# Introduction to CBC and Blood Smear Interpretation

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## Complete blood count

- The most common test used in clinical medicine
- Determine type and severity of blood cell abnormalities
- Nowadays, CBC is fully automated and highly reproducible.
- Correct interpretation of automated CBC can reduce rate of unnecessary blood smear examination
- Provide useful information for provisional diagnosis of RBC and WBC diseases

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## Component of automated CBC

- Blood count basic parameters: Hb, Hct, RBC, WBC, platlet.
- Red cell indices: MCV, MCH, MCHC, RDW
- WBC differentials
- Cytogram or Scattergram
- Reticulocyte count

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## Basic principles of automated blood cell analyzer

- Electrical impedance:
  Coulter Counter , Cell-Dyn®
- Optical impedance and light scatter:
  Technicon-H series
- VCS (volume, conductivity, light scatter) technology
  Beckman-Coulter VCS, MAXM, STKS

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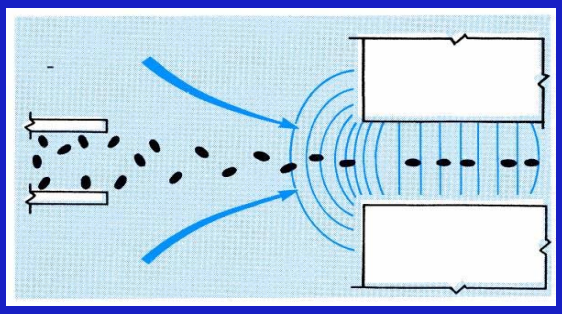
# Coulter Technology

- Cell count and size can be measured by electrical impedance

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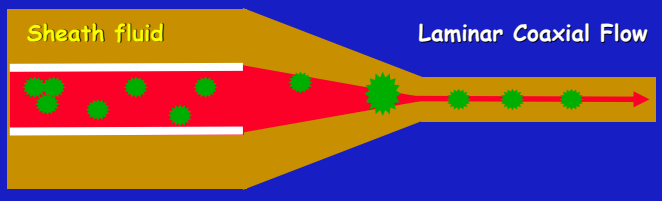
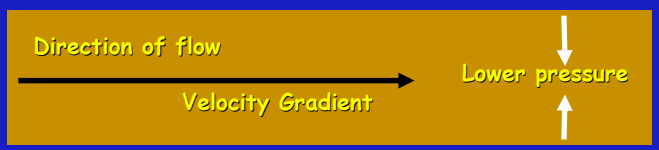
# Hydrodynamic Focusing




The Bernoulli Effect




Viscous drag along walls.

Particles move to low pressure area

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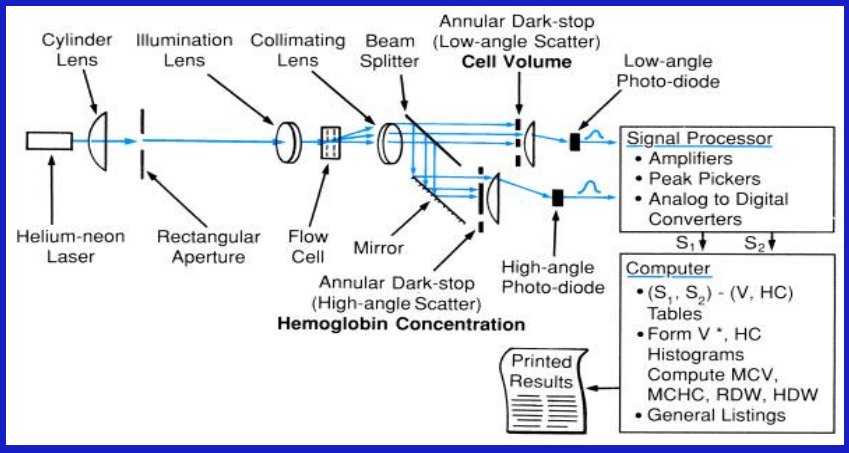
# Technicon Technology

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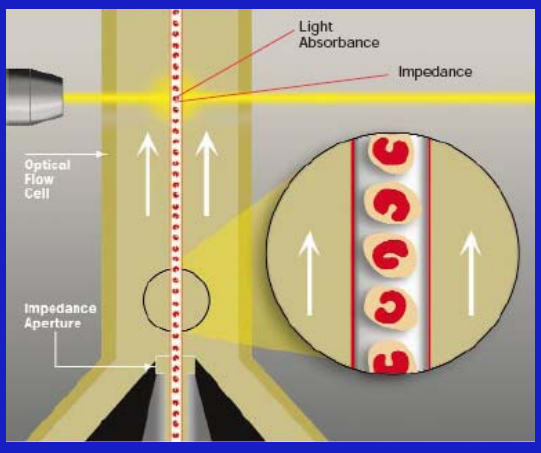
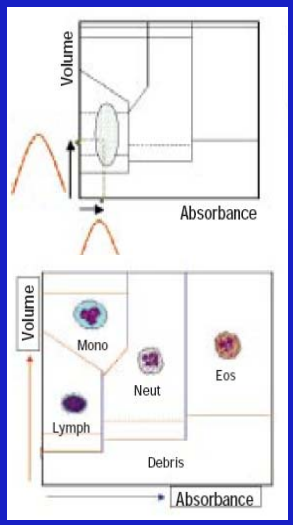
# VCS Technology

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| Instrument | Methodology features |
|---|---|
| Coulter STKS | Impedance, conductivity, laser technology |
| Coulter GEN-S | Impedance, flow cytometry, conductivity, laser light scatter (reticulocyte analysis) |
| Sysmex SE-9000 | Direct current (resistance to cell volume); radio-frequency (cell density, size) |
| Cobas Argos 5-diff (Roche) | Impedance, light absorption (halogen light source) |
| Technicon H-3 (Bayer) | Cytochemistry, flow technology (reticulocyte analysis) |
| Advia 120 (Bayer) | Cytochemistry, flow technology (reticulocyte analysis) |
| Cell Dyn 4000 (Abbott) | Impedance, laser light scatter |

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# Red cell parameters

- Direct Measurement
  - Erythrocyte Concentration (RBC) x $10^6$/ml
  - Mean Corpuscular Volume (MCV) Femtolitre (fl)
  - Hemoglobin (Hb) Gram/decilitre (g/dl)
- Indirect Measurement
  - Hematocrit (Hct) = RBC x MCV/10 %
  - Mean Corpuscular Hemoglobin (MCH) = HB x 10 / RBC (pg)
  - Mean Corpuscular Hemoglobin Concentration (MCHC) = Hb/Hct x 100 (g/dl)
  - Red Cell Distribution Width (RDW) %

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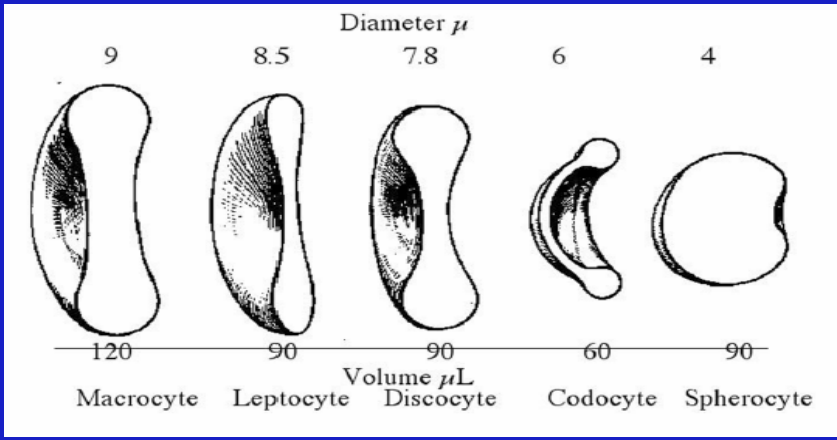
# RBC size and MCV

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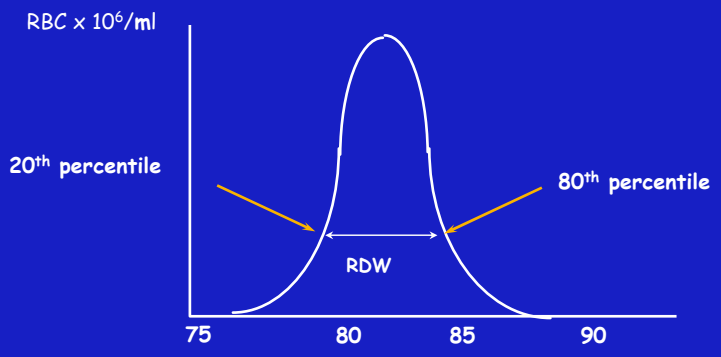
# Red Cell Distribution Width




$$RDW = \frac{80^{th}\text{ percentile} - 20^{th}\text{ percentile}}{80^{th}\text{ percentile} + 20^{th}\text{ percentile}} \times \text{constant}$$

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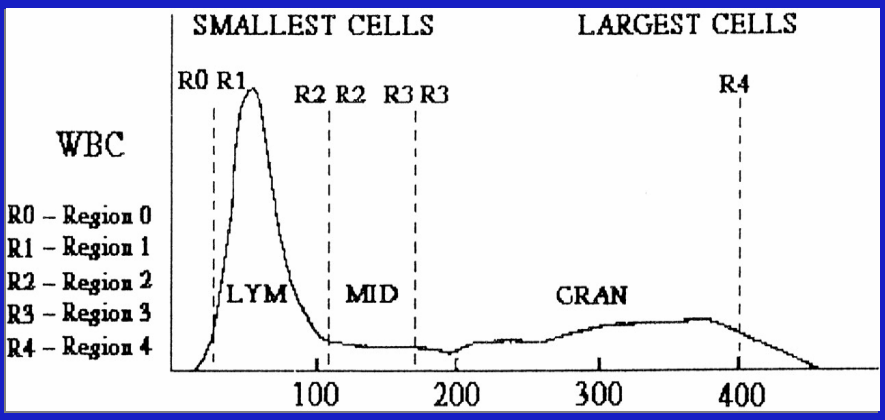
# WBC: three-part differentials




Cell-Dyn 1700CS

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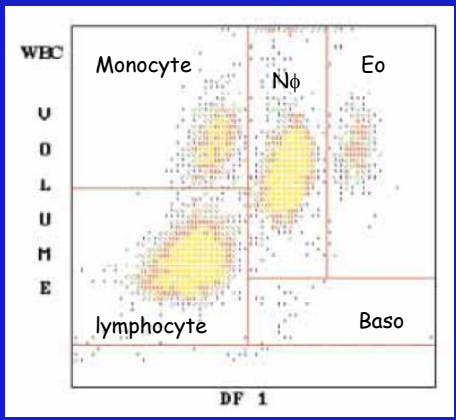
# WBC: Five-part differentials

- Neutrophil
- Lymphocyte
- Monocyte
- Eosinophil
- Basophil




Coulter VCS

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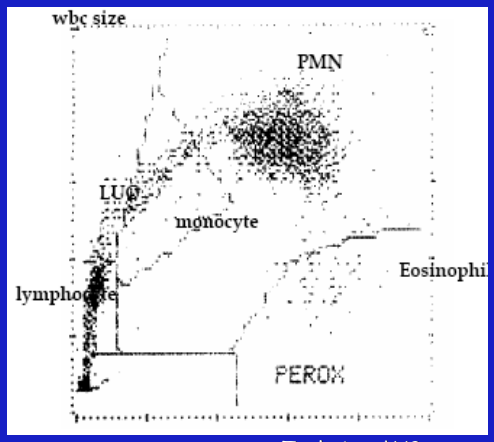
# WBC: Six-part differentials

- Neutrophil
- Lymphocyte
- Monocyte
- Eosinophil
- Basophil
- Large Unstained Cell (LUC)

Peroxidase channel




Technicon*H2

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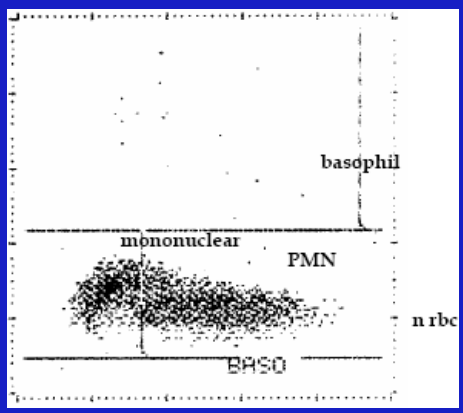
# WBC: Six-part differentials

Basophil/lobularity channel

- Additional parameters in Technicon-H analyzer
  1. Mean peroxidase activity index (MPXI)
  2. Lobularity Index (LI)




Technicon*H2

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# Platelet parameters

- Platelet count
- Mean Platelet Volume (MPV)
- Platelet Distribution Width (PDW)
- Plateletcrit (Pct)

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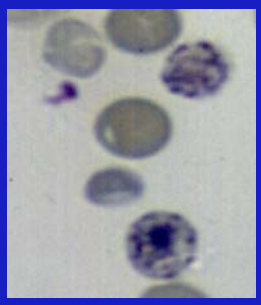
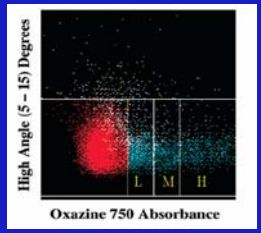
# Reticulocyte count

- Reticulocyte = non-nucleated RBC with polyribosomal RNA as stained by supravital stain (new methylene blue or brilliant cresyl blue)
- Polychromasia underestimates reticulocytes
- Three methods of reticulocyte enumeration
  - Manual count on slide per 1,000 RBC
  - Automated CBC with reticulocyte counter (Coulter VCS, Cell-Dyne 4000, Technicon-H3)
  - Flow cytometry with fluorescent dyes



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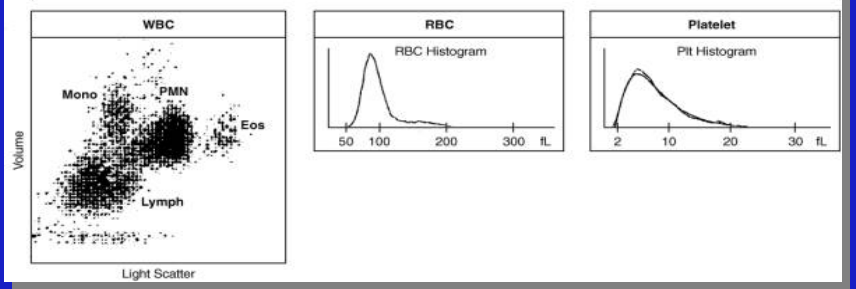
# Report from Coulter™

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **WBC** | | 6.4 | x10³ /µL | **RBC** | 4.17 | L | x10⁶ /µL | **PLT** | 230 | x10³ /µL |
| | | | | **HGB** | 12.3 | L | g/dL | **MPV** | 8.7 | fL |
| **NE** | % | 55.4 | % | **HCT** | 37.7 | L | % | | | |
| **LY** | % | 32.0 | % | **MCV** | 90.3 | | fL | | | |
| **MO** | % | 9.0 | % | **MCH** | 29.5 | | pg | | | |
| **EO** | % | 2.8 | % | **MCHC** | 32.7 | L | g/dL | | | |
| **BA** | % | 0.8 | % | **RDW** | 16.6 | aH | % | | | |
| **NE** | # | 3.56 | x10³ /µL | | | | | | | |
| **LY** | # | 2.06 | x10³ /µL | **RET** | % | | | | | |
| **MO** | # | 0.58 | x10³ /µL | **RET** | # | | | | | |
| **EO** | # | 0.18 | x10³ /µL | | | | | | | |
| **BA** | # | 0.05 | x10³ /µL | | | | | | | |

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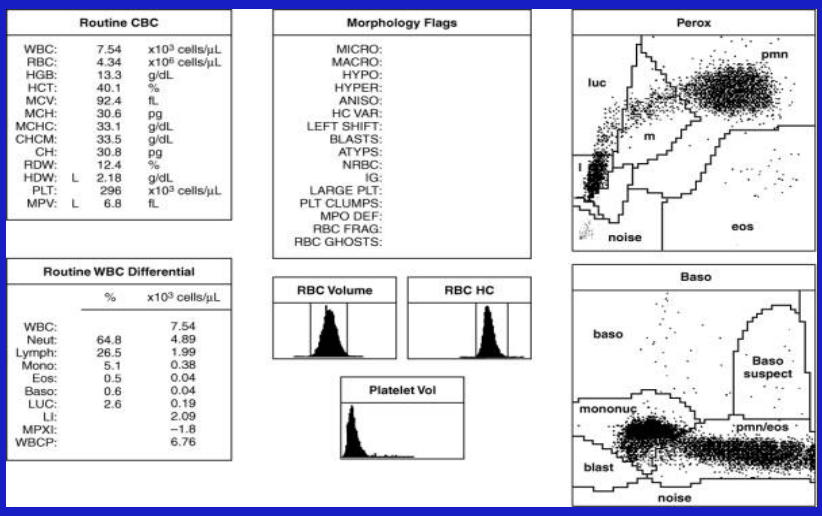
# Report from Technicon-H™

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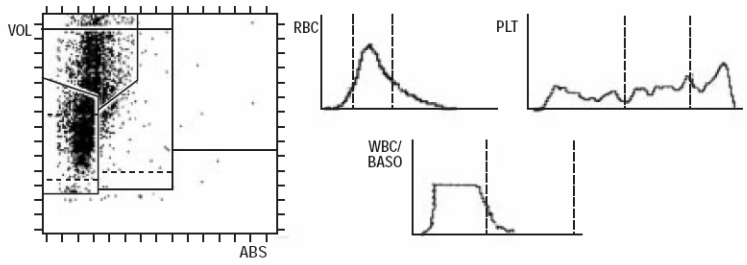
| | | | Range (1) | | | | | Range (1) |
|---|---|---|---|---|---|---|---|---|
| WBC | 8.6 | $10^3/\mu L$ | 6.0 / 11.0 | NE | 6.1 RLL | % | 50.0 / 80.0 | FLAGS |
| RBC | 3.99 L | $10^6/\mu L$ | 4.00 / 6.20 | LY | 58.0 HH | % | 25.0 / 50.0 | WBC: UM,ATL,IMM |
| HGB | 11.1 L | g/dL | 11.0 / 18.8 | MO | 33.6 RHH | % | 2.0 / 10.0 | RBC: |
| HCT | 33.9 L | % | 35.0 / 55.0 | EO | 0.5 | % | 0.0 / 5.0 | PLT: SCH |
| MCV | 85 | fL | 80.0 / 100.0 | BA | 1.8 | % | 0.0 / 5.0 | |
| MCH | 27.9 | pg | 26.0 / 34.0 | | | | | |
| MCHC | 32.9 | g/dL | 31.0 / 35.0 | | | | | |
| RDW | 13.9 | % | 10.0 / 20.0 | NE# | 0.53 RLL | $10^3/\mu L$ | 2.0 / 8.0 | Lymphocytosis, |
| | | | | LY# | 5.01 H | $10^3/\mu L$ | 1.0 / 5.0 | Neutropenia, |
| PLT | 18 RLL | $10^3/\mu L$ | 150 / 400 | MO# | 2.90 RHH | $10^3/\mu L$ | 0.1 / 1.0 | Immature Cells, |
| MPV | 8.4 R | fL | 6.0 / 10.0 | EO# | 0.04 | $10^3/\mu L$ | 0.0 / 0.4 | Atypical Lymph, |
| | | | | BA# | 0.16 | $10^3/\mu L$ | 0.0 / 0.2 | Monocytosis |
| | | | | | | | | Cold Agglutinins |
| | | | | | | | | Plt interpretation impossible |

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# Clinical Utility of automated CBC

- Differential diagnosis of RBC disorders
  - Microcytic RBC
- Use WBC differential cytogram to guide WBC morphology and phenotypes

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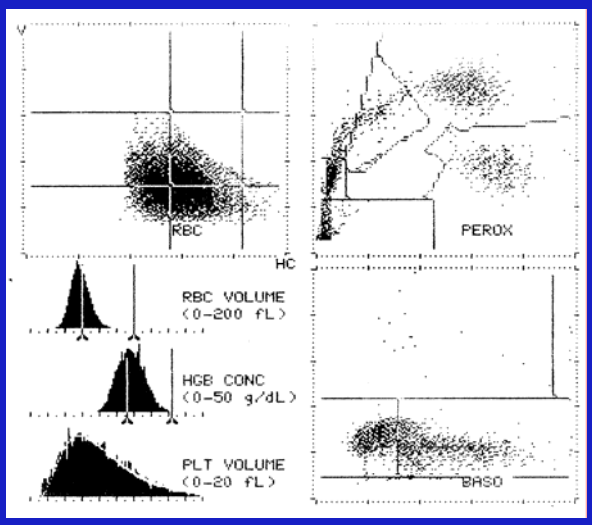
# What is your diagnosis?

| | | |
|---|---|---|
| WBC | 5.00 | $x10^3/\mu L$ |
| RBC | 3.56 | $x10^6/\mu L$ |
| Hb | 6.0 | g/dL |
| HCT | 21.0 | % |
| MCV | 59.0 | fL |
| MCH | 17.0 | pg |
| MCHC | 28.8 | g/dL |
| RDW | 19.4 | % |
| HDW | 4.02 | g/dL |
| PLT | 427 | $x10^3/\mu L$ |

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# Differential diagnosis of anemia using MCV and RDW

| | Low MCV | Normal MCV | High MCV |
|---|---|---|---|
| RDW <15 | Thalassemia trait<br>Heterozygous HbE, HbC, etc.<br>Anemia of chronic disease (ACD) | ACD<br>Heterozygous HbS, HbCS, HbE, etc.<br>Hereditary spherocytosis<br>Acute hemorrhage | Aplastic anemia<br>MDS<br>Myeloma<br>Liver disease<br>Hyperthyroidism |
| RDW >15 | Iron deficiency anemia<br>Thalassemia intermedia<br>Sideroblastic anemia<br>Severe ACD<br>RBC fragmentation | Early or combined nutritional deficiency<br>Myelodysplasia<br>Myelophthisis<br>Sickle cell anemia or Homozygous HbCS | B12 deficiency<br>Folate deficiency<br>AIHA<br>Drugs: HU, ARV, AZA, etc. |

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## Factors known to cause spurious laboratory results in hematology analyzers.

| Parameter | Spuriously increased | Spuriously decreased |
|---|---|---|
| RBCs | WBC >50 000/mm$^3$ | Clotting |
| Hb | hyperlipidemia, hyperbilirubinemia | Clotting |
| MCV | Cold agglutinins, hyperglycemia, WBC >50 000/mm$^3$ | Cryoglobulins |
| MCHC | Hyperlipidemia, cold agglutinins | WBC >50 000/mm$^3$ |
| RDW | Post transfusion | |
| WBCs | Nucleated red cells, platelet clumps, unlysed red cells, cryoglobulins | Clotting |
| Platelets | WBC fragmentation, severe microcytosis, cryoglobulins | Satellitism, clumping |

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## Basic Principles for Blood Smear Interpretation

- Assess quality of smears
  - Specimen preparation & staining
- Estimate cell numbers
  - RBC: evenly dispersed with minimal intercellular space
  - WBC: 10-20/LPF
  - Platelets: 7-20/OF
- Determine predominant cell populations
- Carefully examine cellular morphology

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## RBC disorders

- Hypochromic microcytic anemia
  - Iron deficiency anemia
  - Thalassemia and hemoglobinopathy
- Macrocytic anemia
  - Megaloblastic anemia
  - Non-megaloblastic macrocytic anemia
- Hemolytic anemia
  - Immune hemolytic anemia: AIHA, DHTR
  - Microangiopathic hemolytic anemia (MAHA)
  - Red cell enzymopathies: G-6-PD deficiency
  - RBC membrane defects: spherocytosis, ovalocytosis, elliptocytosis, stomatocytosis
- RBC inclusion bodies and parasites

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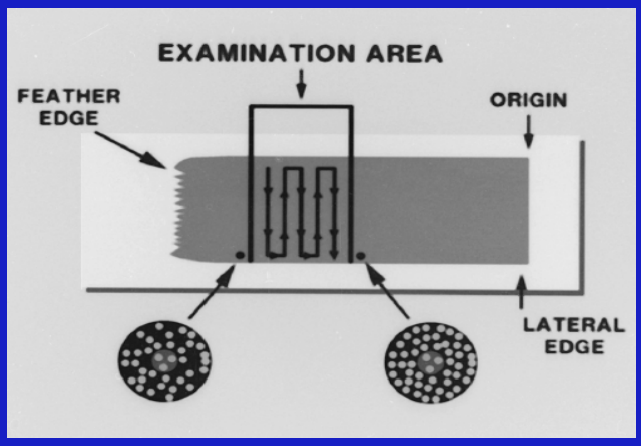
## Area of blood film examination

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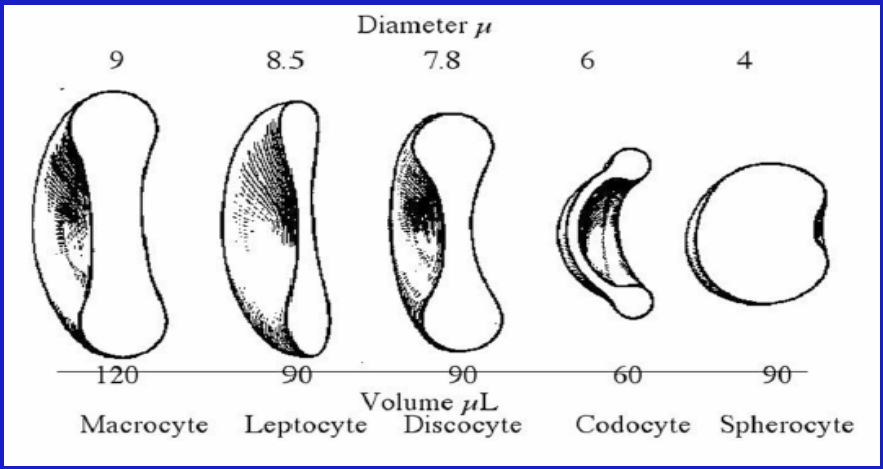
# RBC Size and MCV

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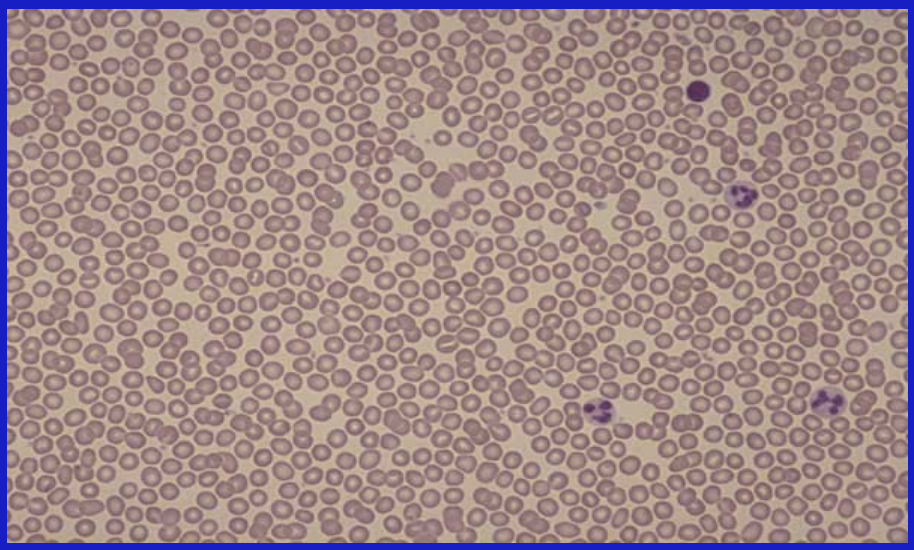

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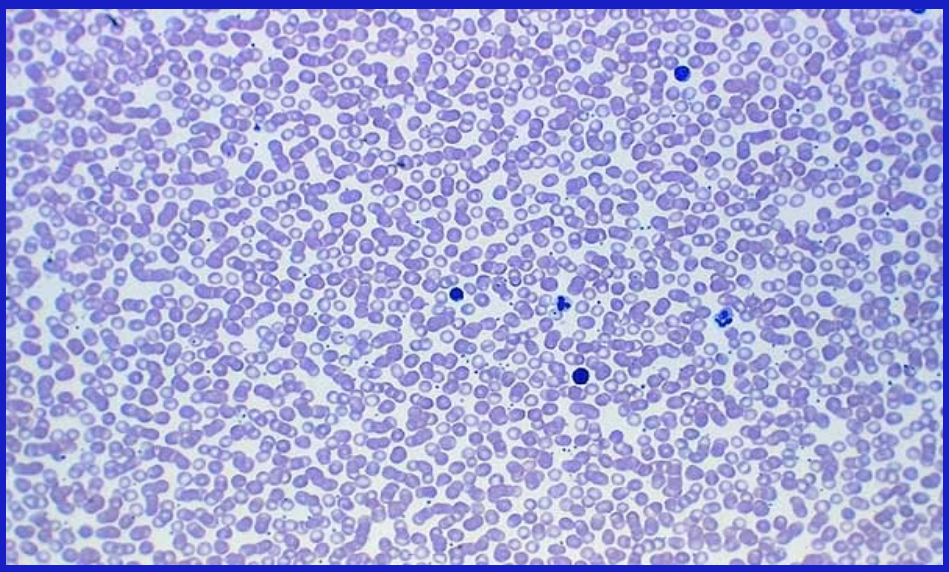

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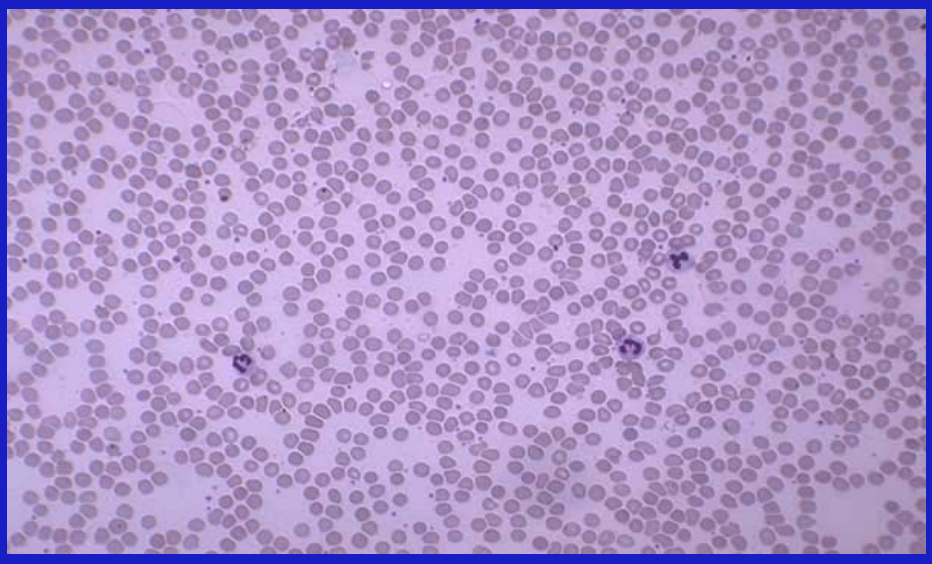

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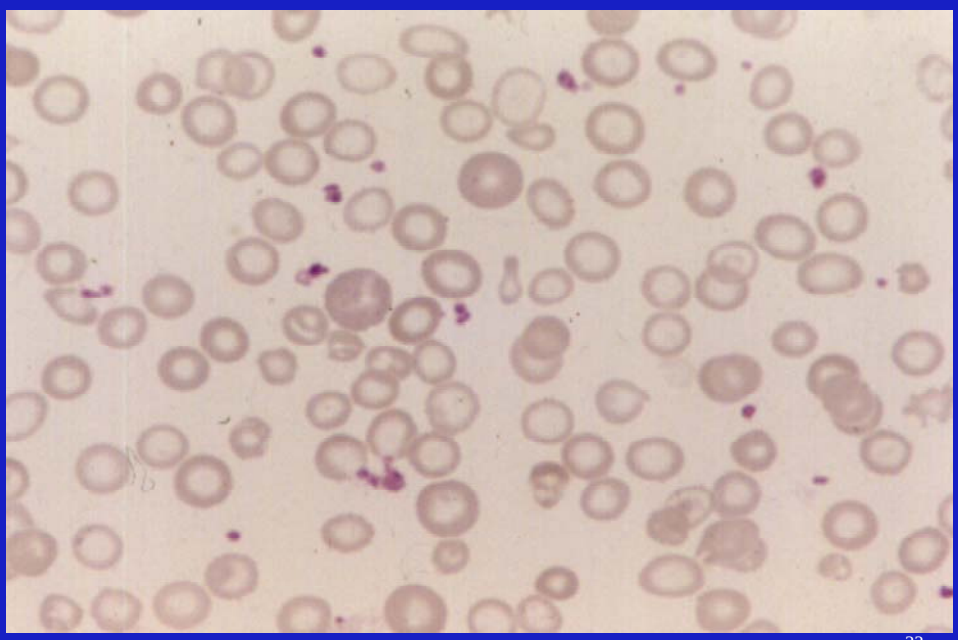

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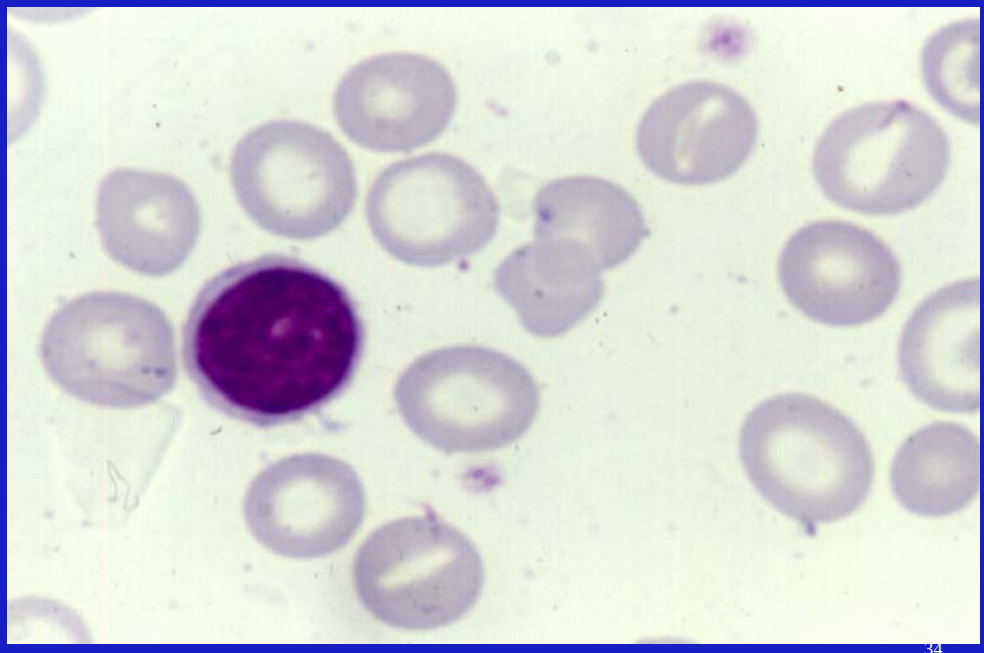

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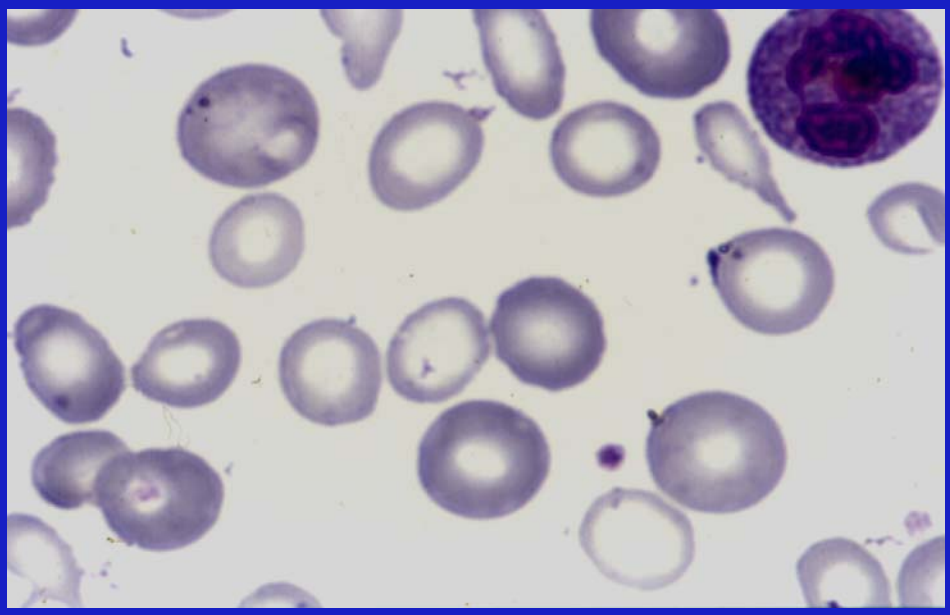

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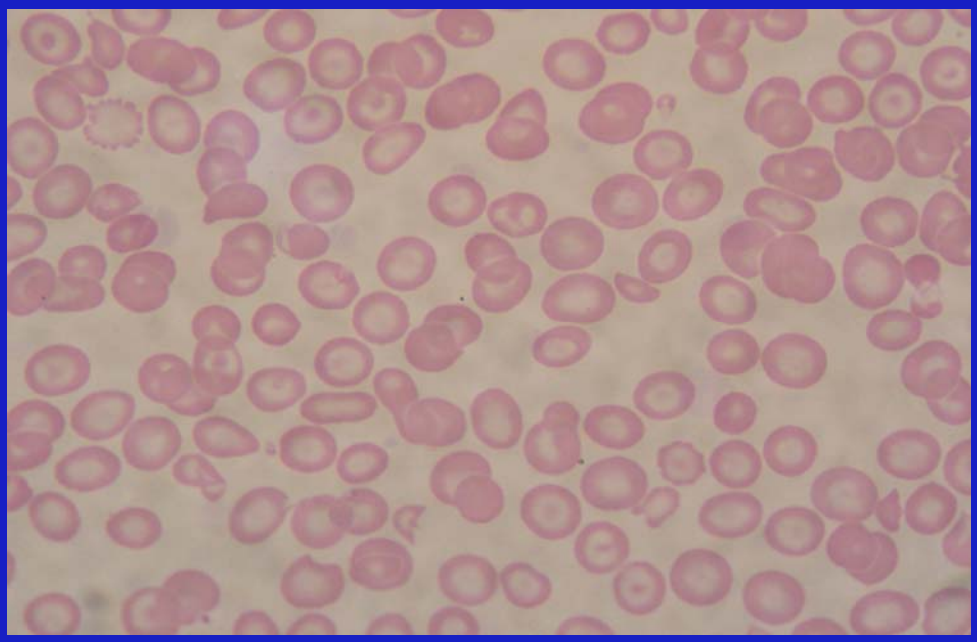

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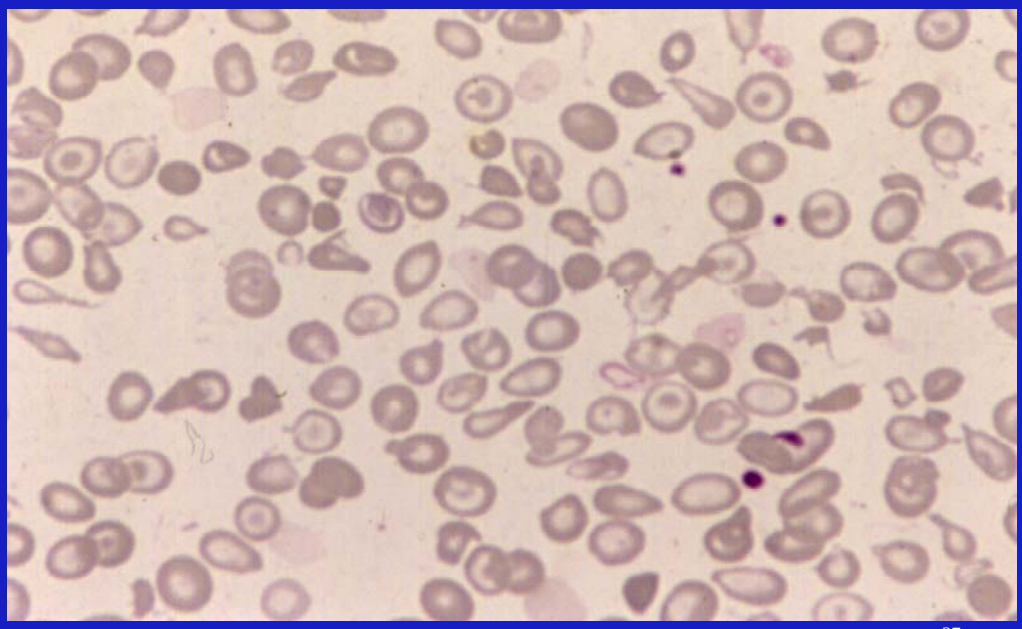

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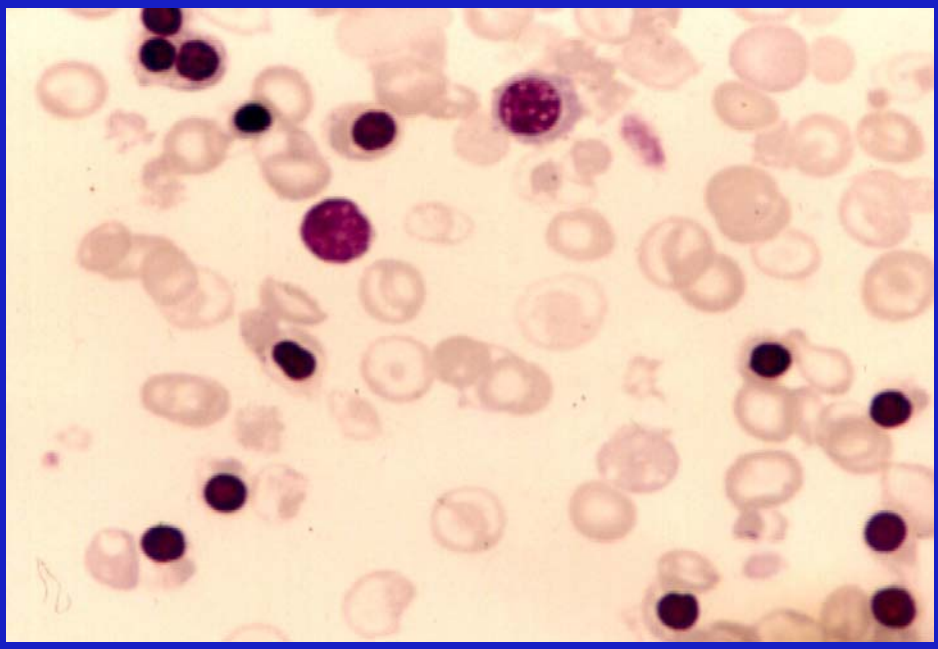

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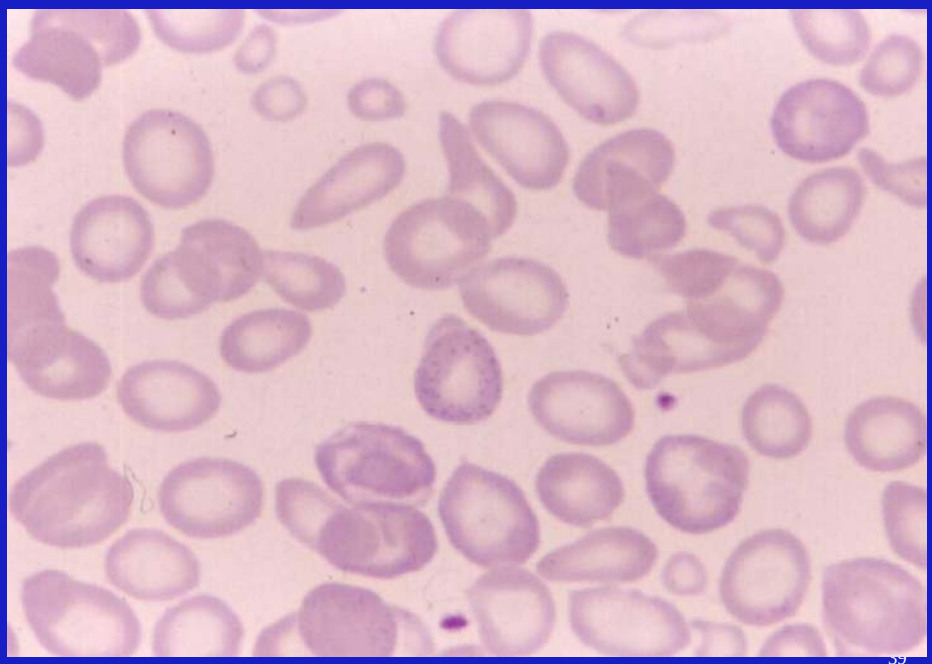

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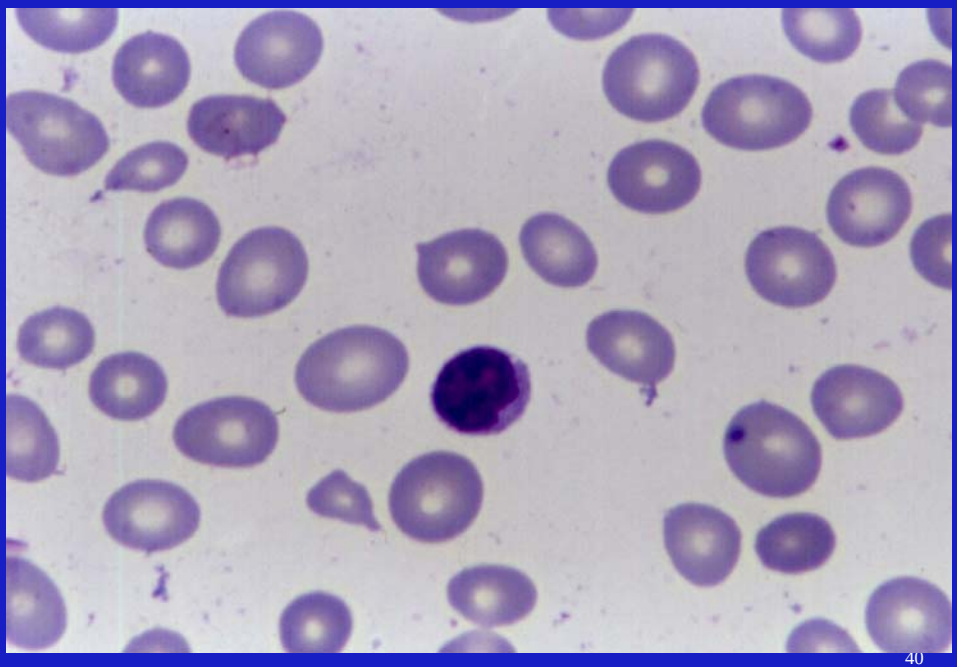

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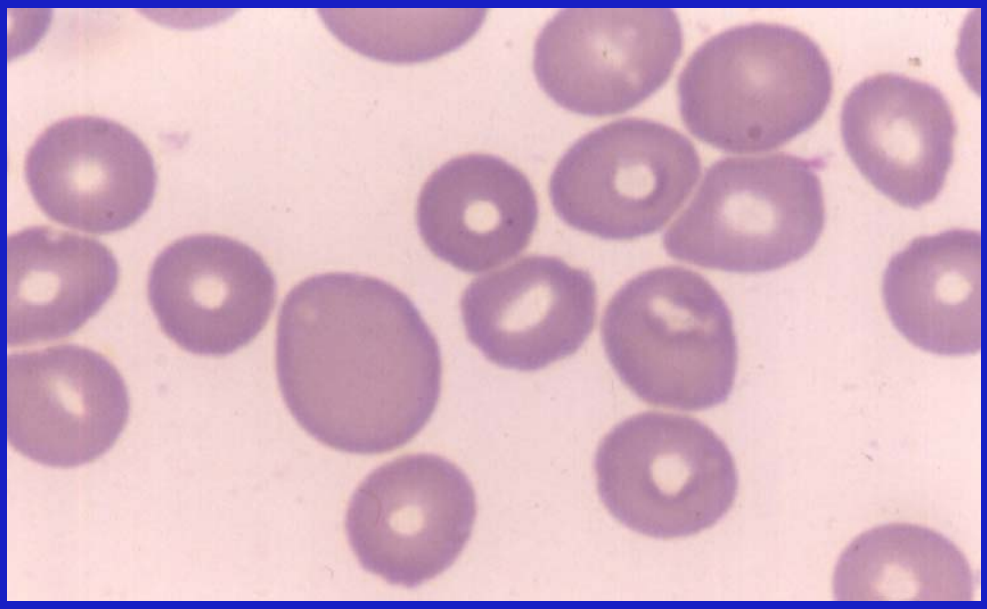

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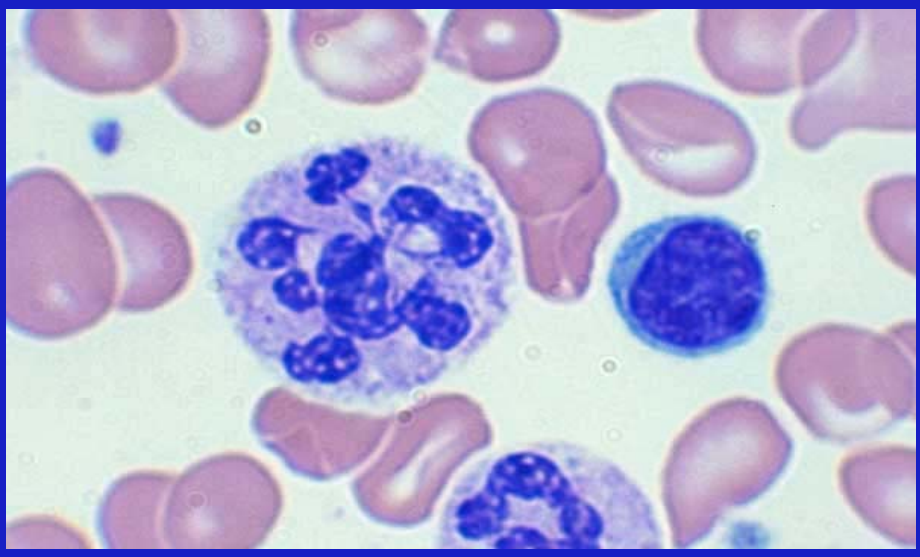

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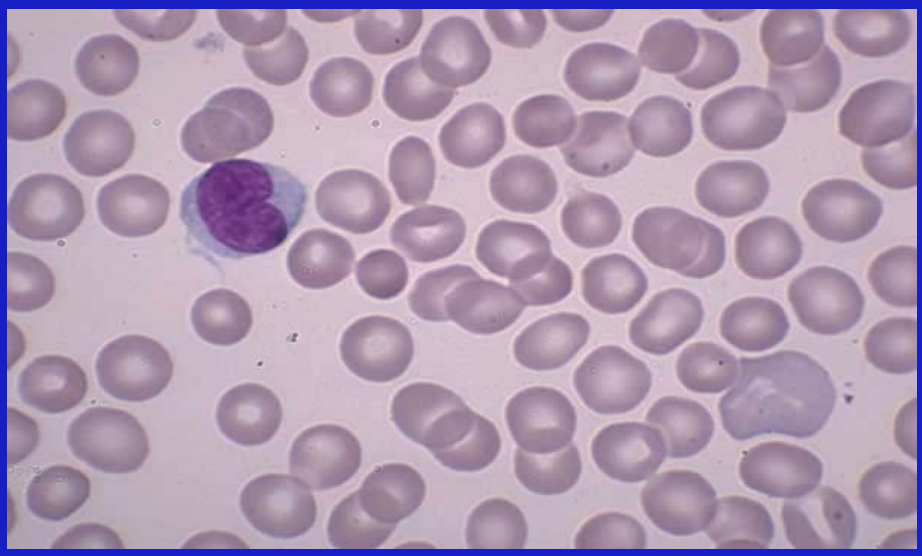

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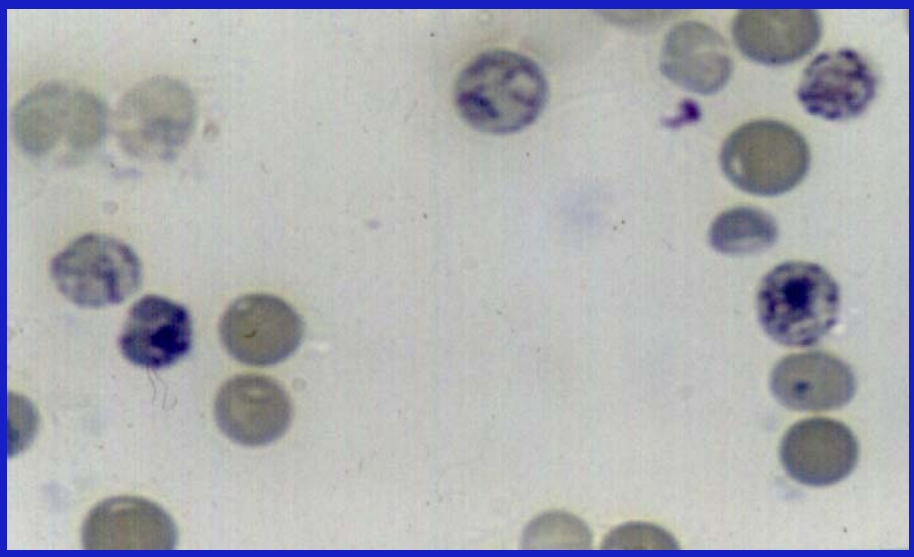

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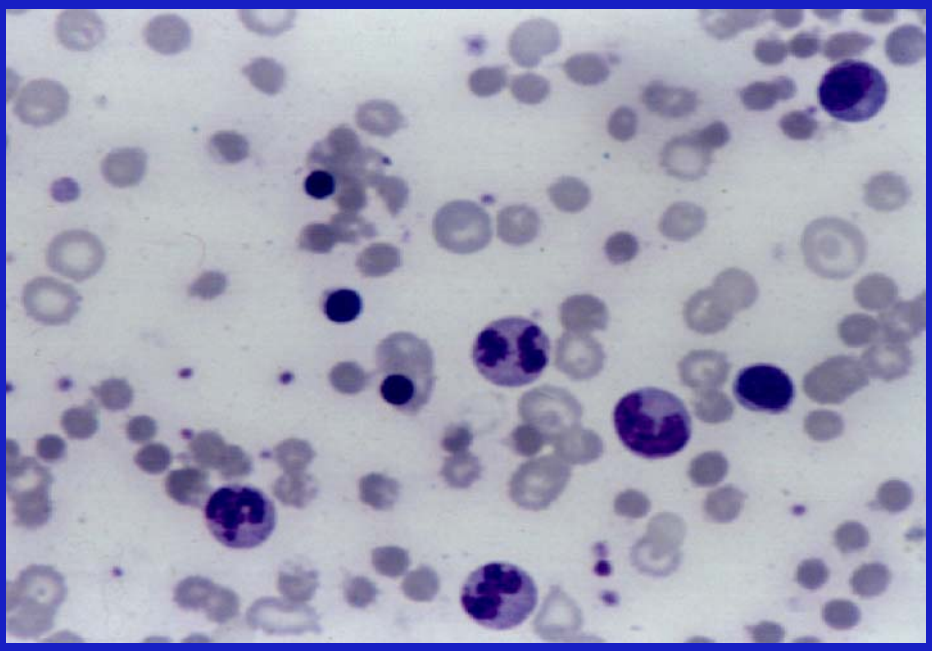

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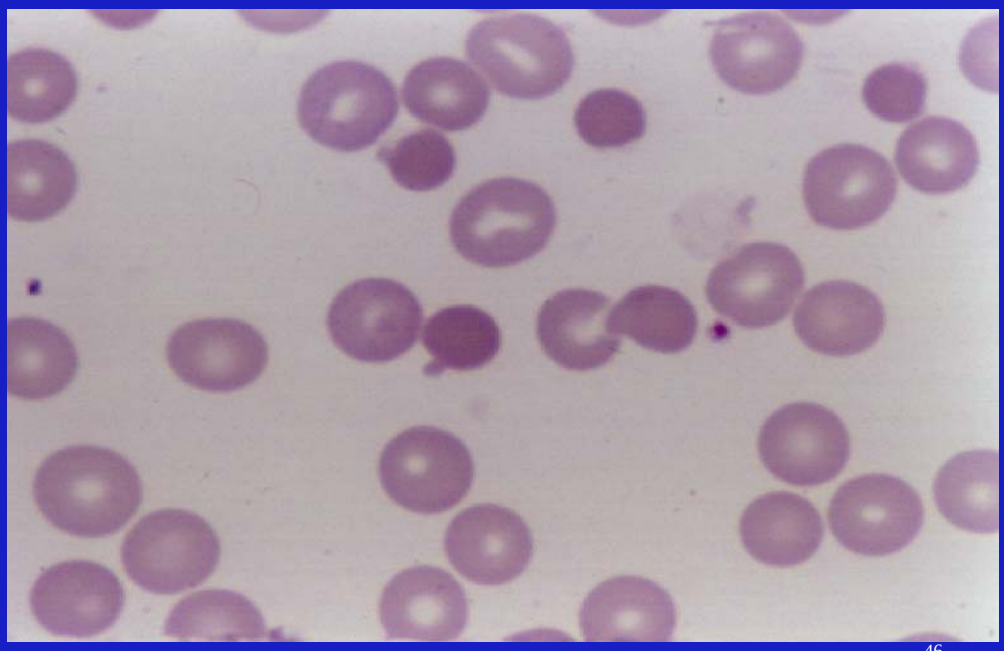

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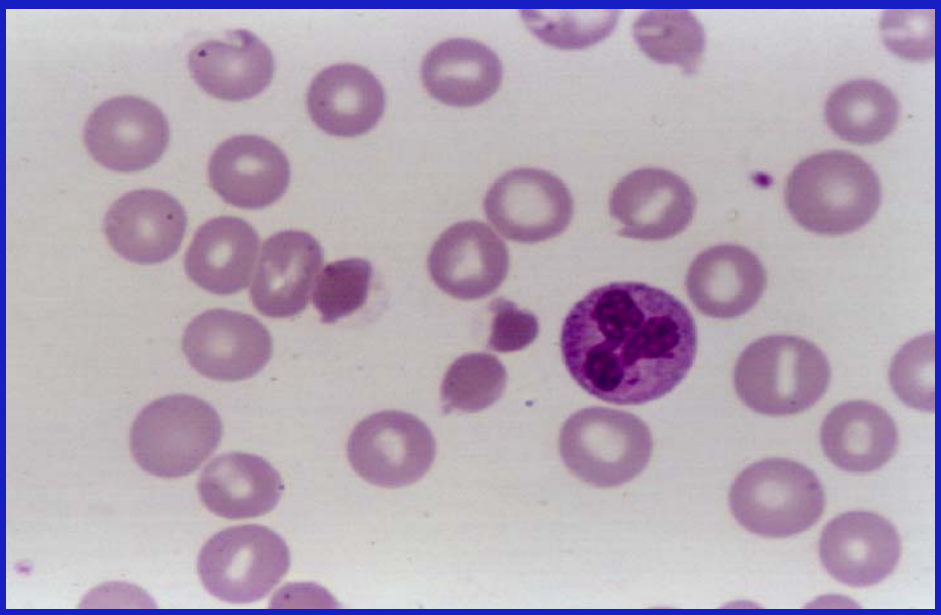

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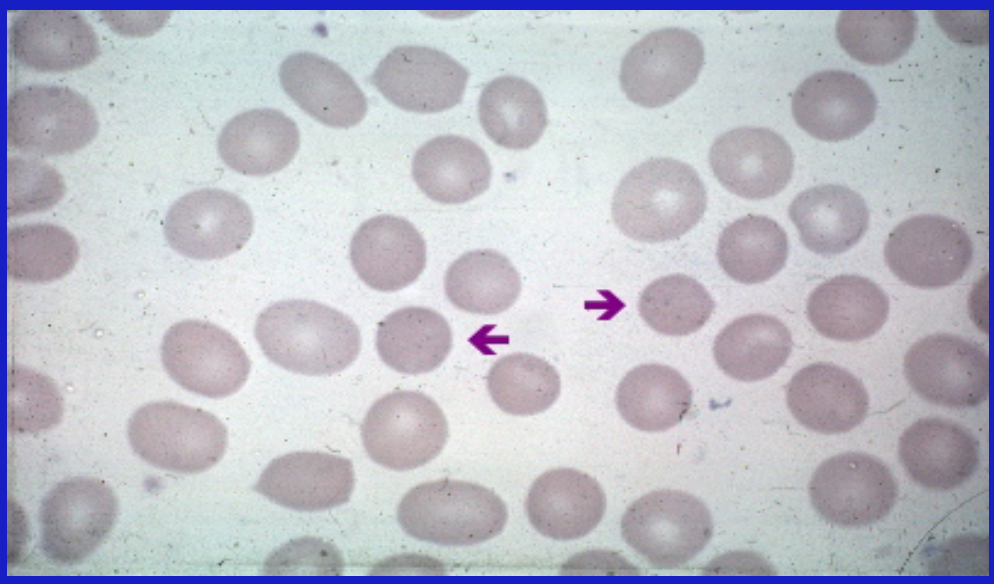

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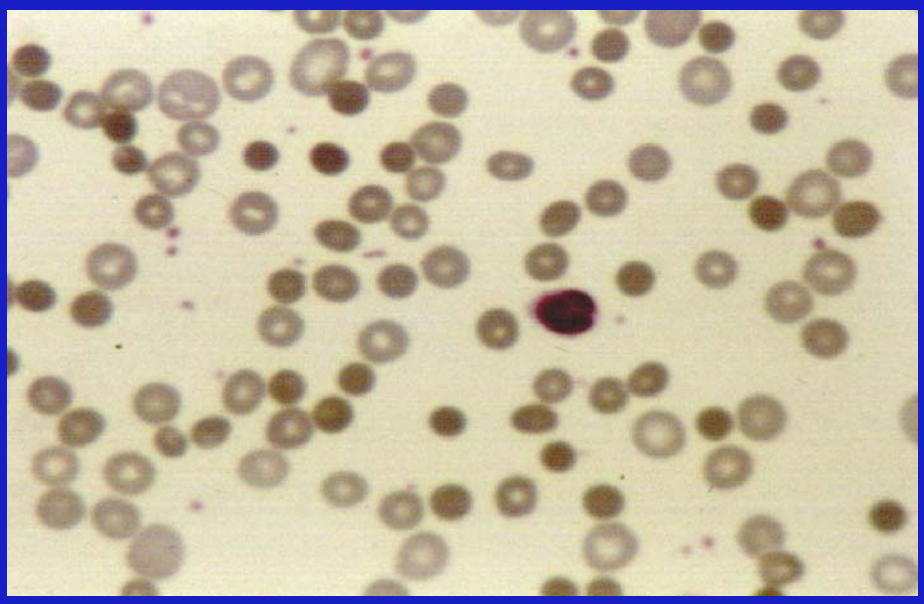

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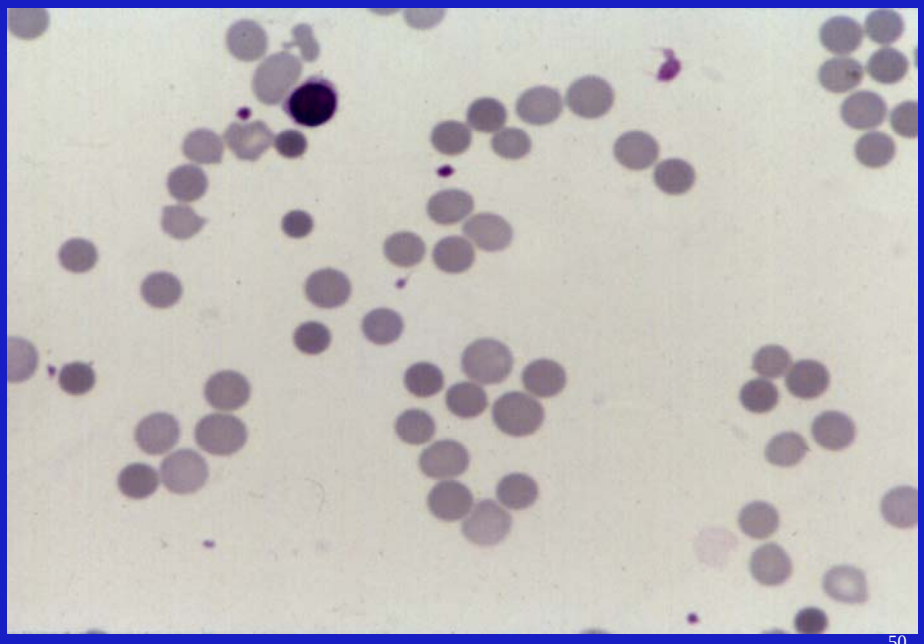

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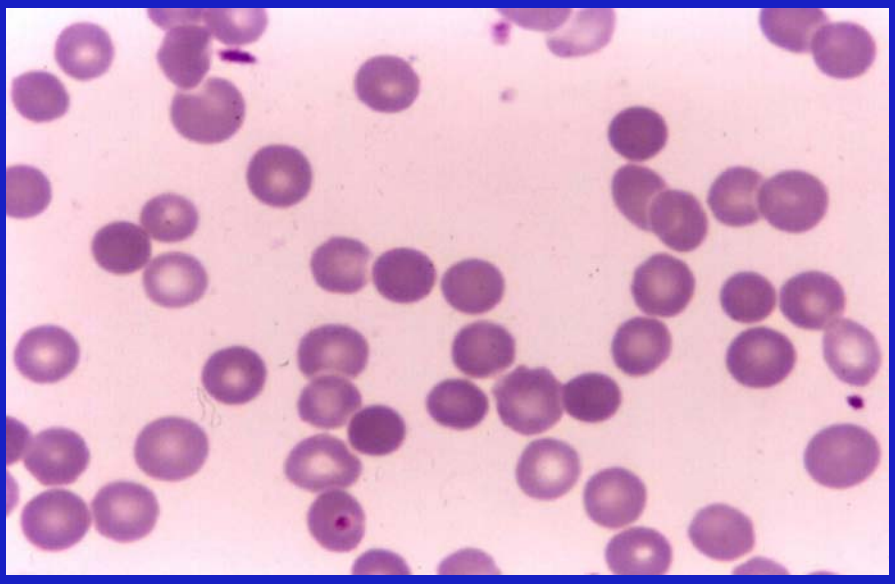

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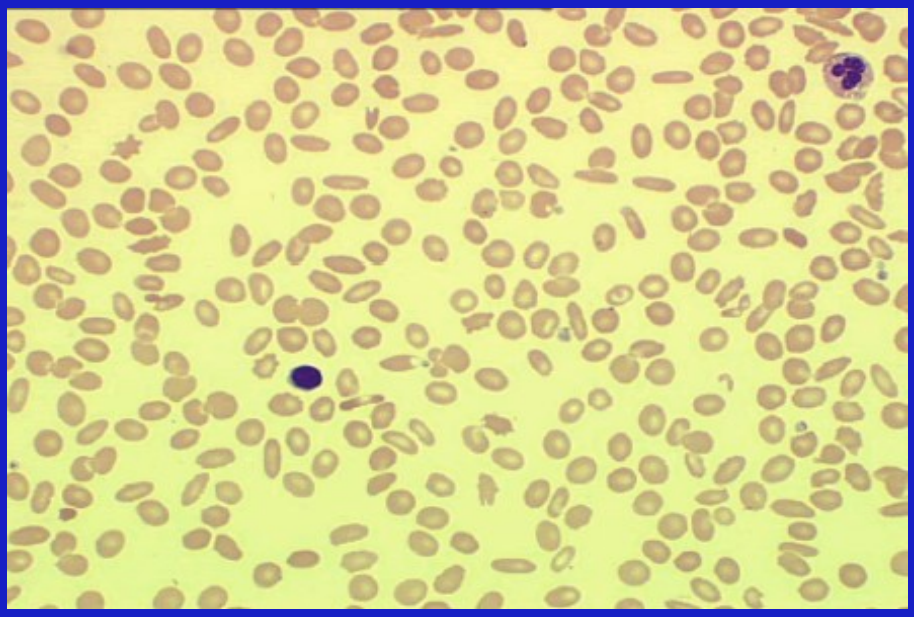

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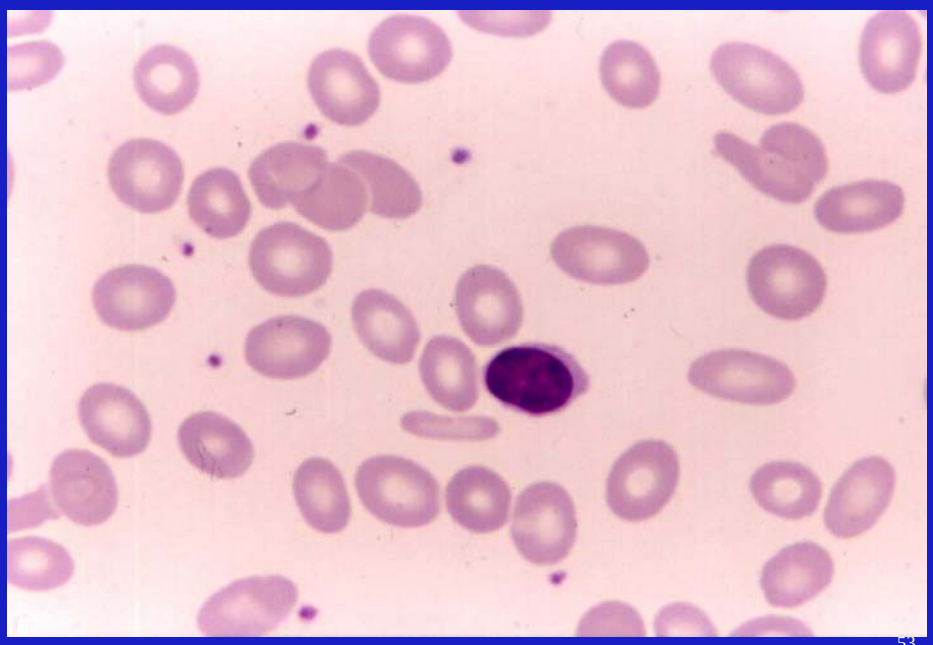

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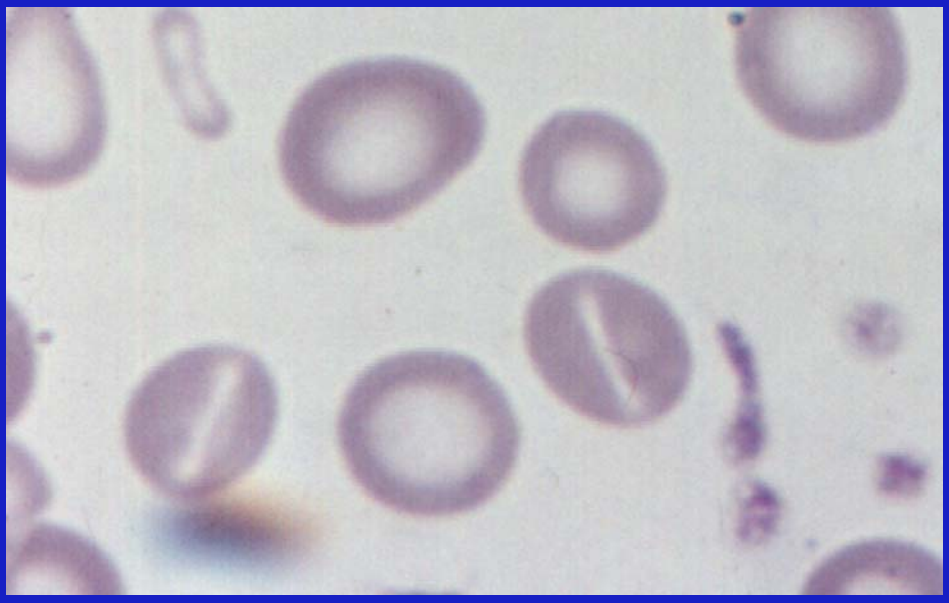

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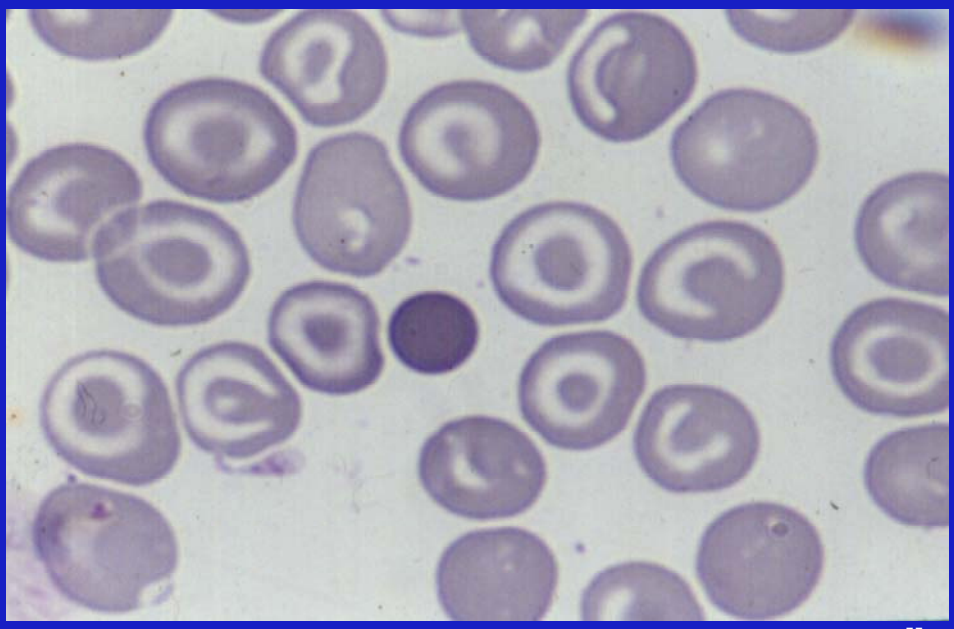

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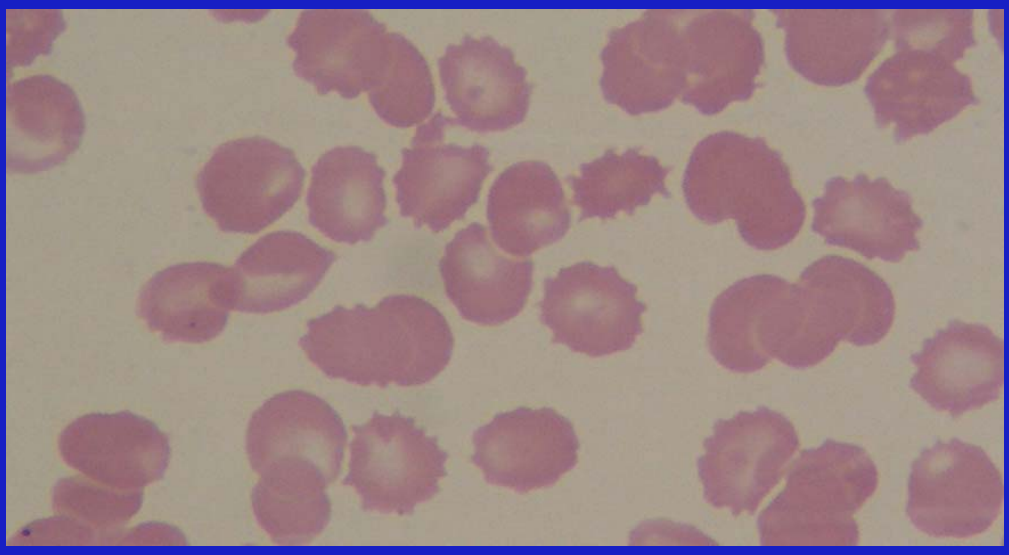

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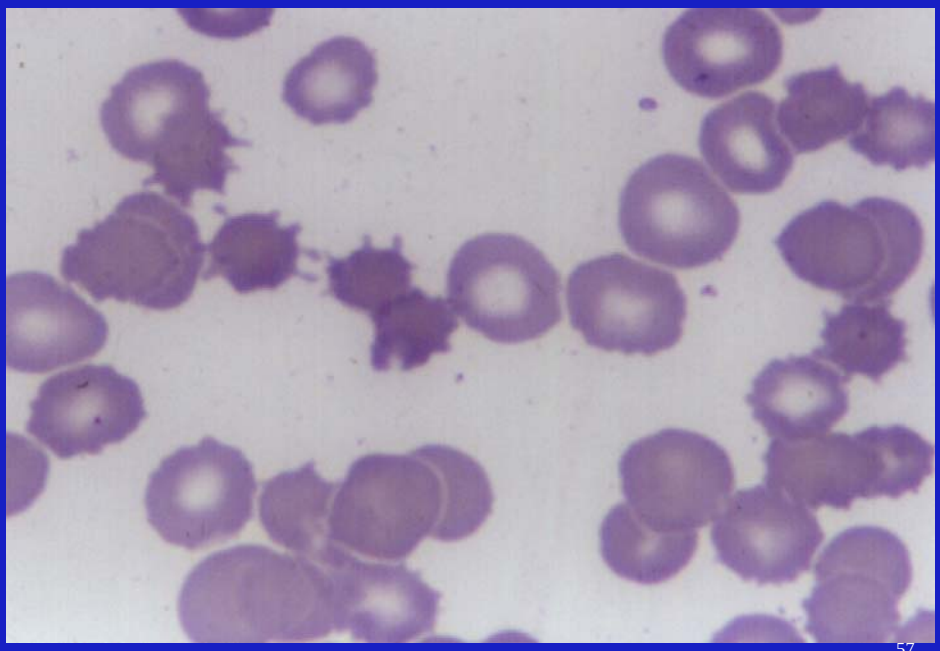

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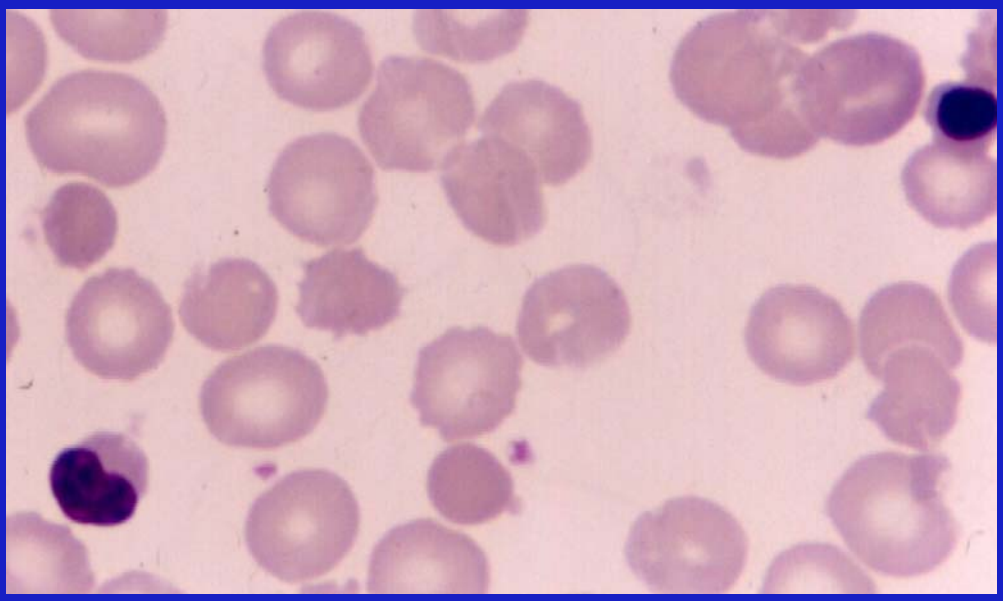

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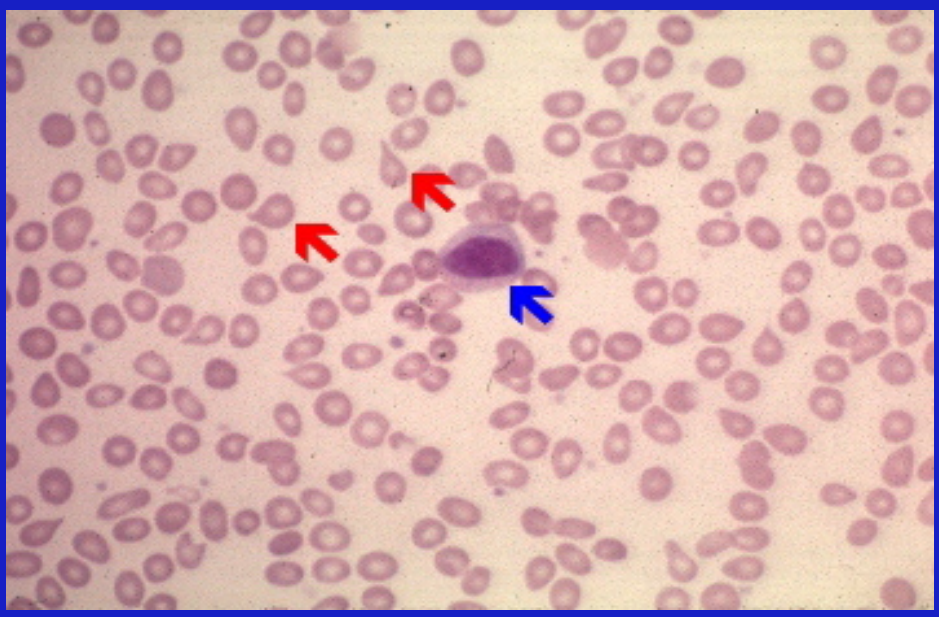

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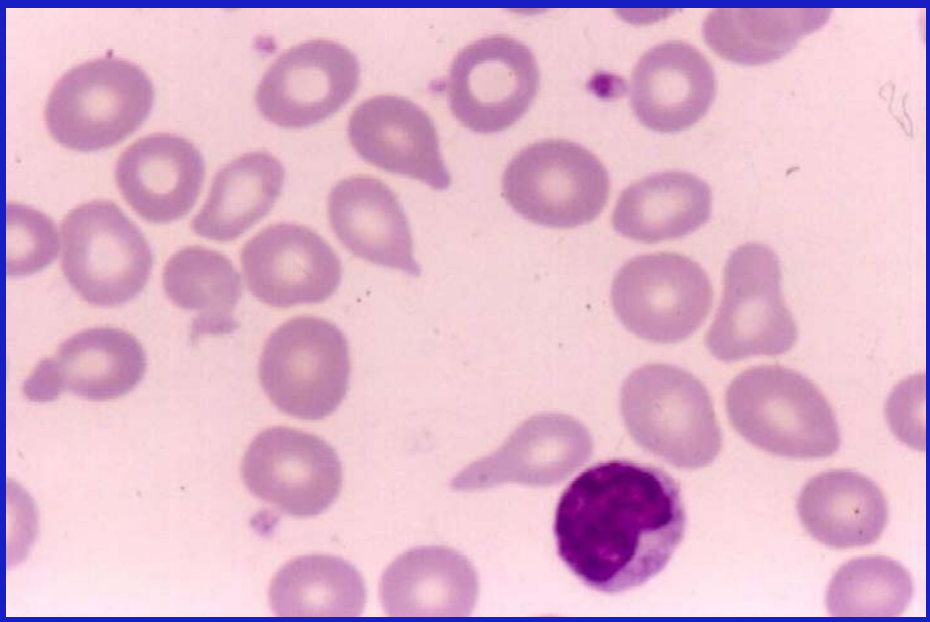

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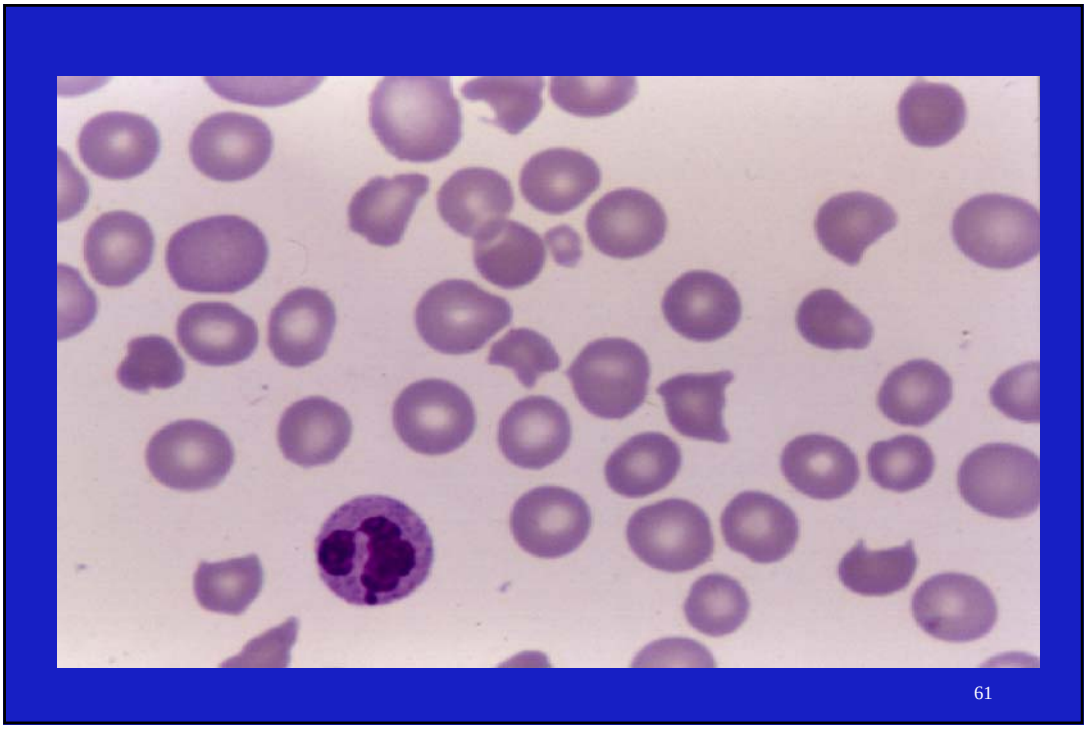

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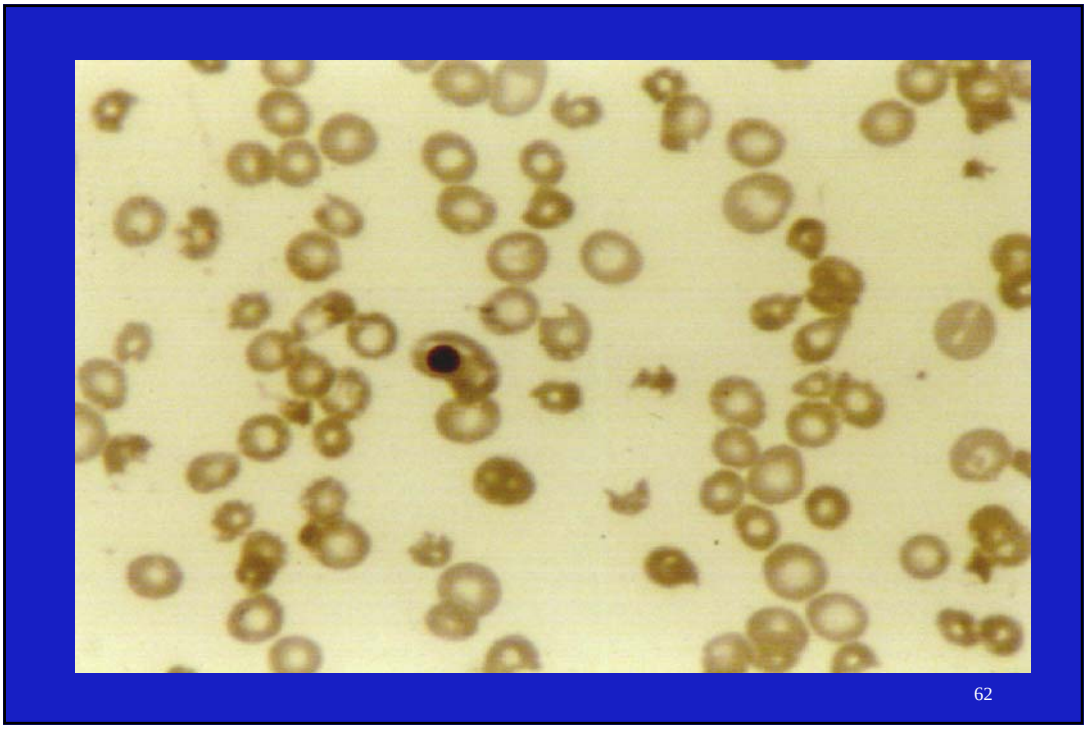

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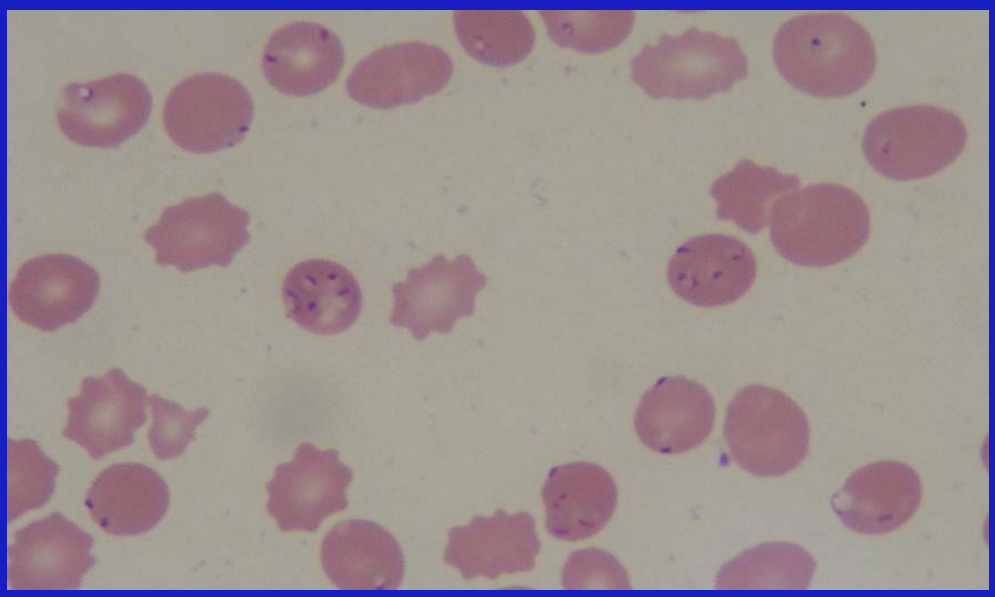

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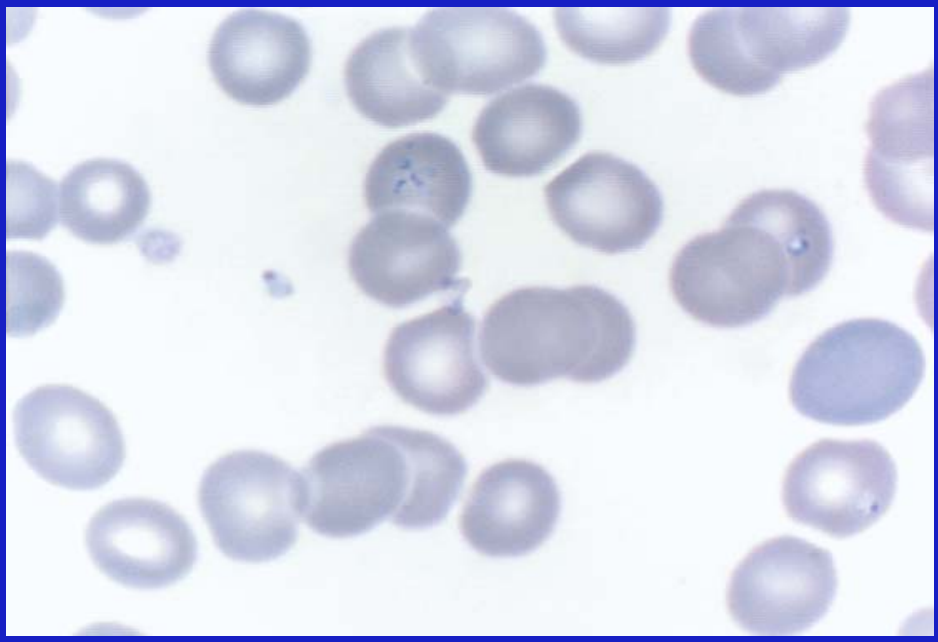

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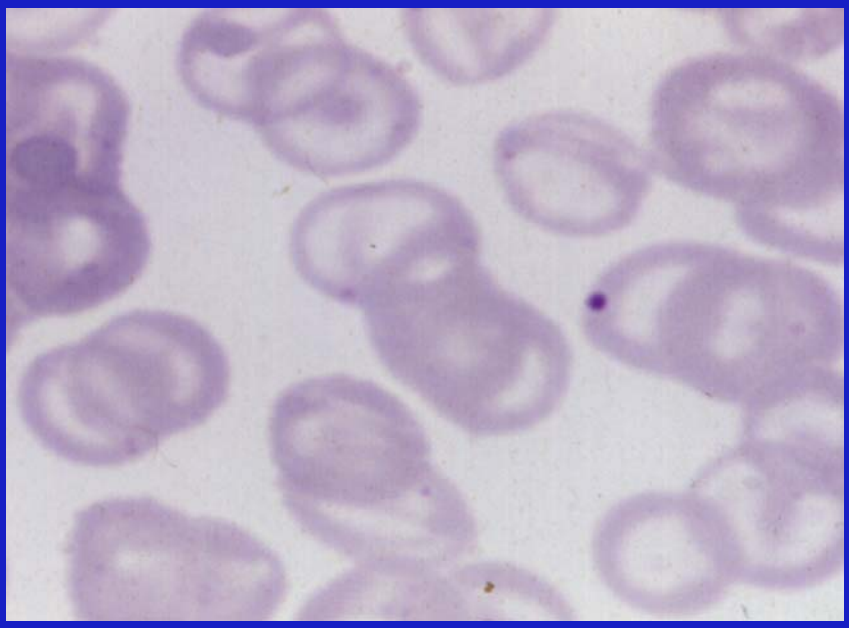

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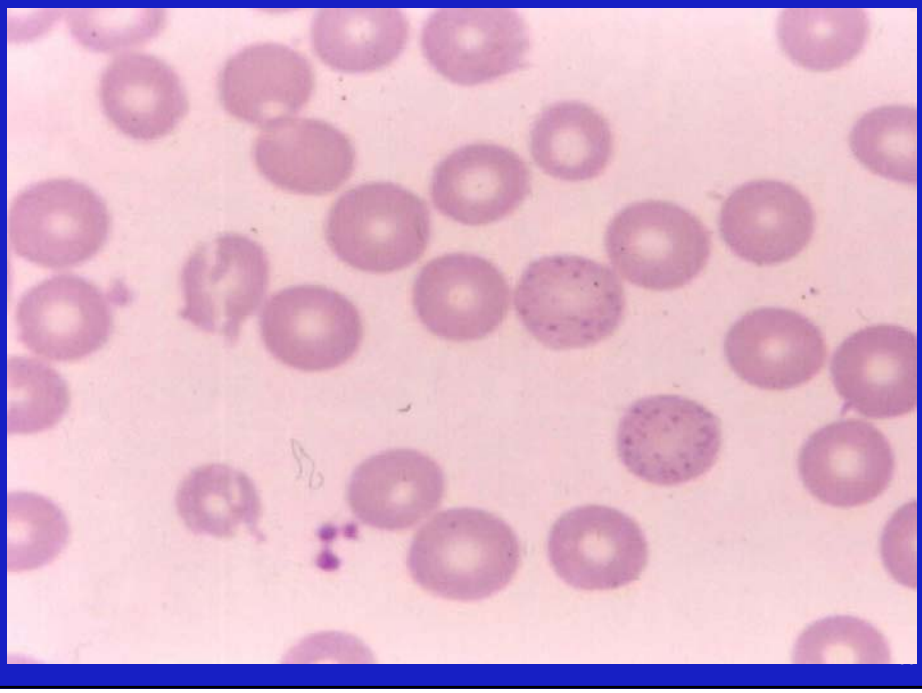
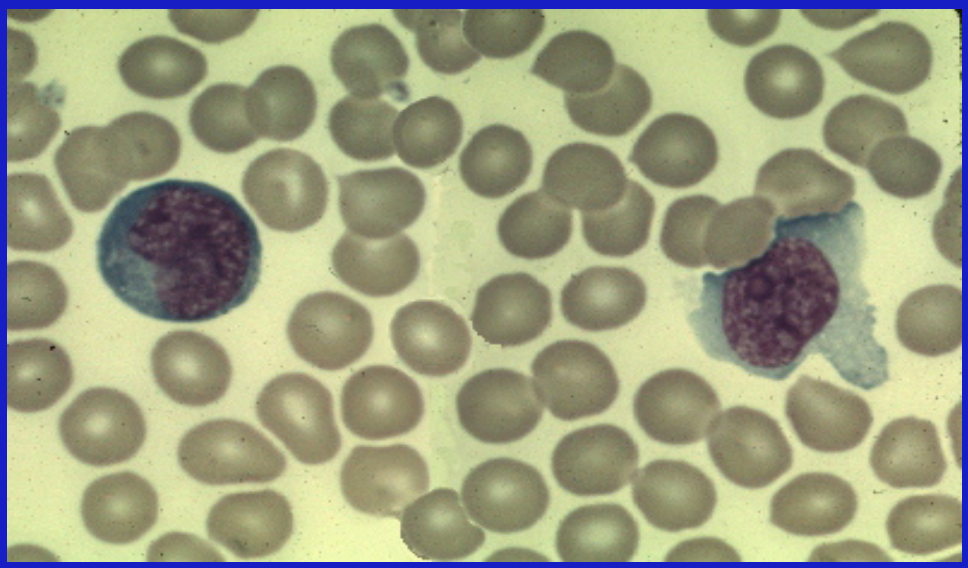


## WBC disorders

- Leukopenia
  - with absolute neutropenia: bone marrow failure, agranulocytosis
  - with atypical lymphocytes: viral infection, chronic lymphoproliferative disorders
  - with immature myeloid cells: acute leukemia, MDS or myelopthisis
- Leukocytosis
  - Reactive leukocytosis: leukemoid reaction
  - Acute leukemia: AML vs. ALL
  - Chronic myeloproliferative disorders
  - Chronic lymphoproliferative disorders
- Leukoerythroblastosis

67



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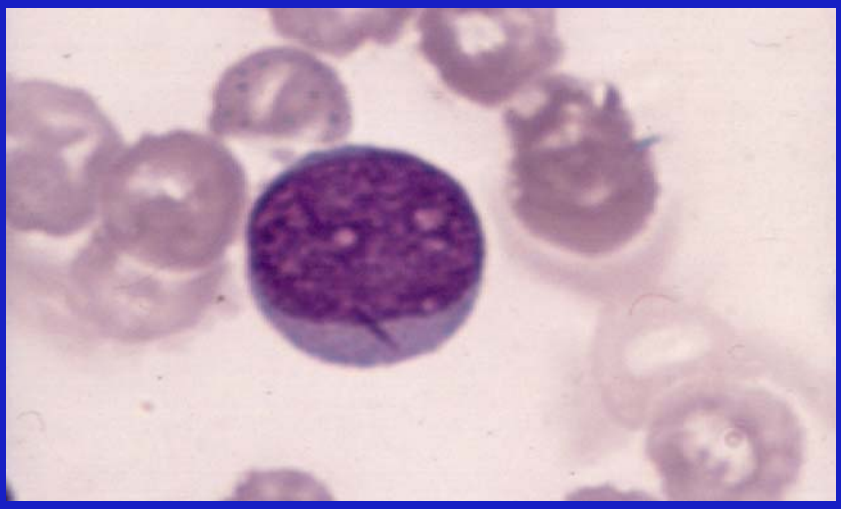

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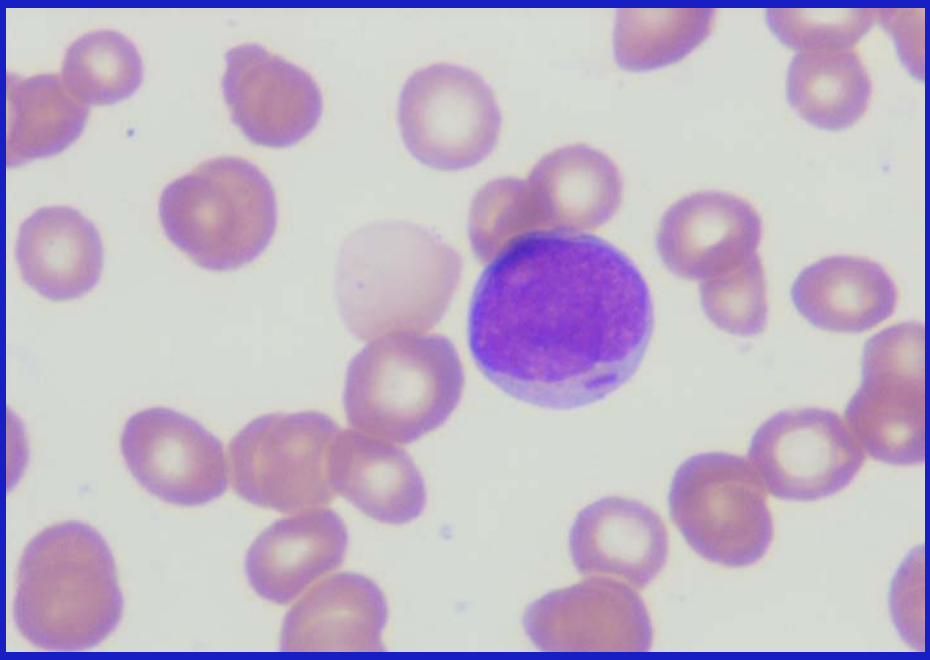

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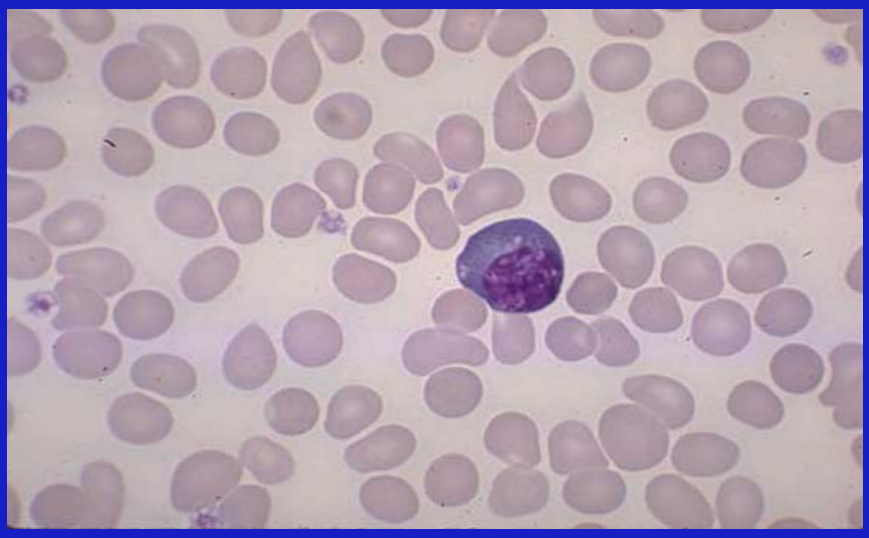

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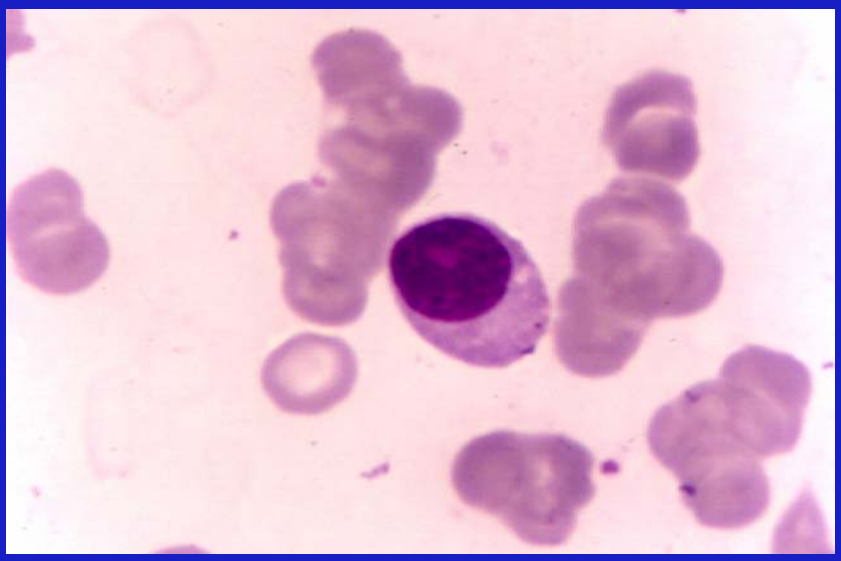

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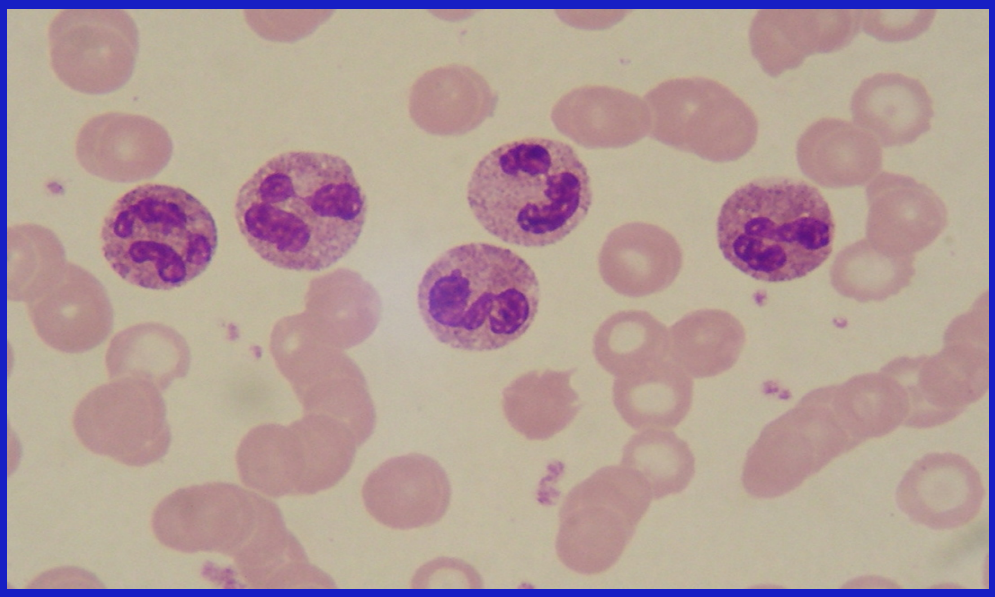

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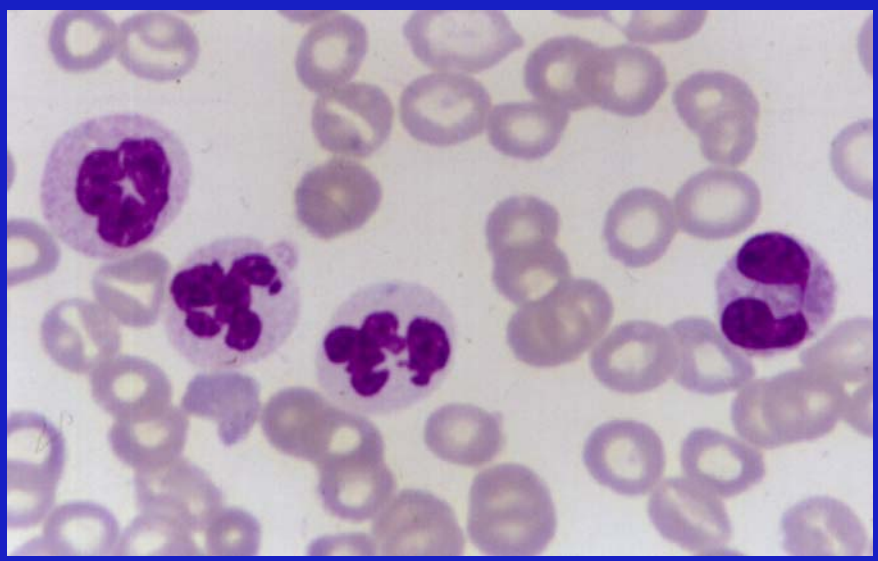

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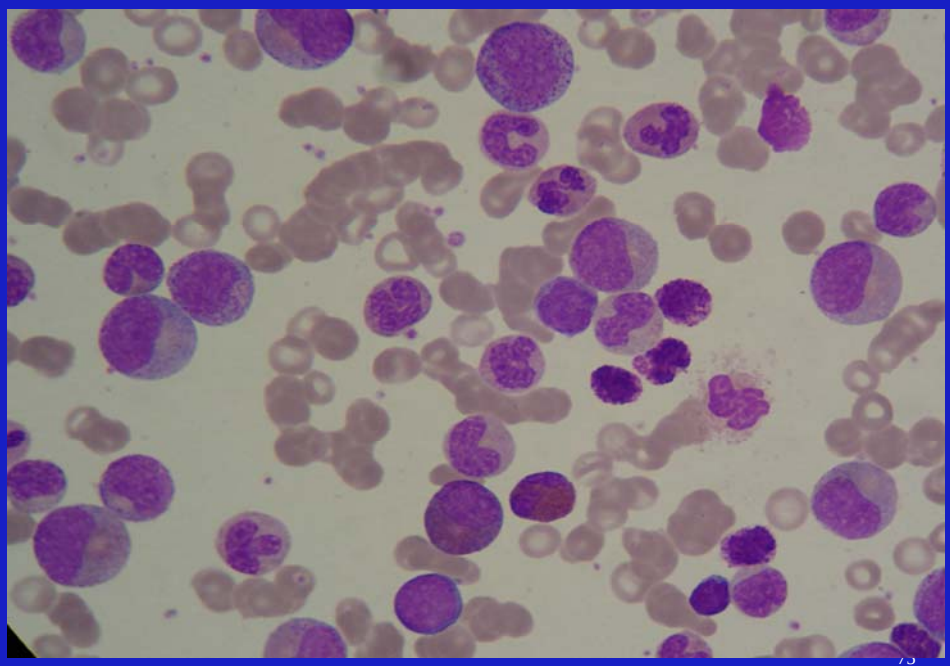

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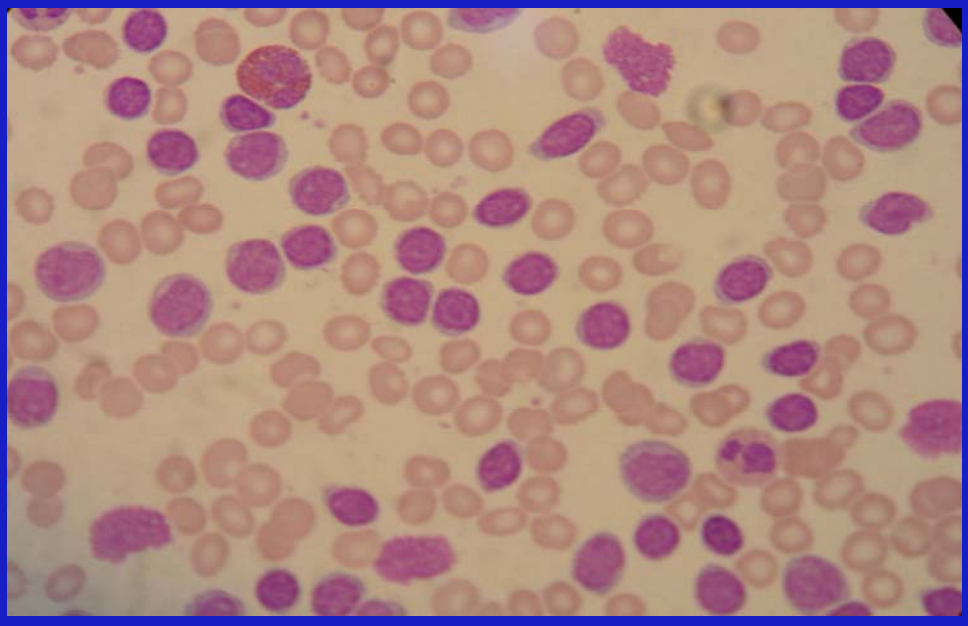

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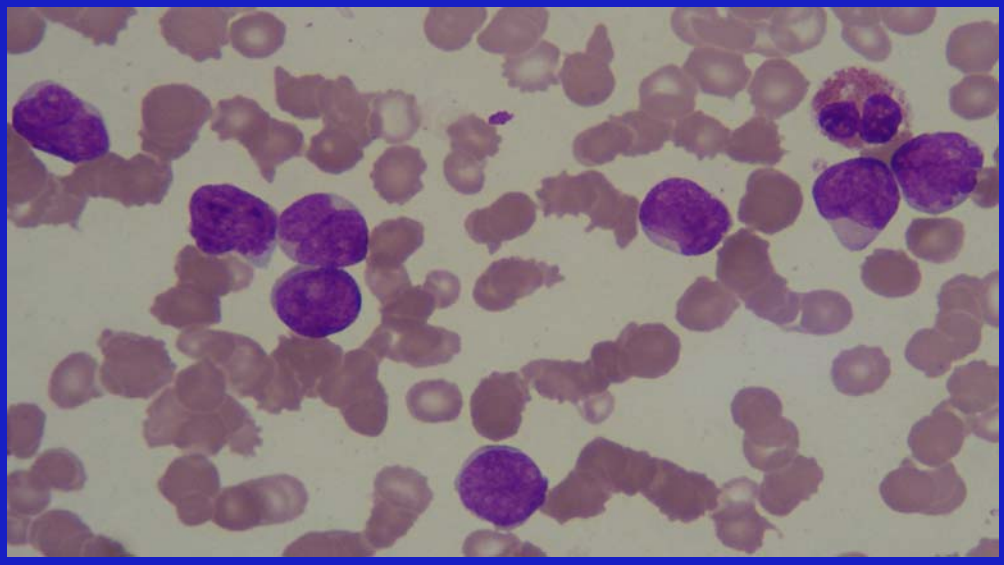

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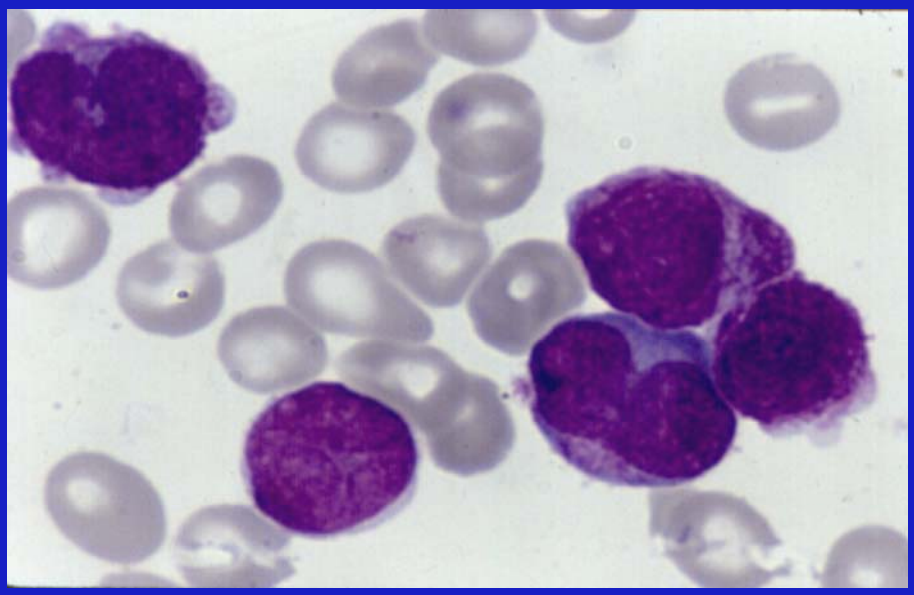

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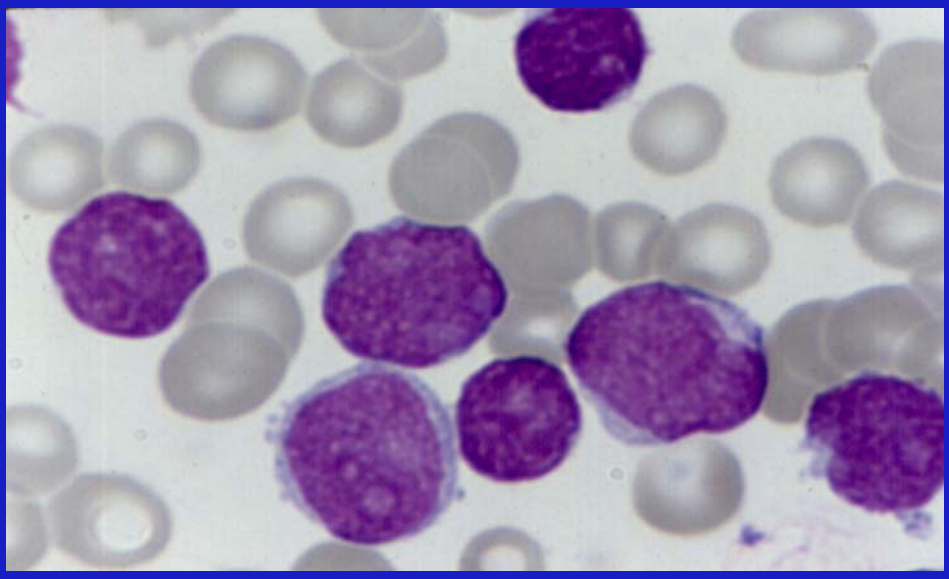

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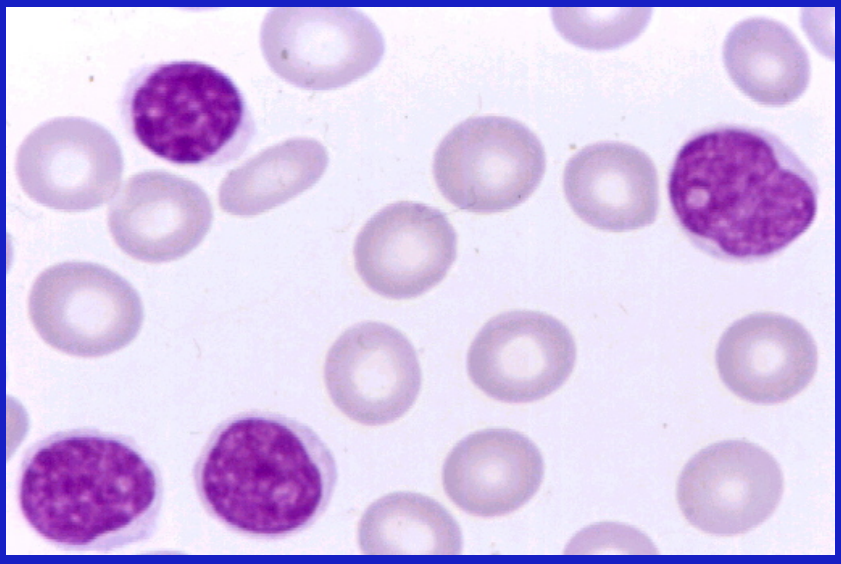

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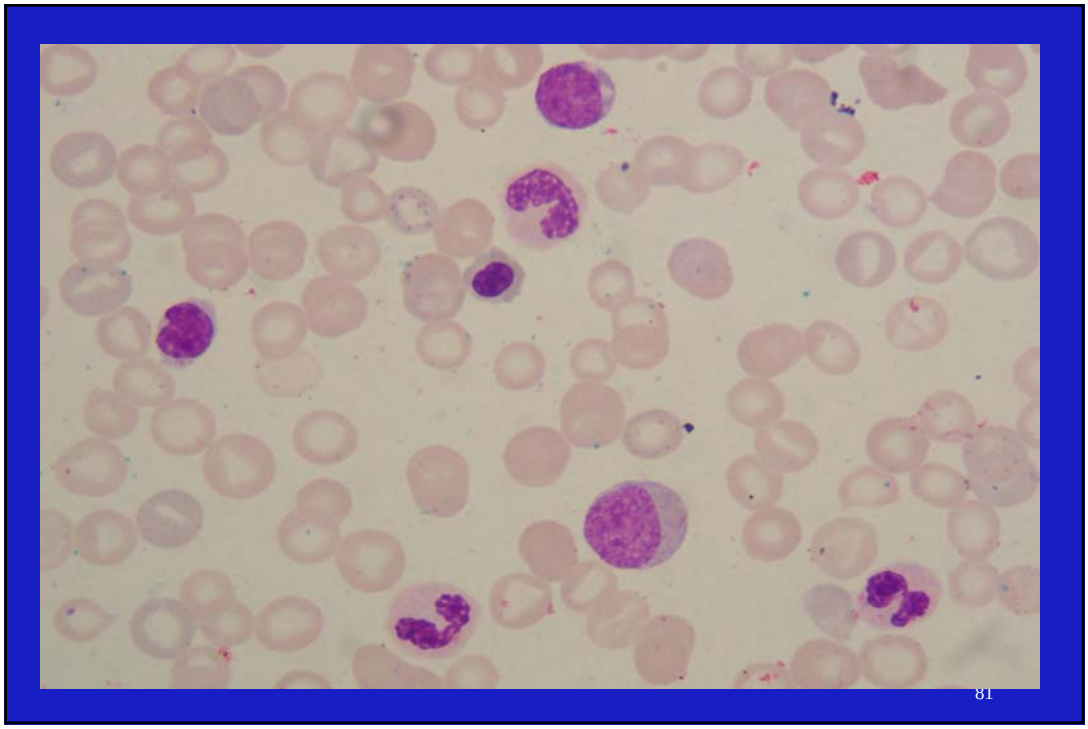

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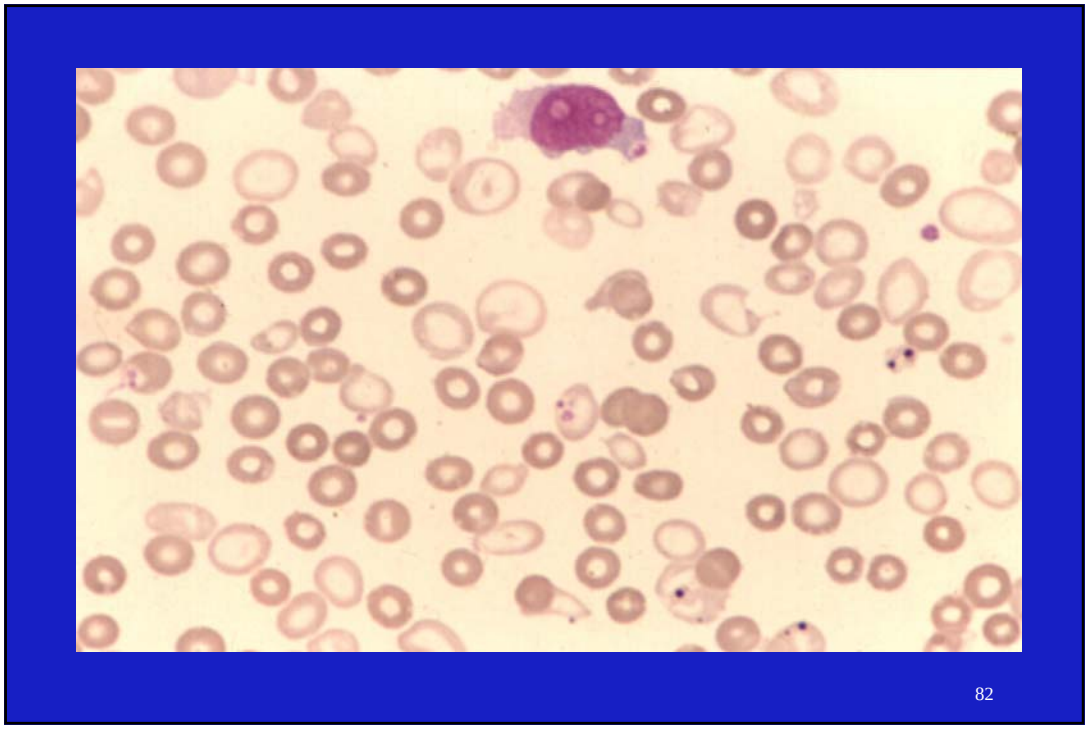
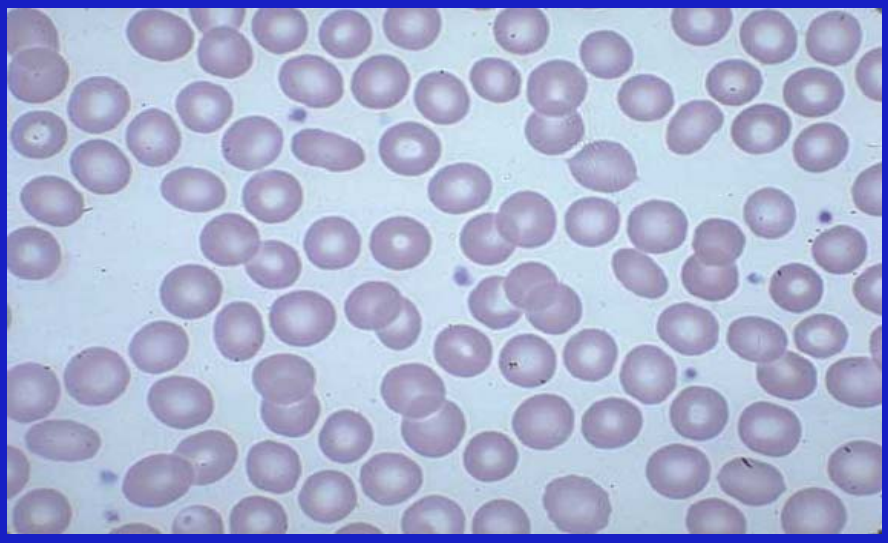


82

## Platelet disorders

- Quantitative disorders
  - Isolated thrombocytopenia: Immune vs. non-immune
  - Thrombocytopenia associated with other hematologic abnormalities
  - Thrombocytosis
- Qualitative disorders
  - Giant platelets (megathrombocytes)
  - Platelet inclusion or granule abnormality
  - Bizarre in shape and size
  - Megakaryocytes or megakaryoblasts

83



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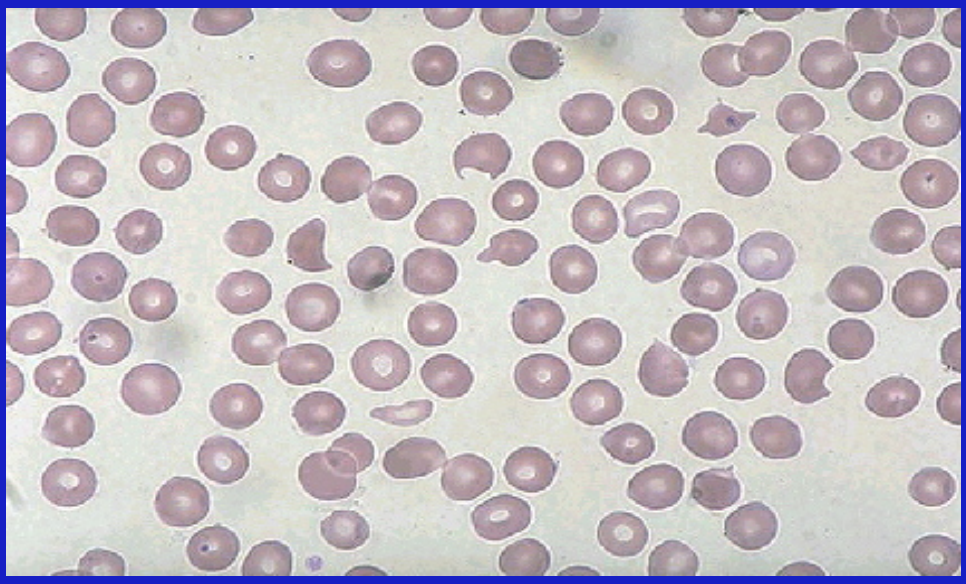

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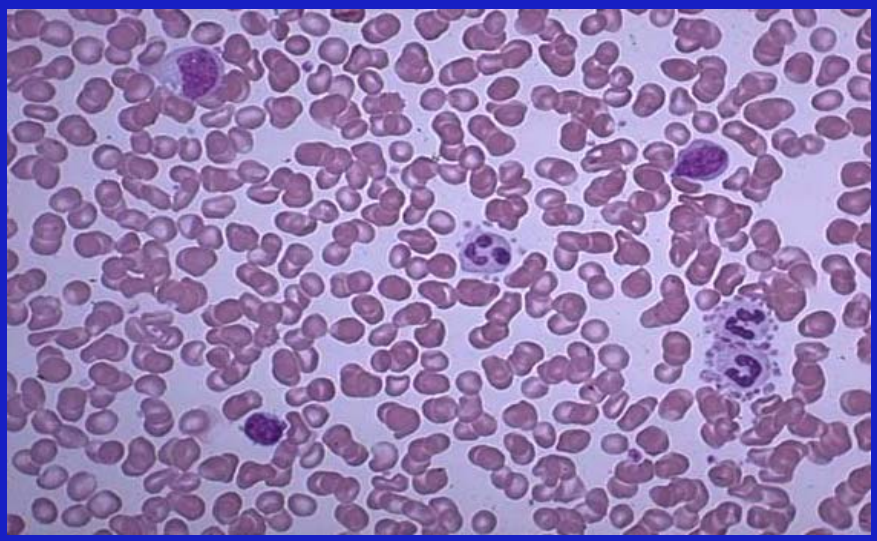

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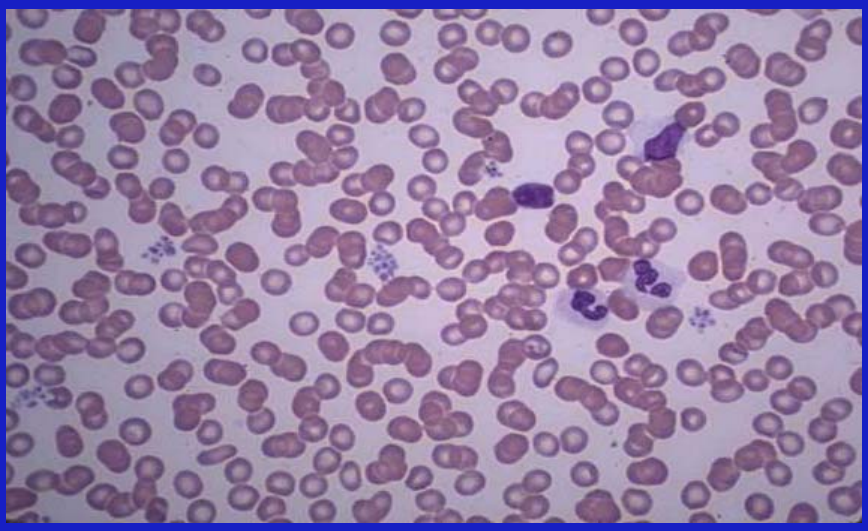

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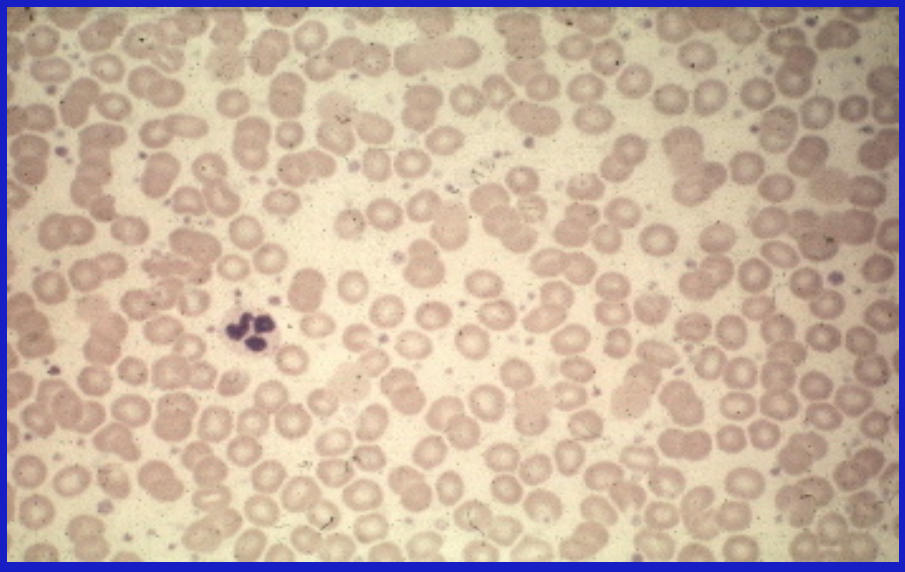

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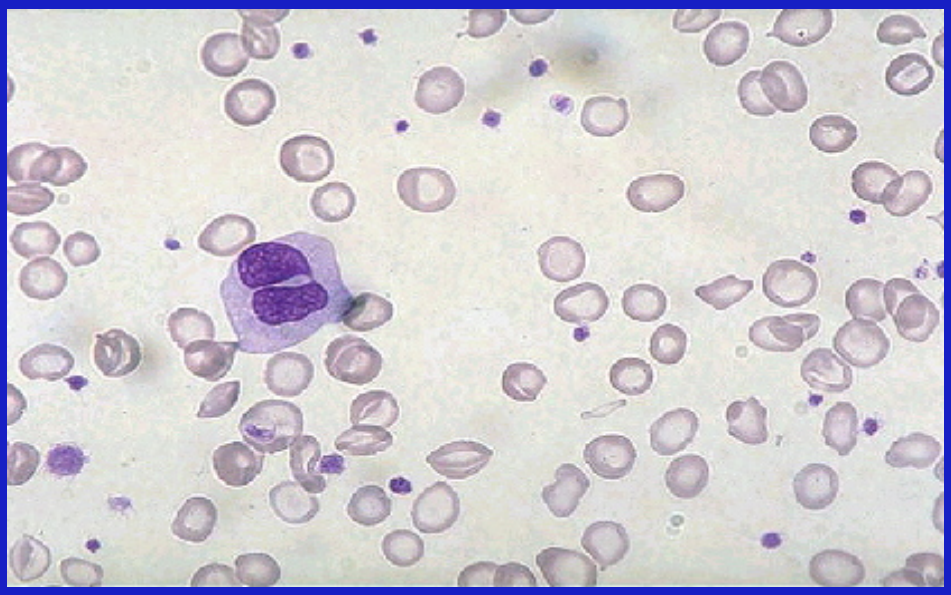

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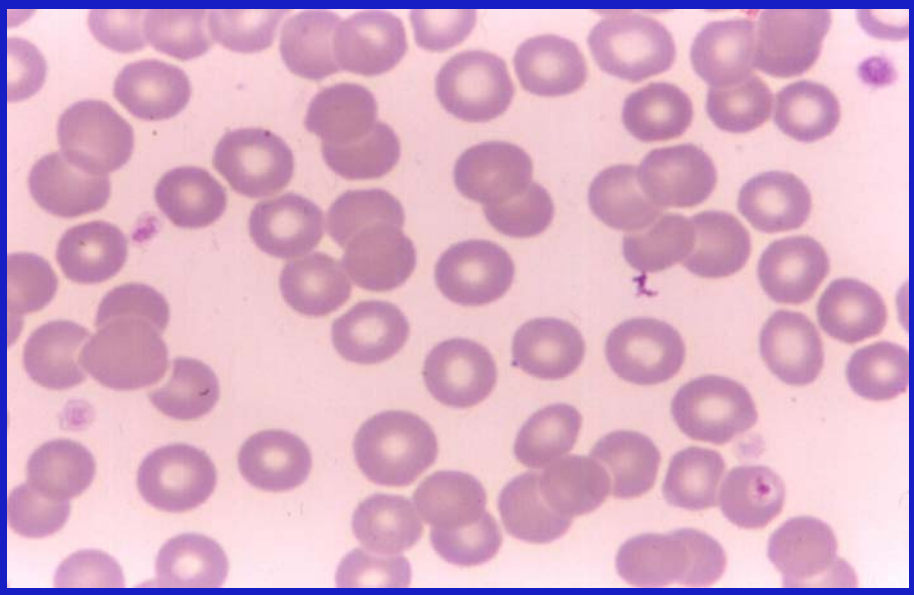

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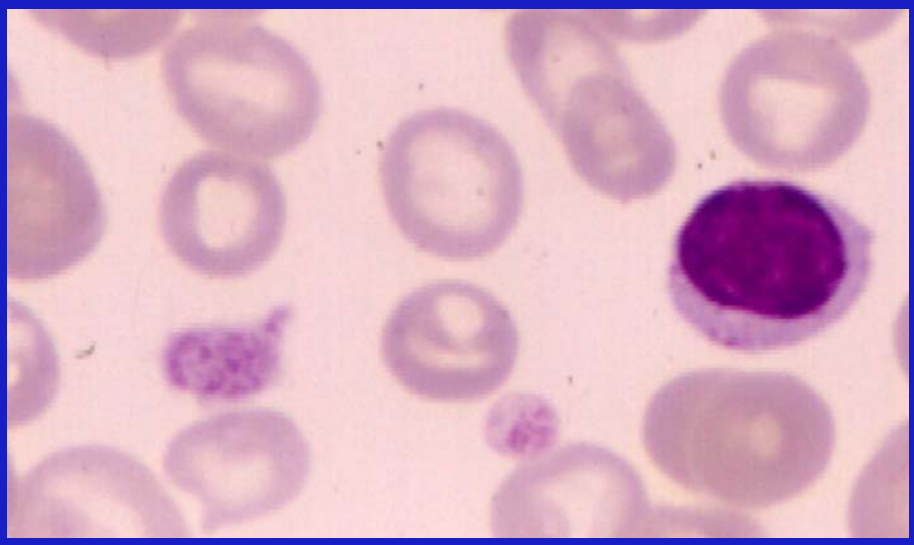

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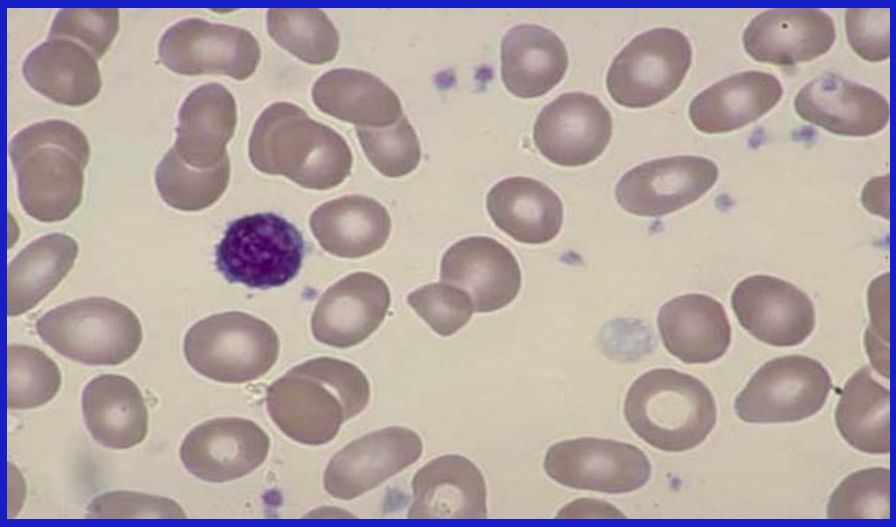

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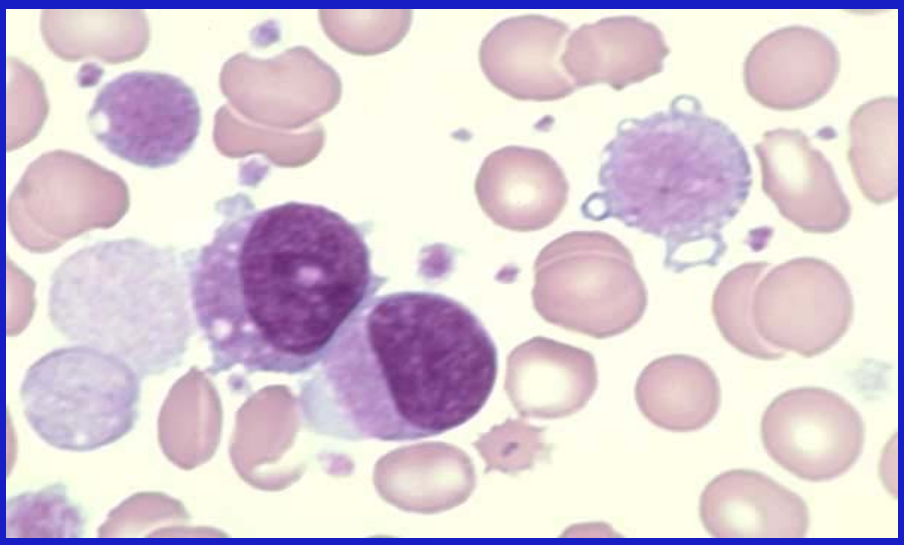

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