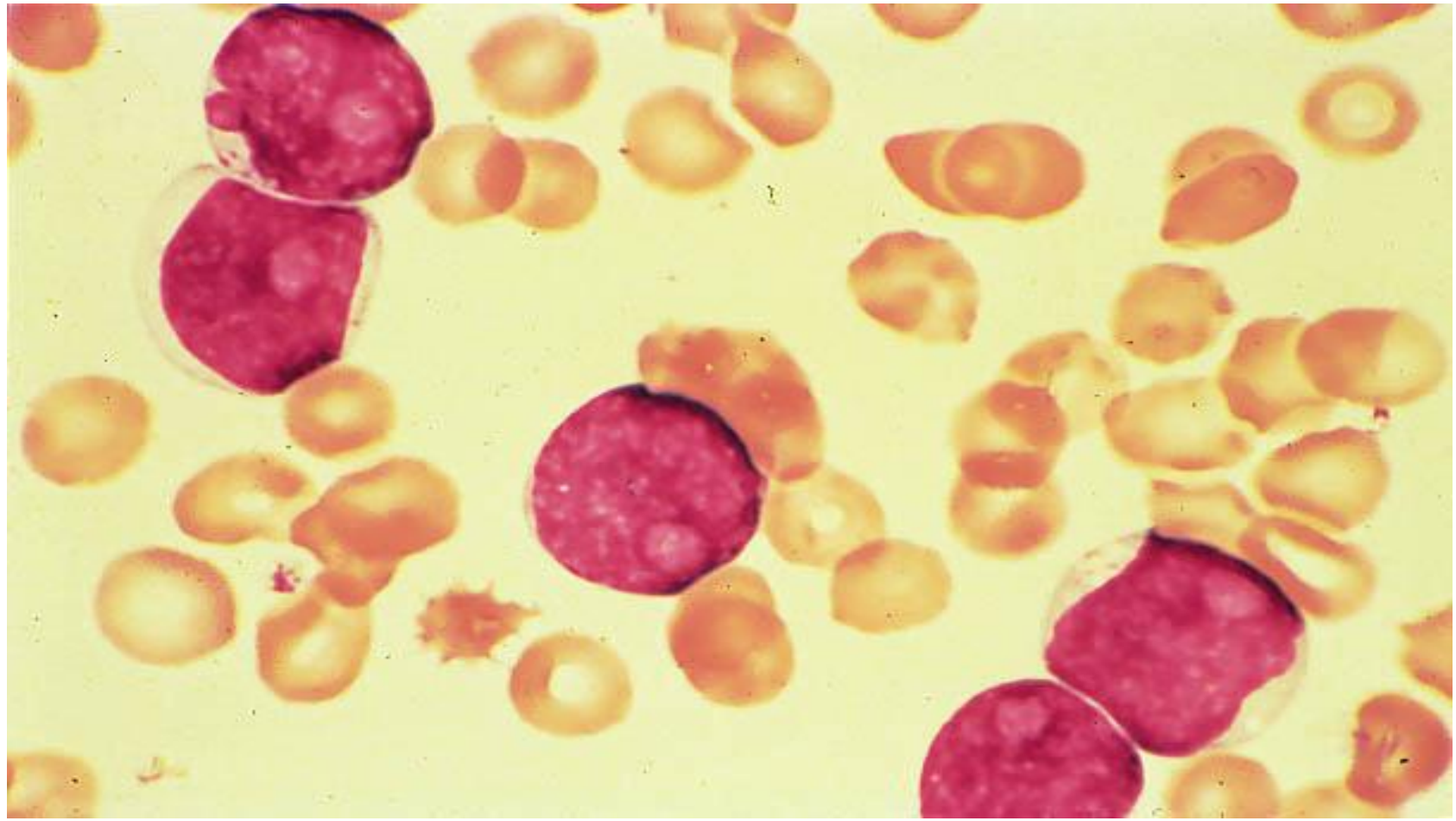
Neutrophilia: CML



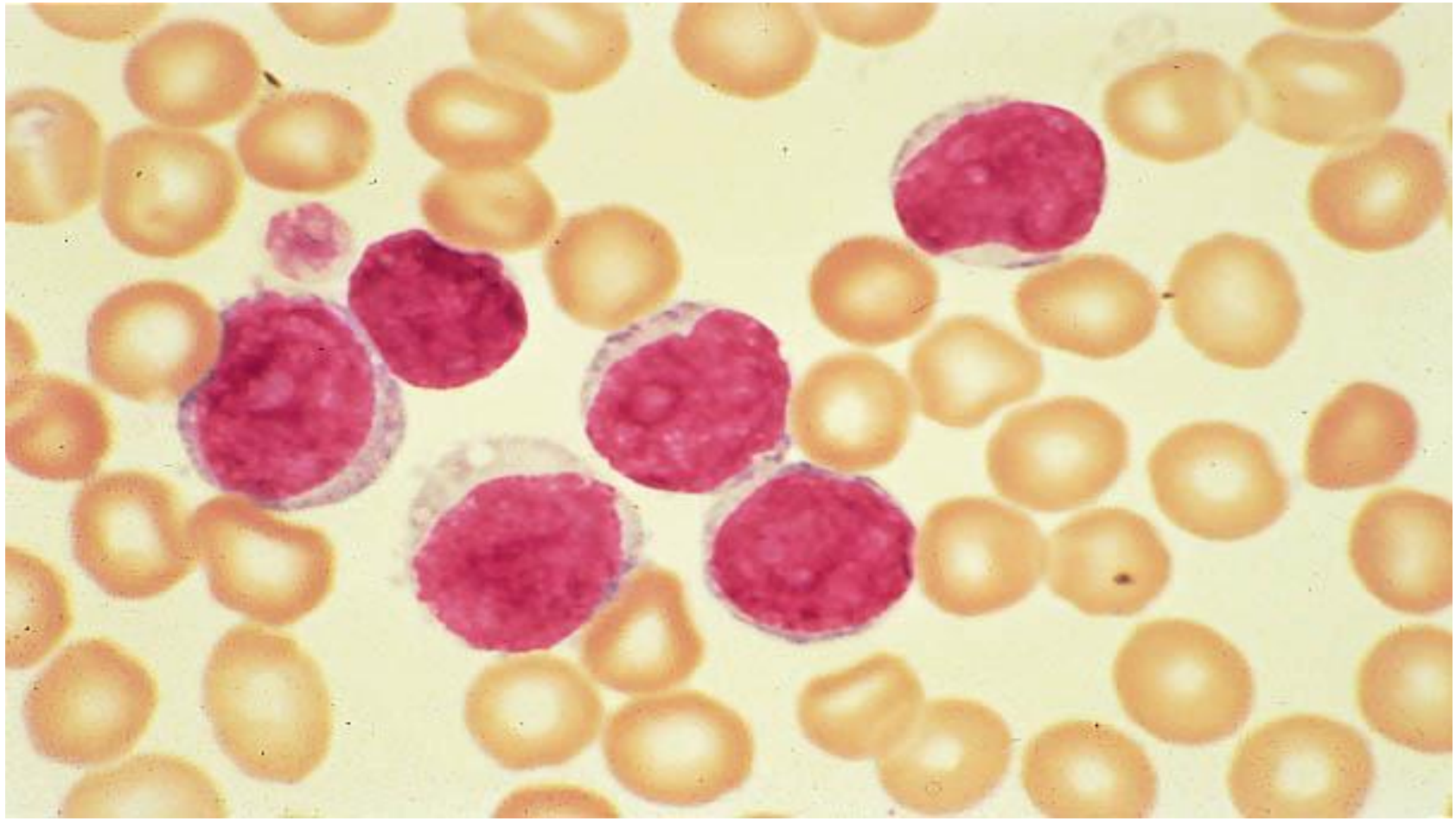
Pelger-Huet Abnormality



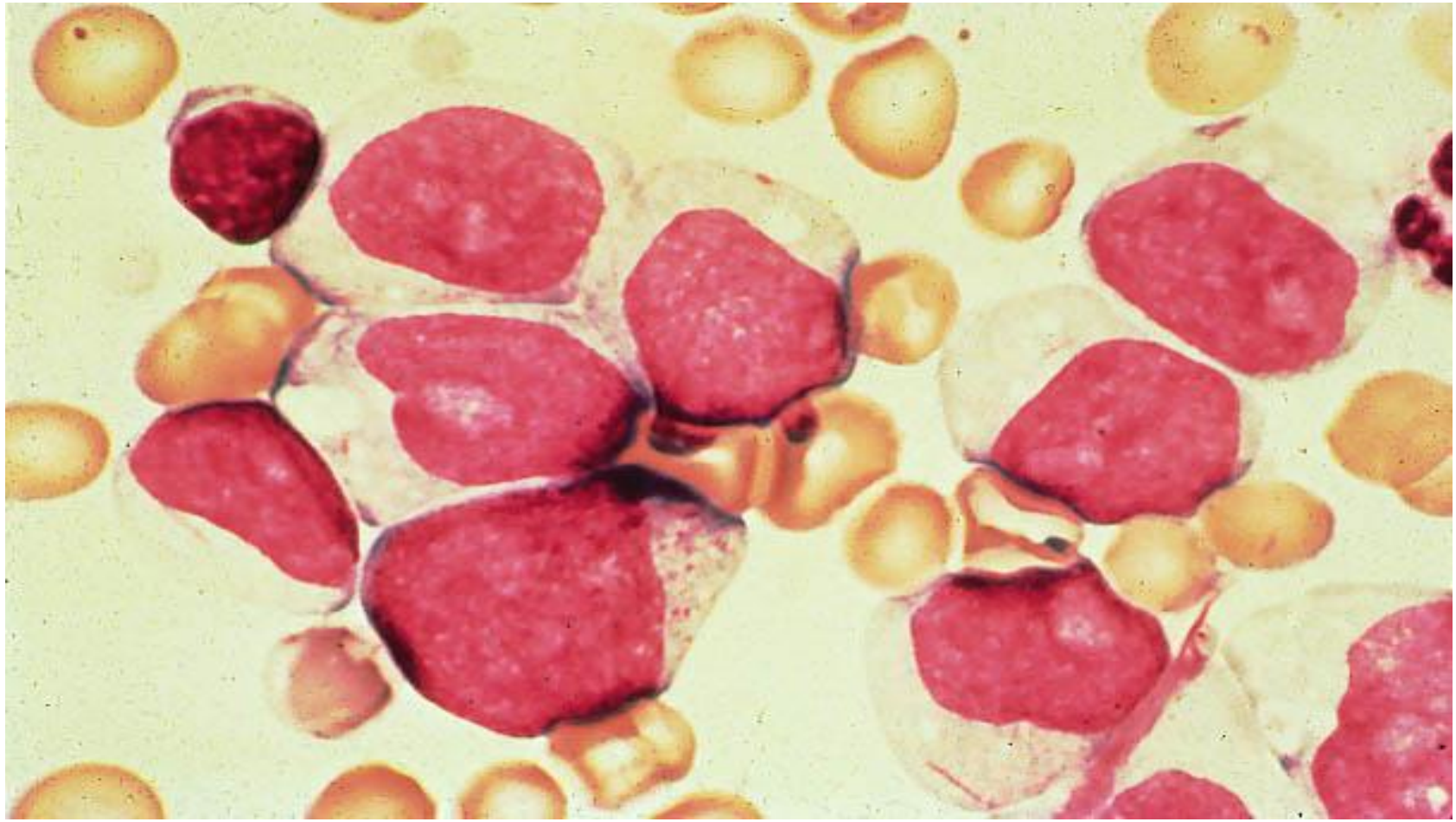
Acute Myeloid Leukemia: M1 Myeloblasts without Differentiation



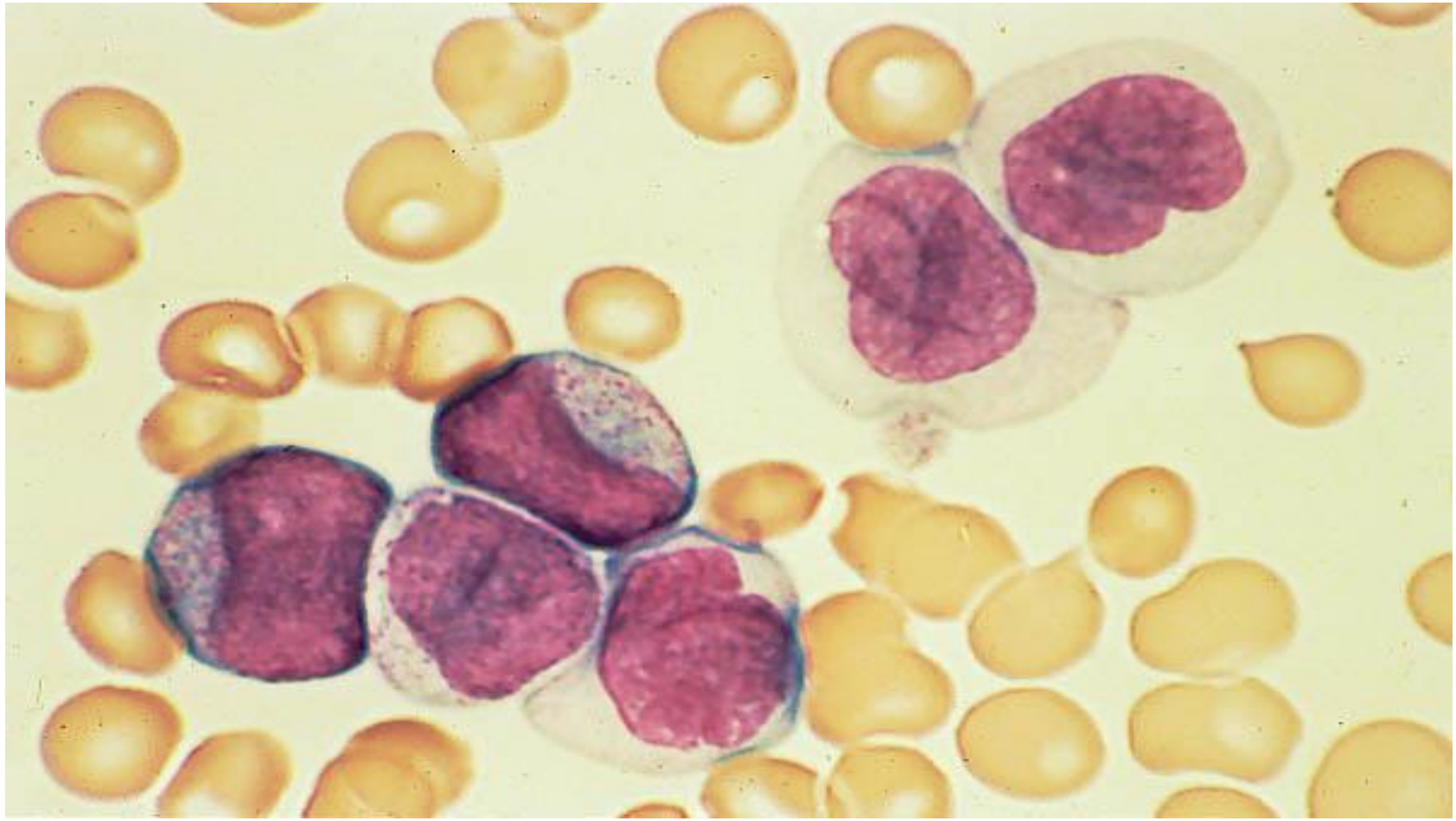
Acute Myeloid Leukemia: M2 Myeloblasts with Some Differentiation



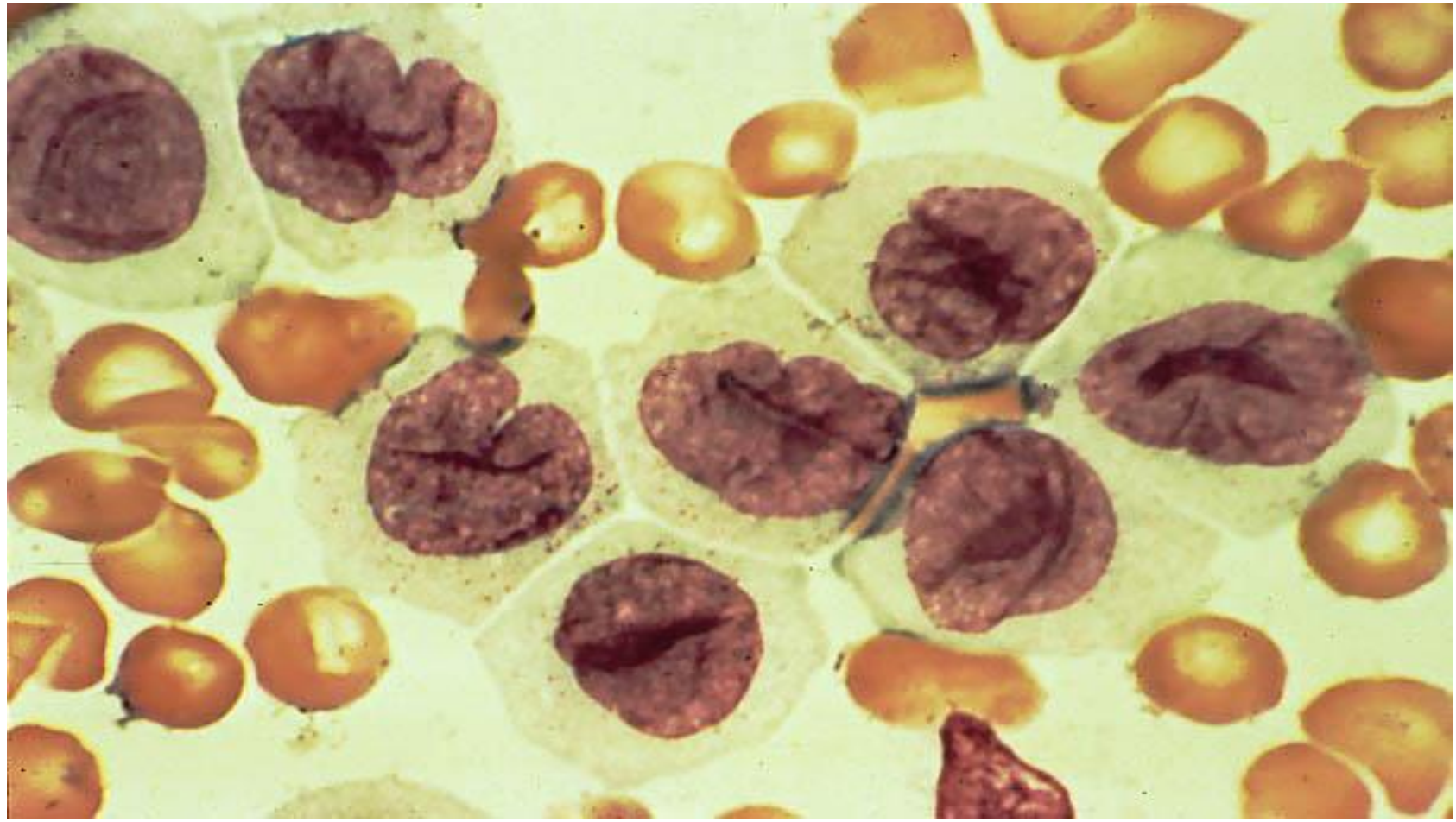
Acute Myeloid Leukemia: M3 Promyelocytic Leukemia



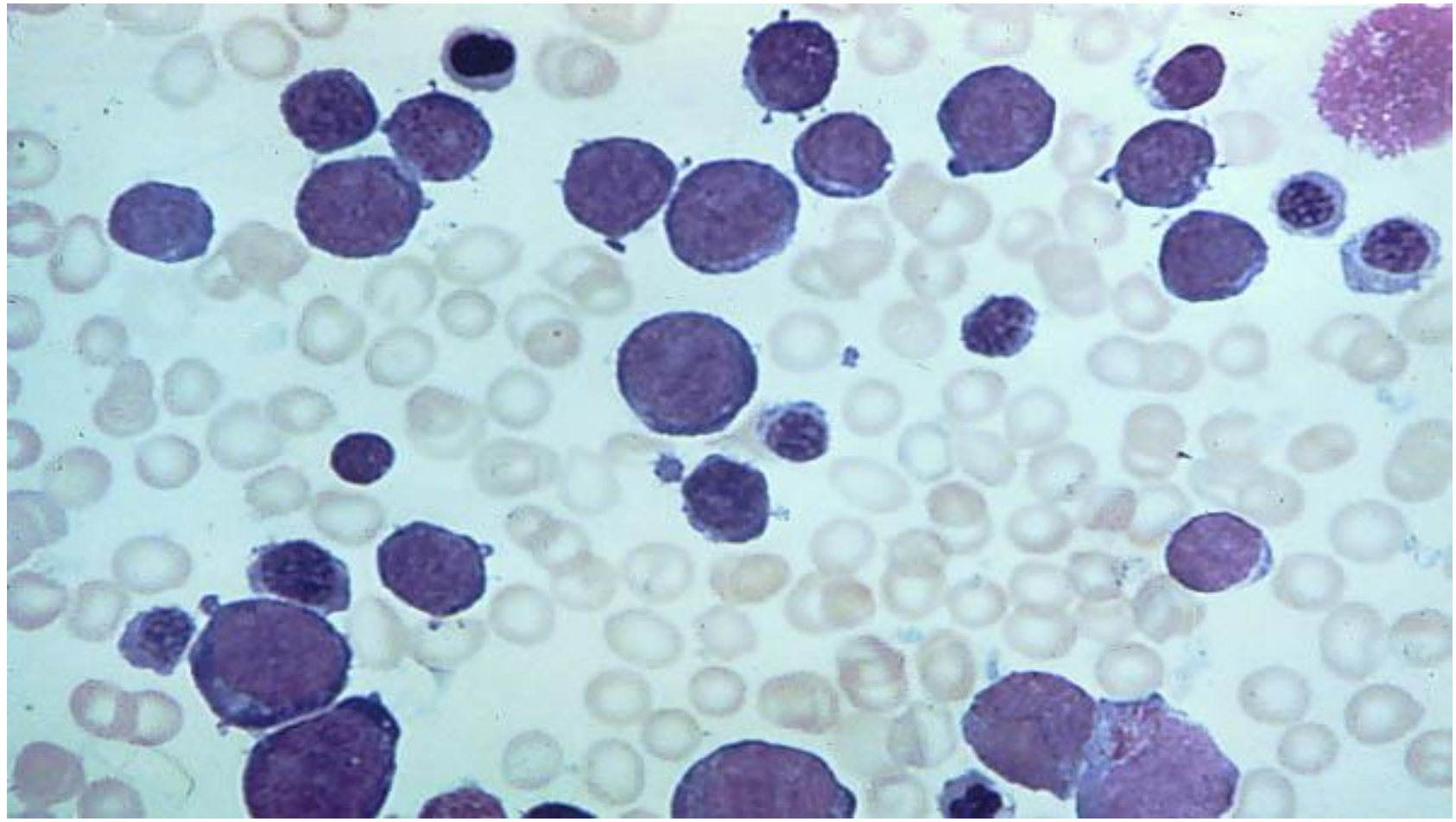
Acute Myeloid Leukemia: M4 Myelomonocytic Leukemia



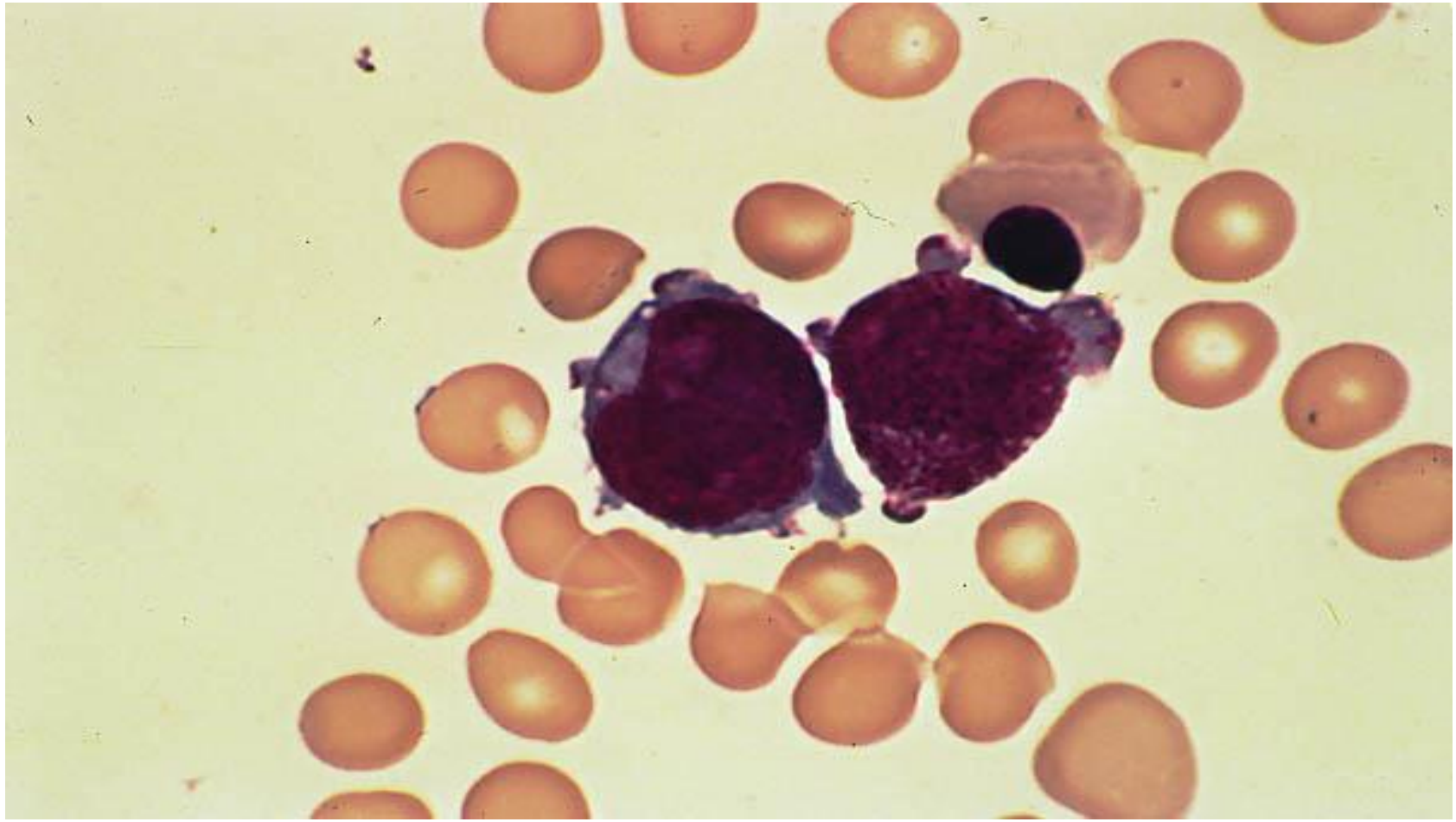
Acute Myeloid Leukemia: M5 Monocytic Leukemia



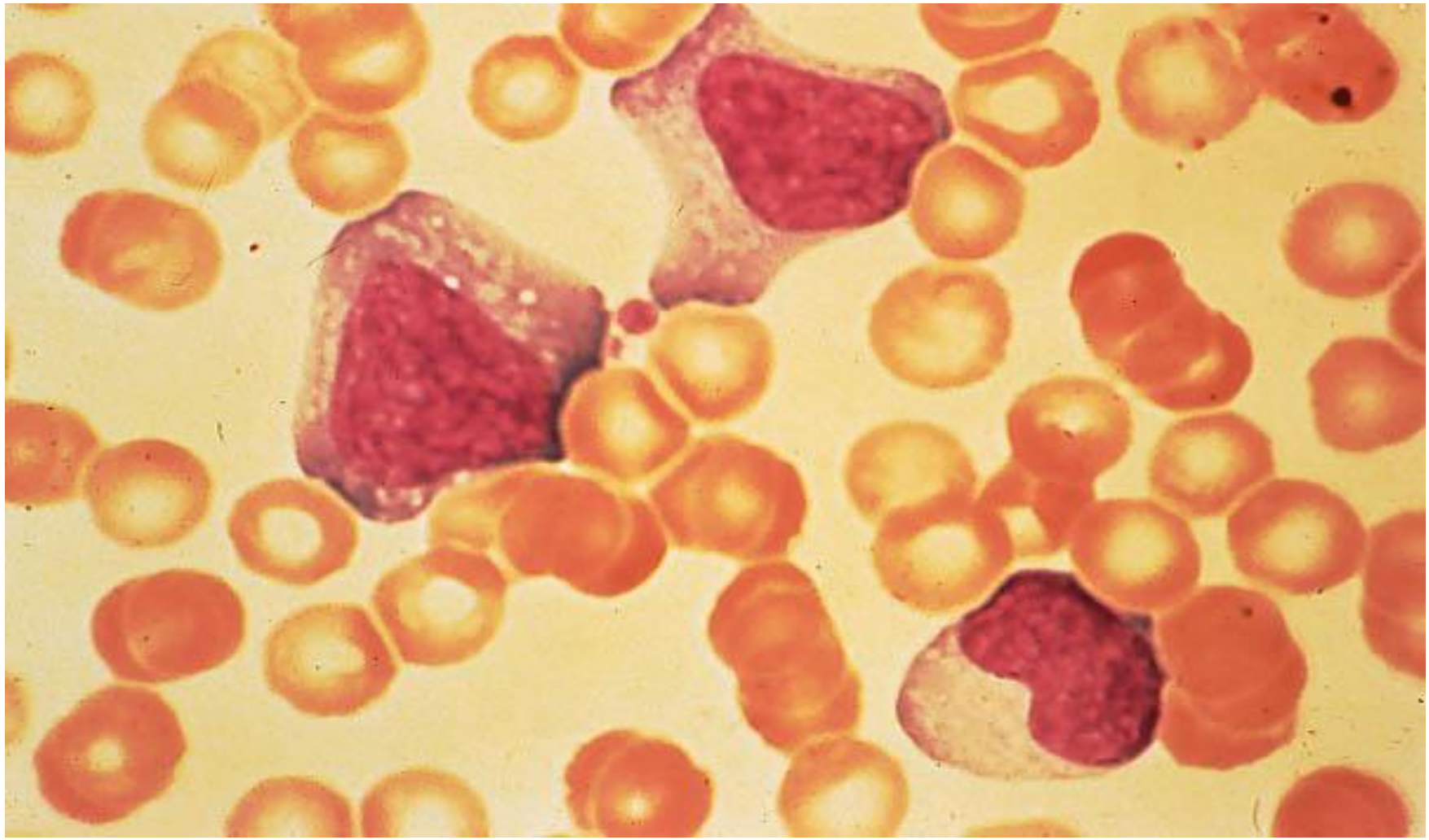
Acute Myeloid Leukemia: M6 Erythroleukemia



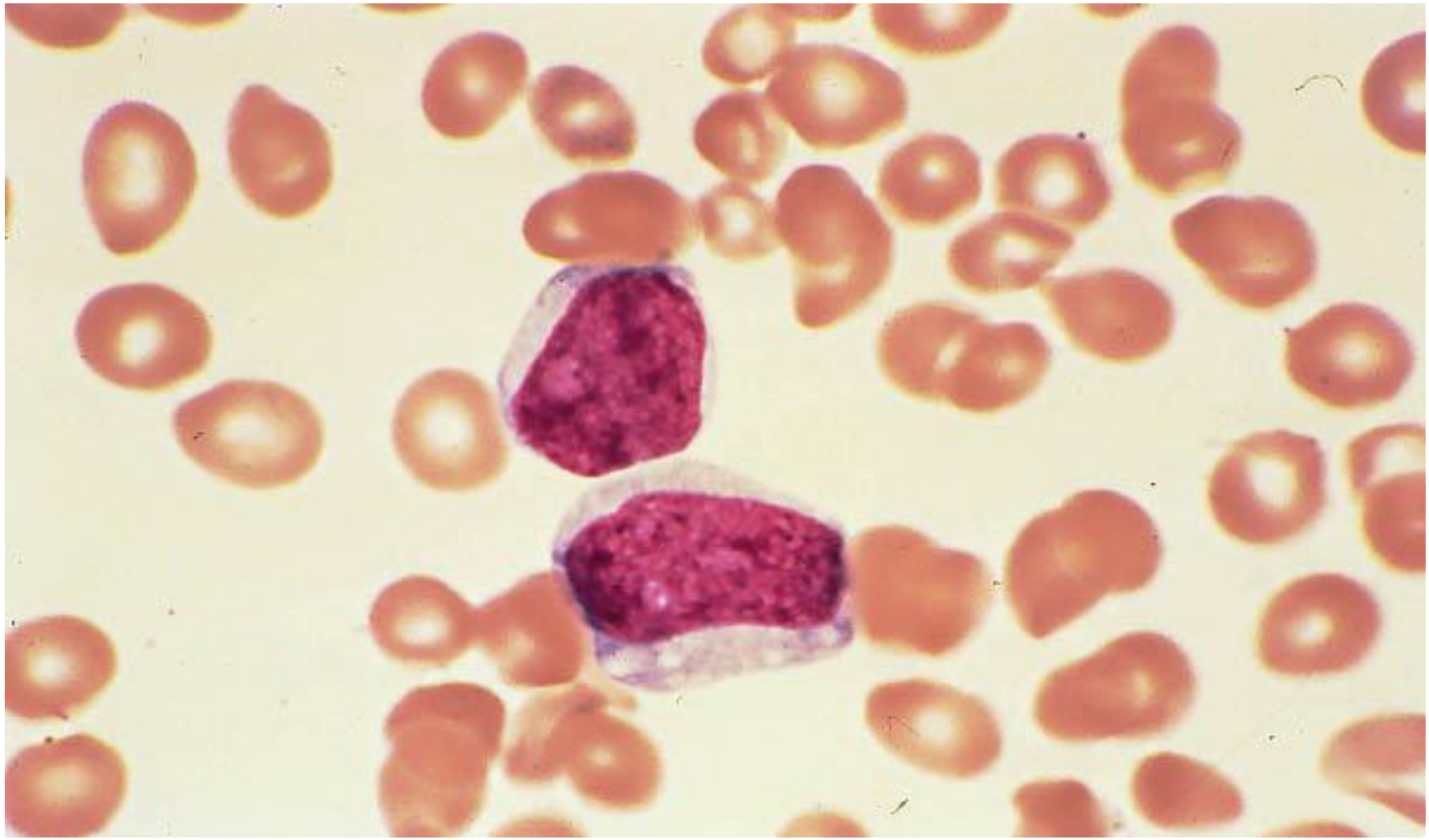
Acute Myeloid Leukemia: M7 Megakaryocytic Leukemia



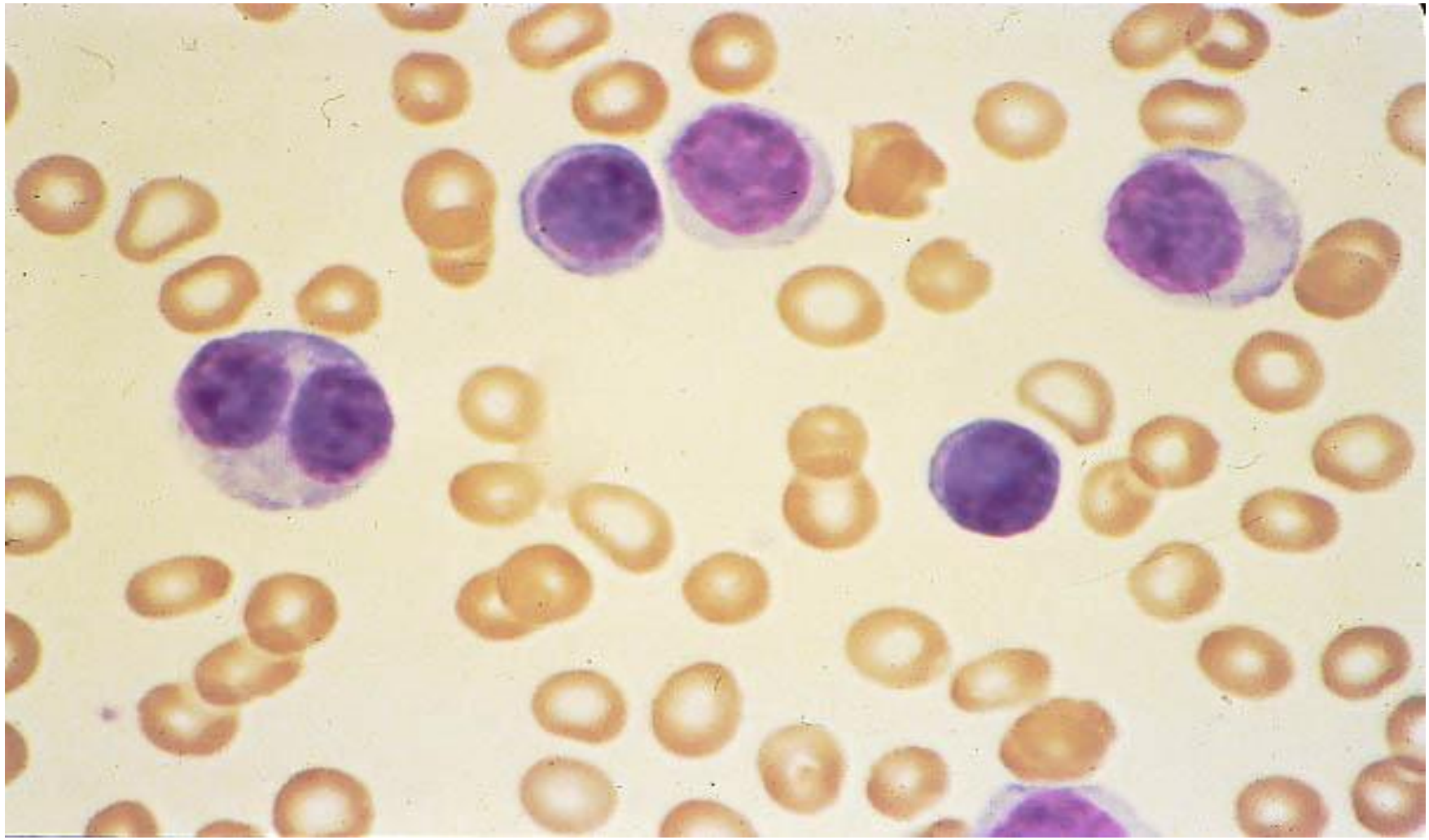
Atypical (Reactive) Lymphocytes



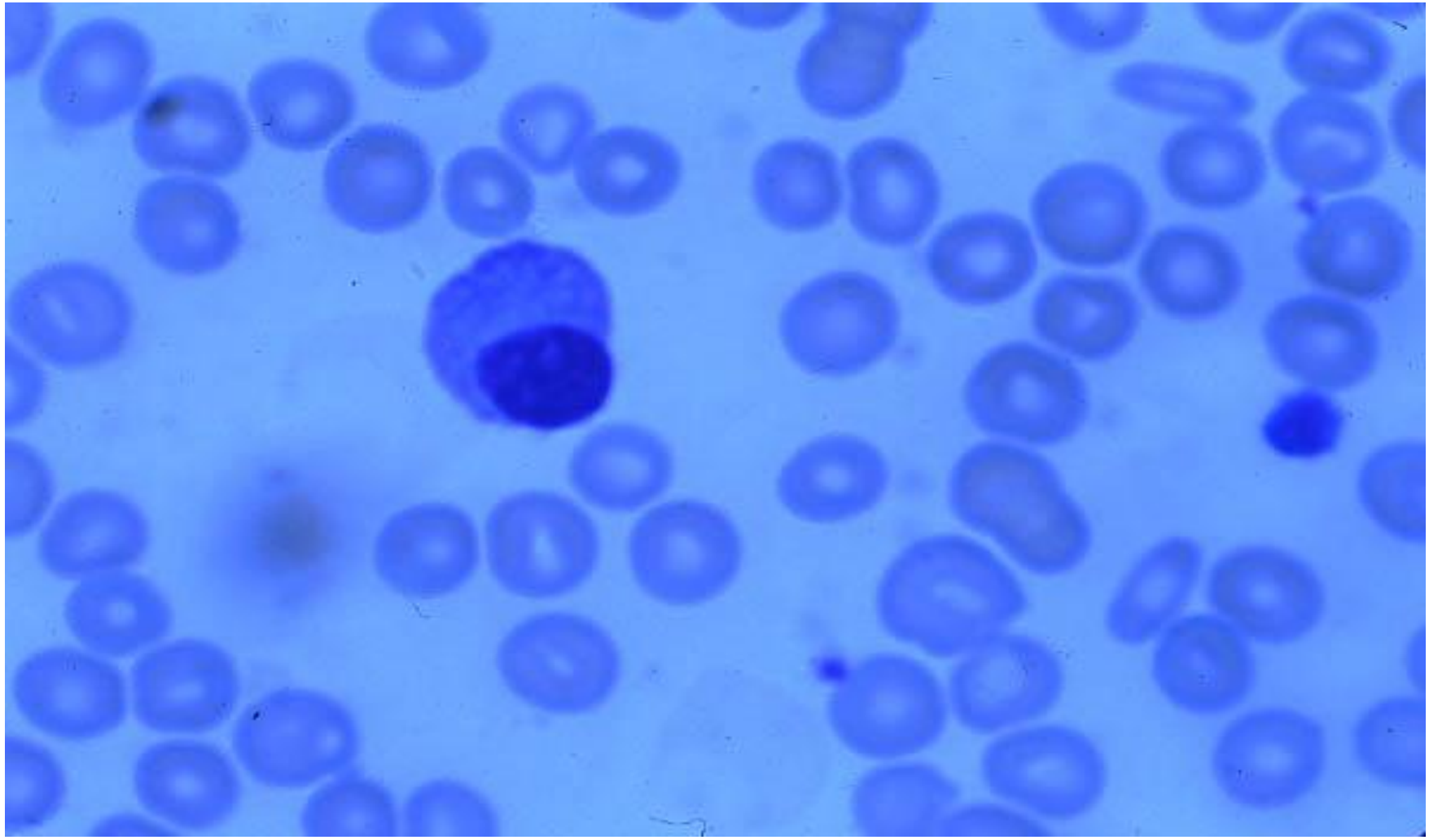
Atypical (Reactive) Lymphocytes



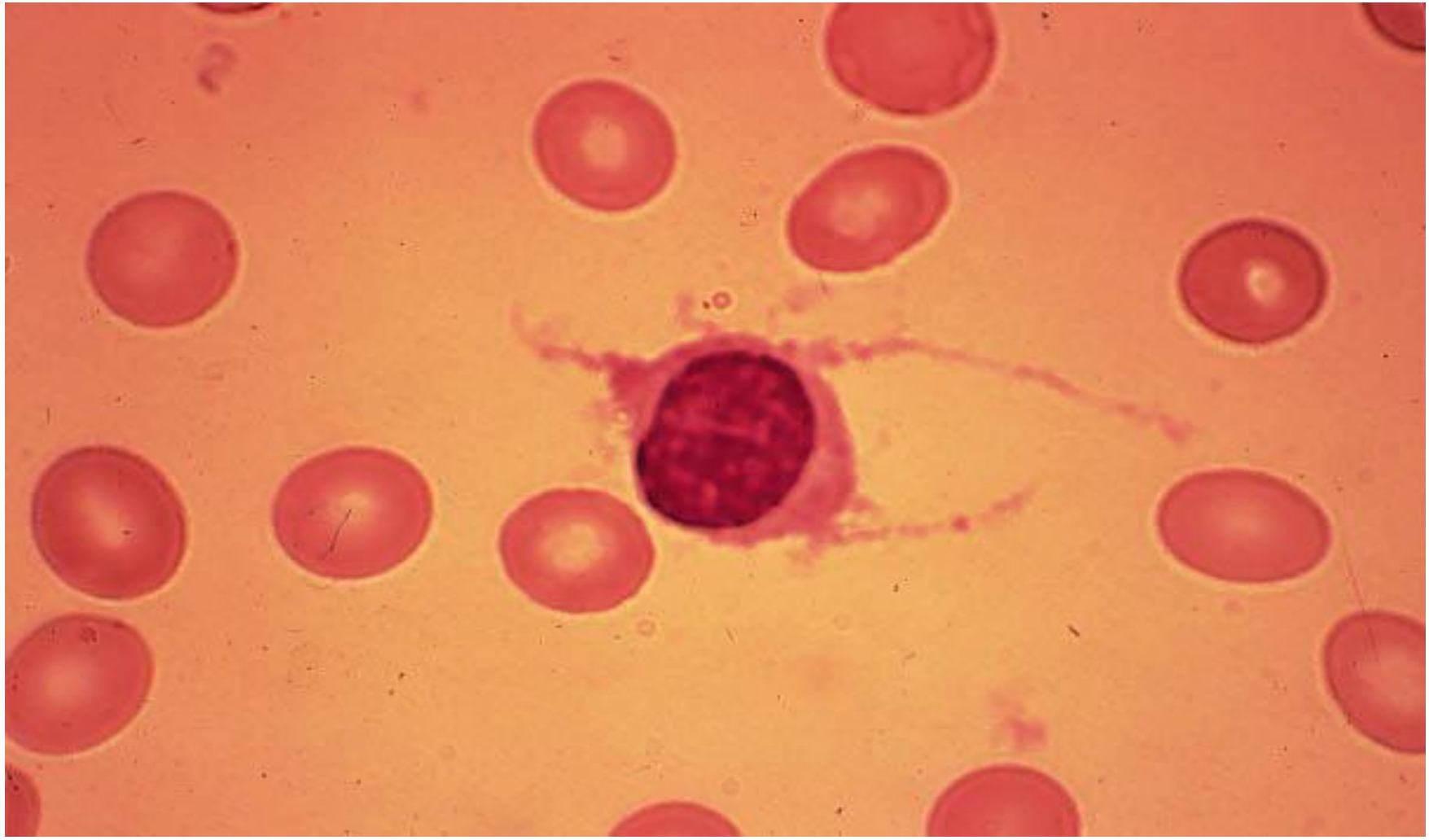
Plasmacytoid Lymphocytes



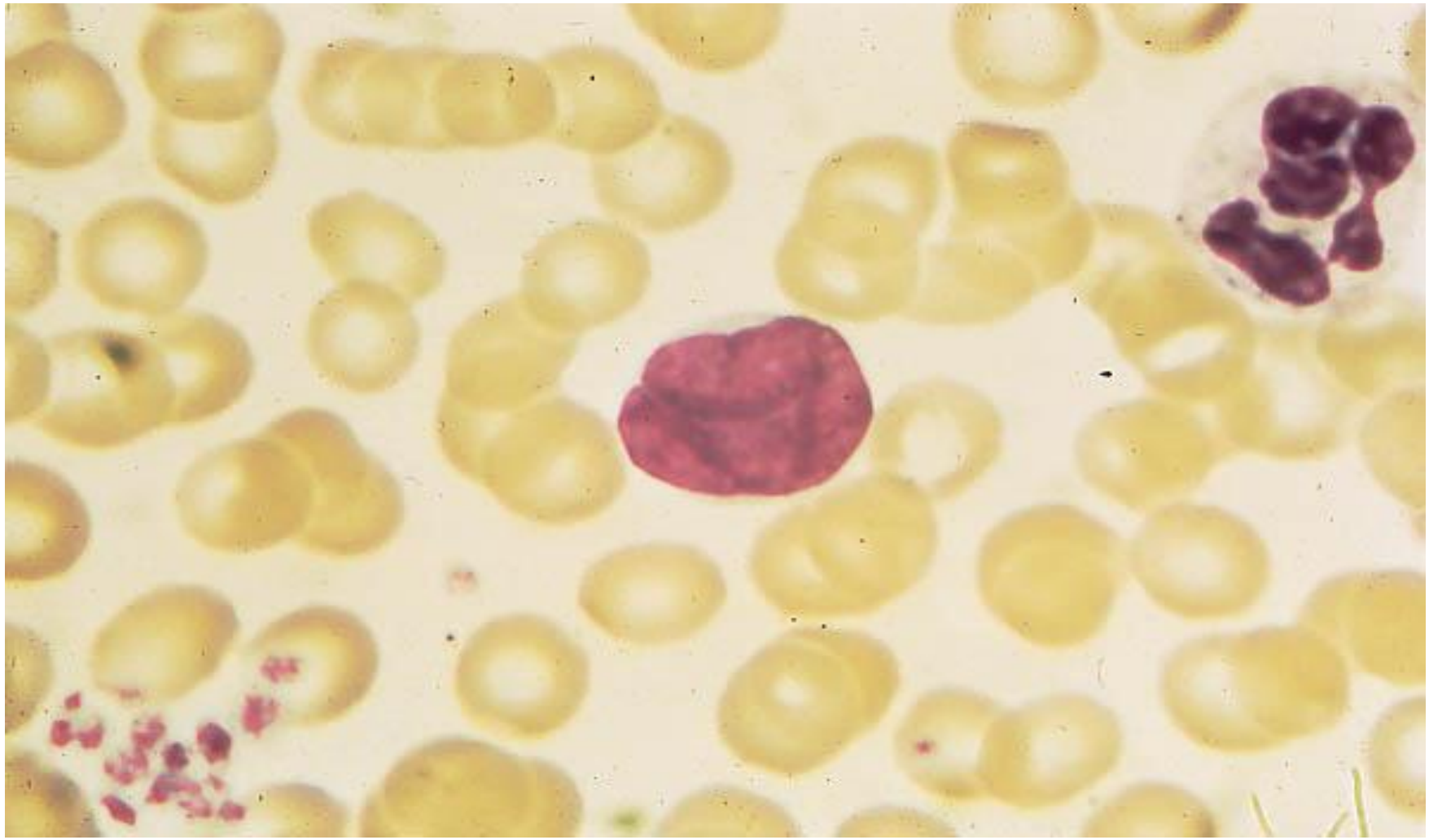
Plasma Cell: Plasma Cell Leukemia



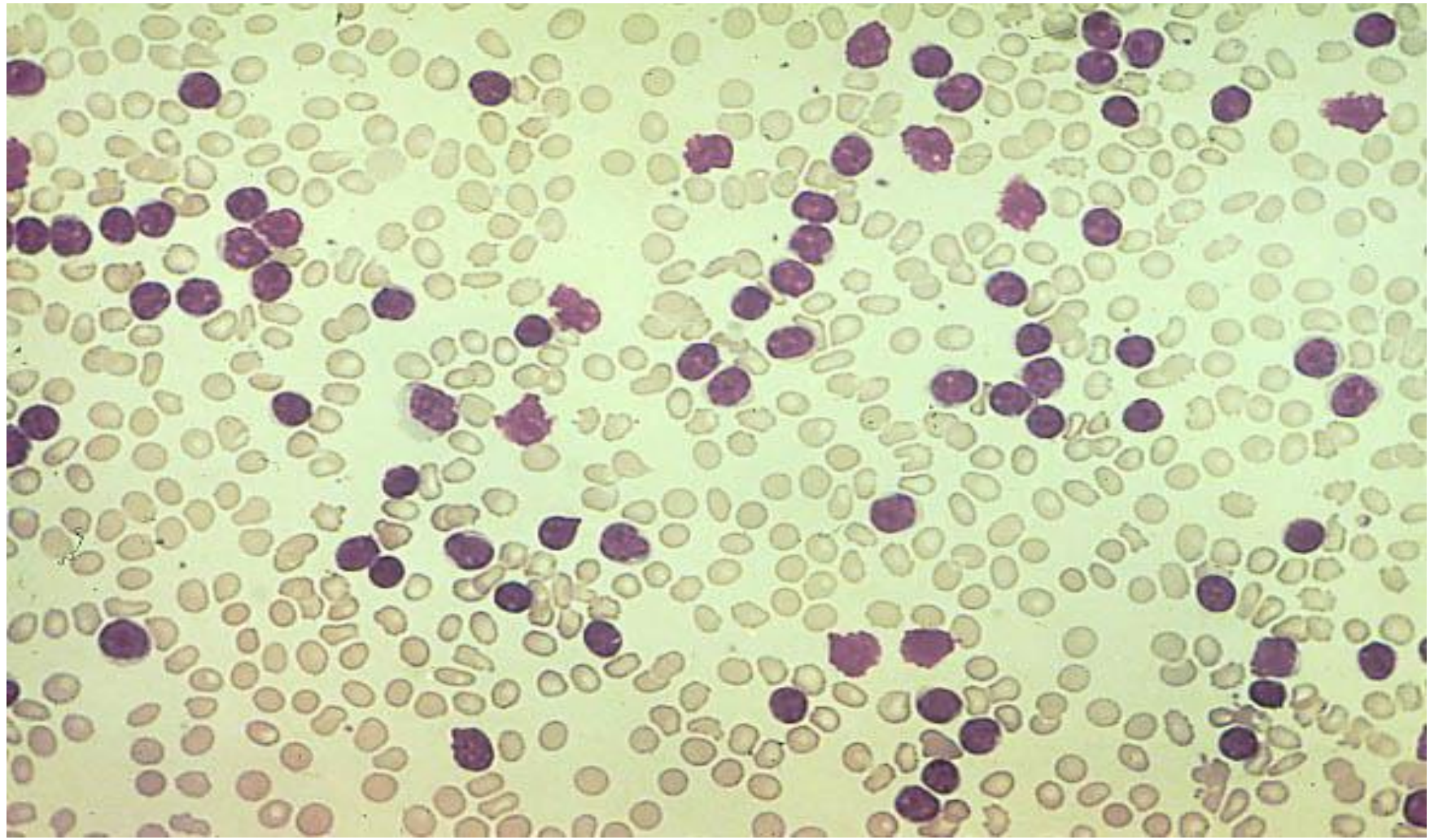
Hairy Cell: Hairy Cell Leukemia



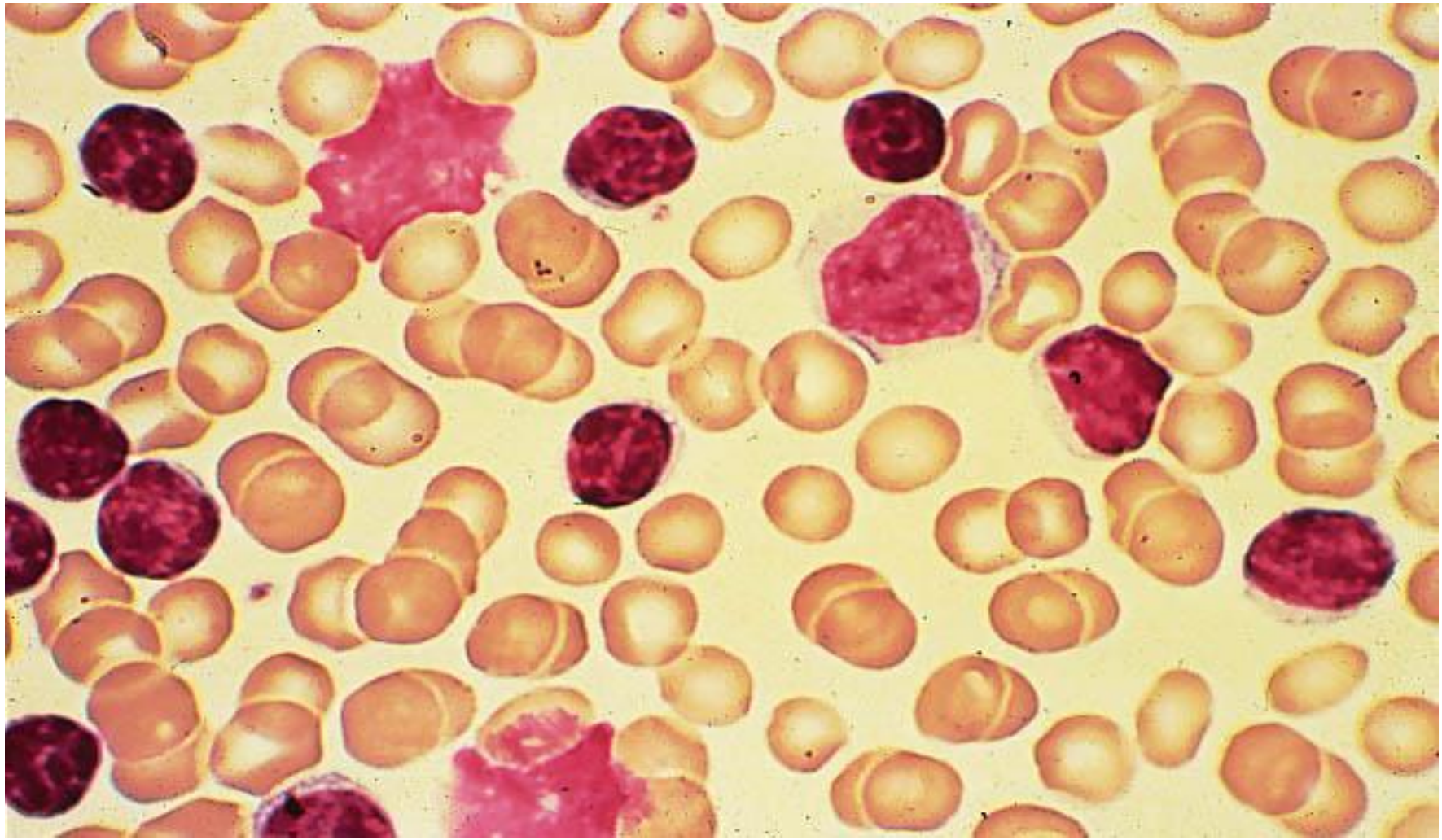
Sezary Cell



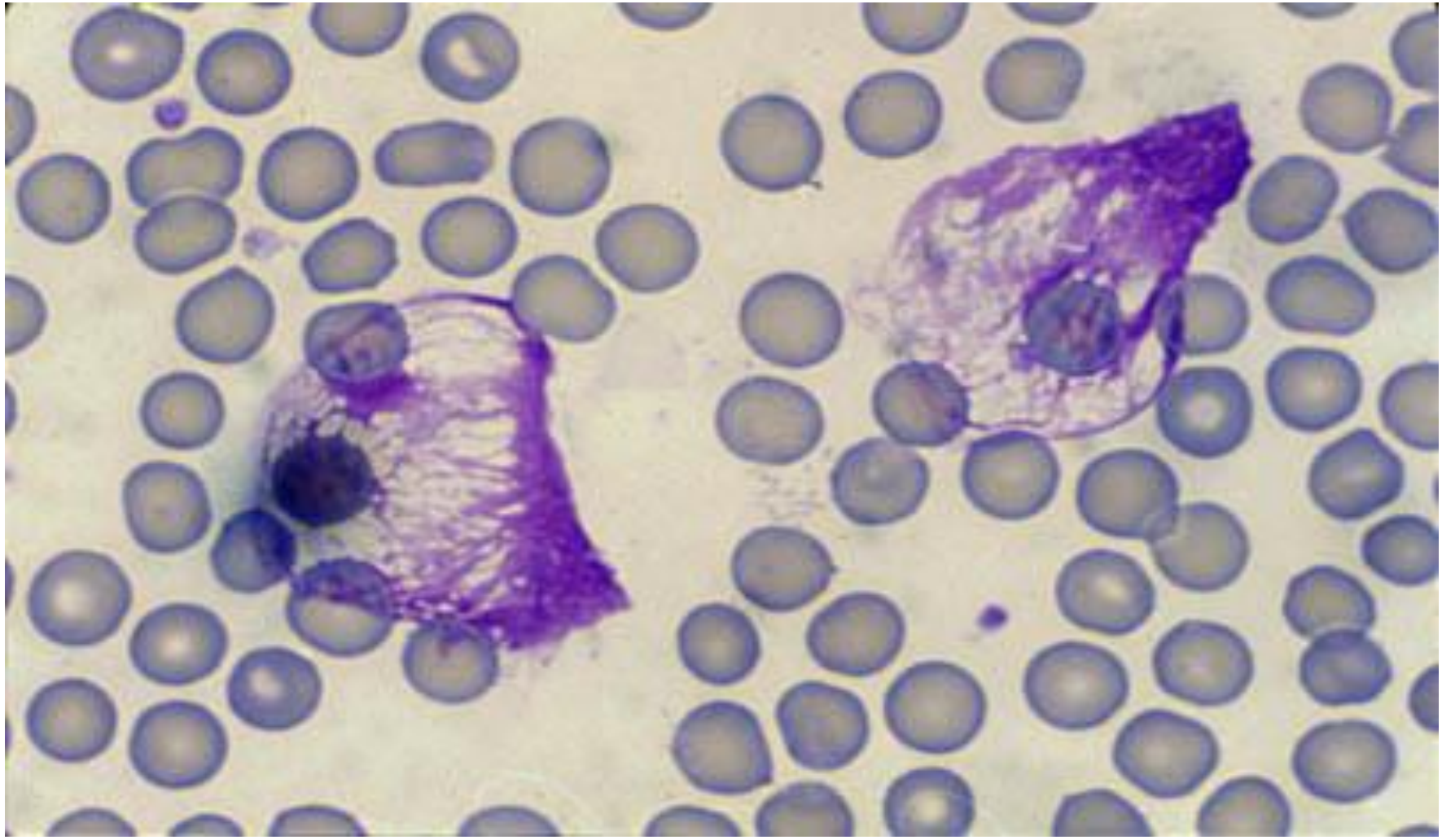
Chronic Lymphocytic Leukemia (CLL)



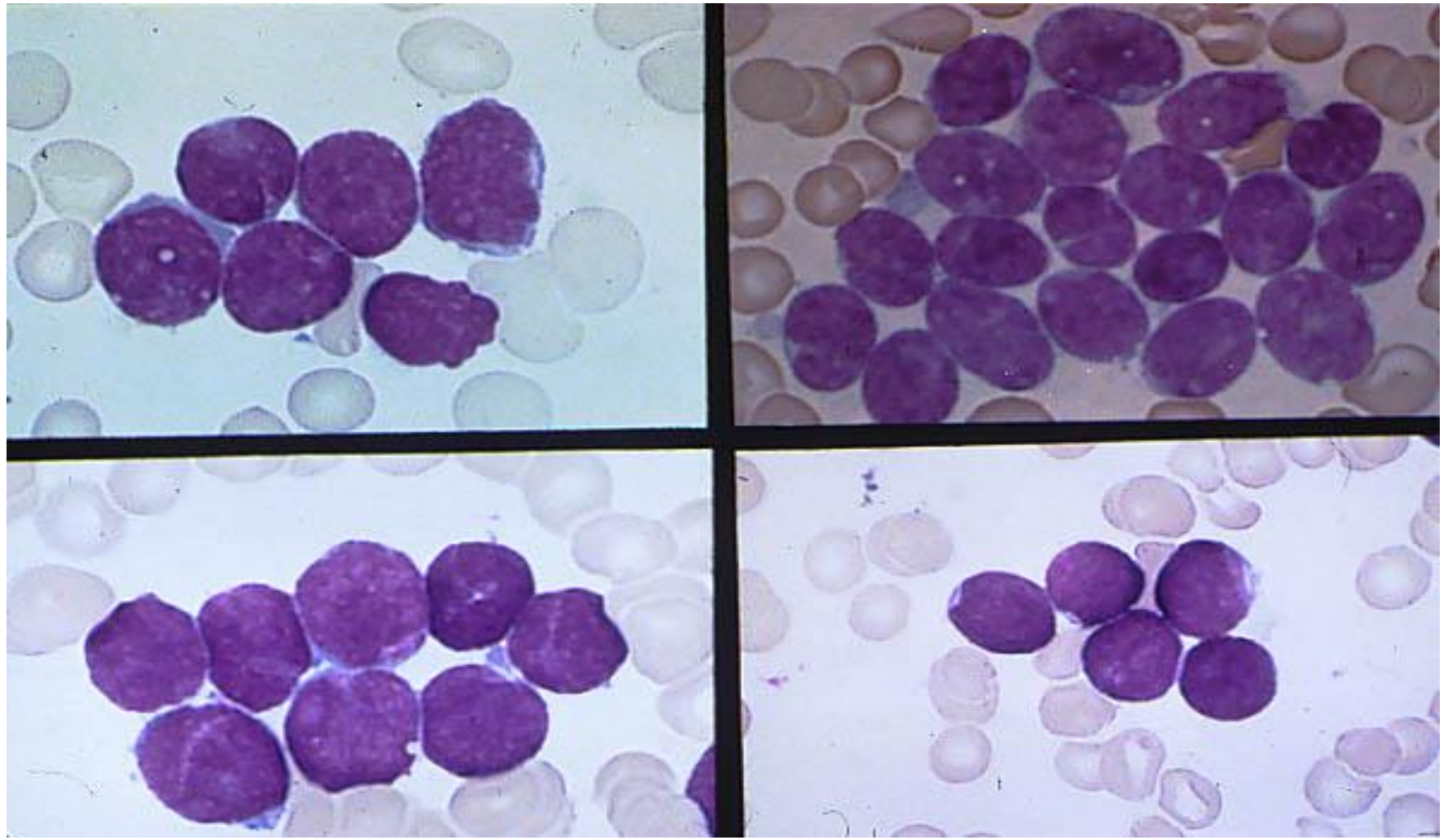
CLL: Smudge Cells



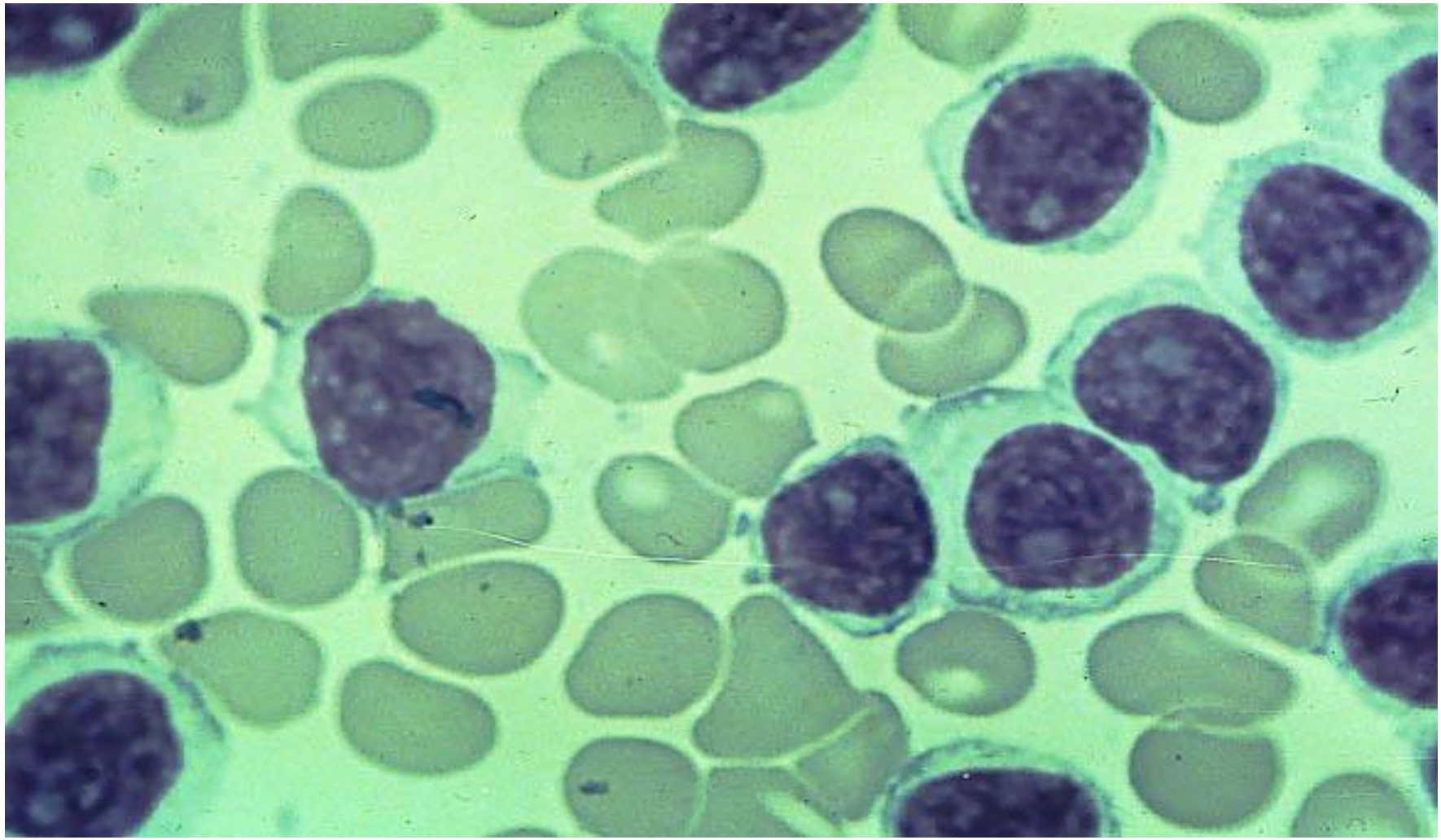
CLL: Balloon Cells



Acute Lymphocytic Leukemia: L1



Acute Lymphocytic Leukemia: L2



Acute Lymphocytic Leukemia: L3 (Burkitts)