

**Bone Marrow Differentiation
in Toxicity Studies – Sense and Non-sense**

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RCC Ltd Itingen, Switzerland**

Guidelines



The European Agency for the Evaluation of Medicinal Products
Evaluation of Medicines for Human Use

London, 27 July 2000
CPMP/SWP/1042/99 corr.

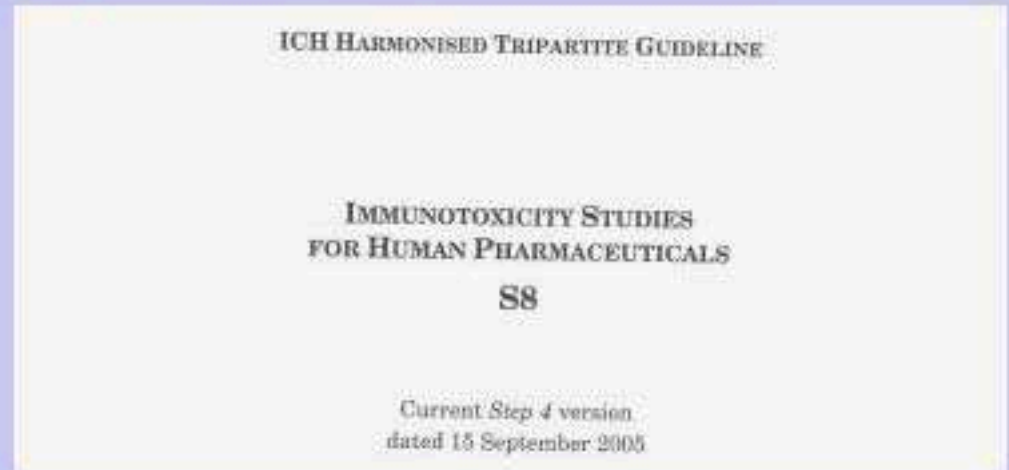
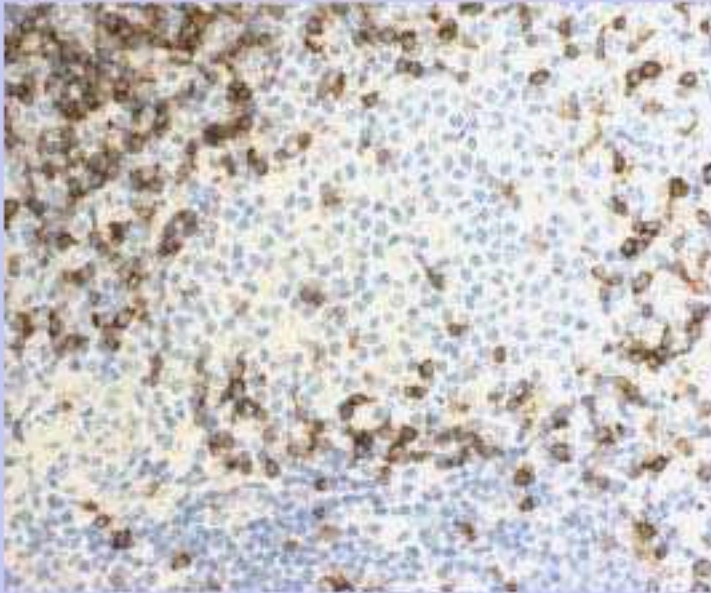
**COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS
(CPMP)**

NOTE FOR GUIDANCE ON REPEATED DOSE TOXICITY

Guidelines

- ✓ Refer to **ICH M3** and other relevant guidelines
- ✓ All new medical products should be screened for **immunotoxic potential** in at least one repeated dose toxicity study....The interpretation....**integrative analysis** of changes in lymphoid tissues and immune cell populations as well as other types of toxicity
- ✓ Bone marrow cellularity, lymphocyte subsets and NK cell activity or the primary antibody response to T-cell dependent antigen (**Appendix B**)
- ✓ Tissue list specified (**Appendix A**)

Guidelines

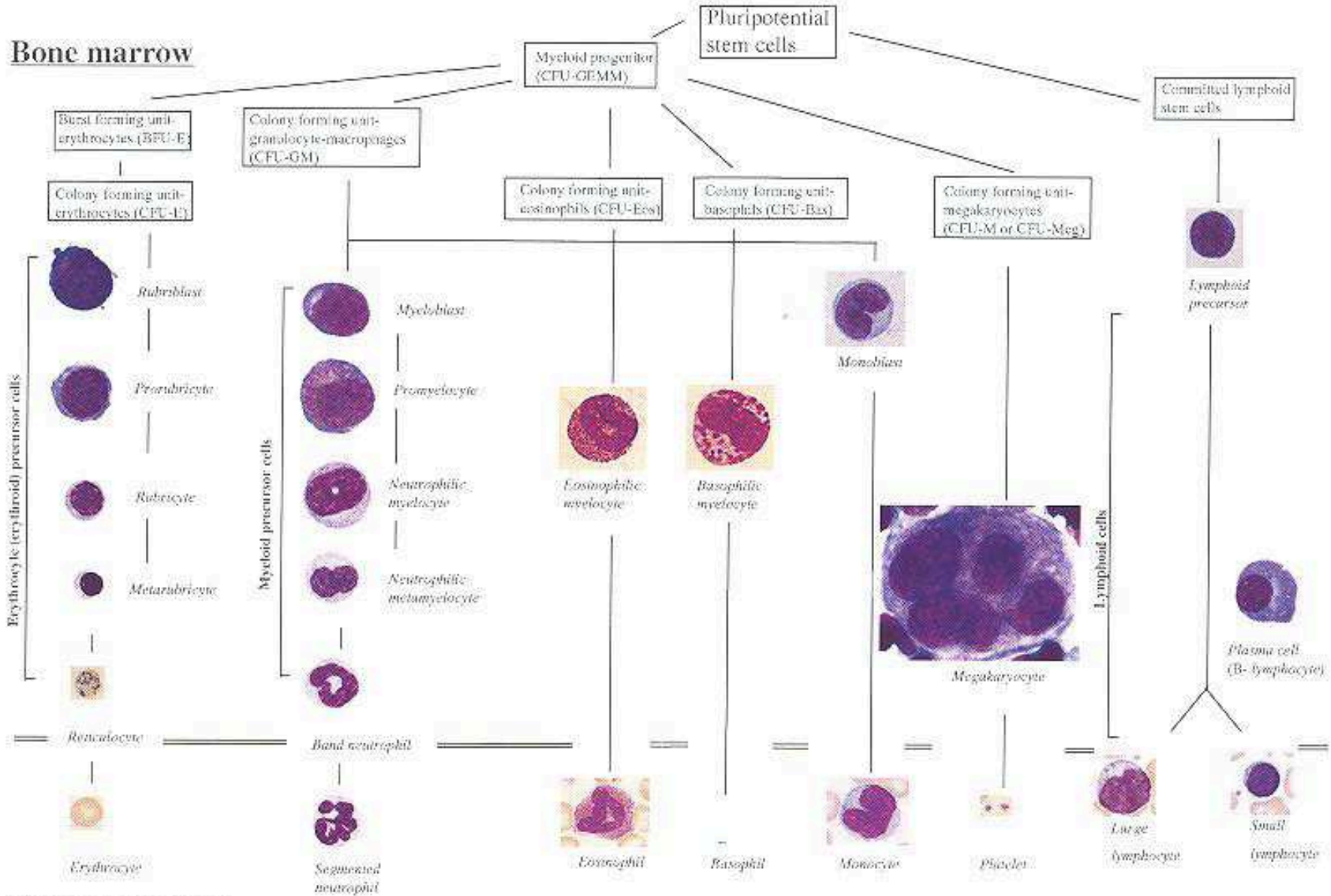


- ✓ Detailed strategy!
- ✓ **all lymphoid tissues** to be examined (incl. Peyer's patches)
- ✓ **immunohistochemistry** superior to FACScan
- ✓ interpretation of **stress-related effects**

Guidelines

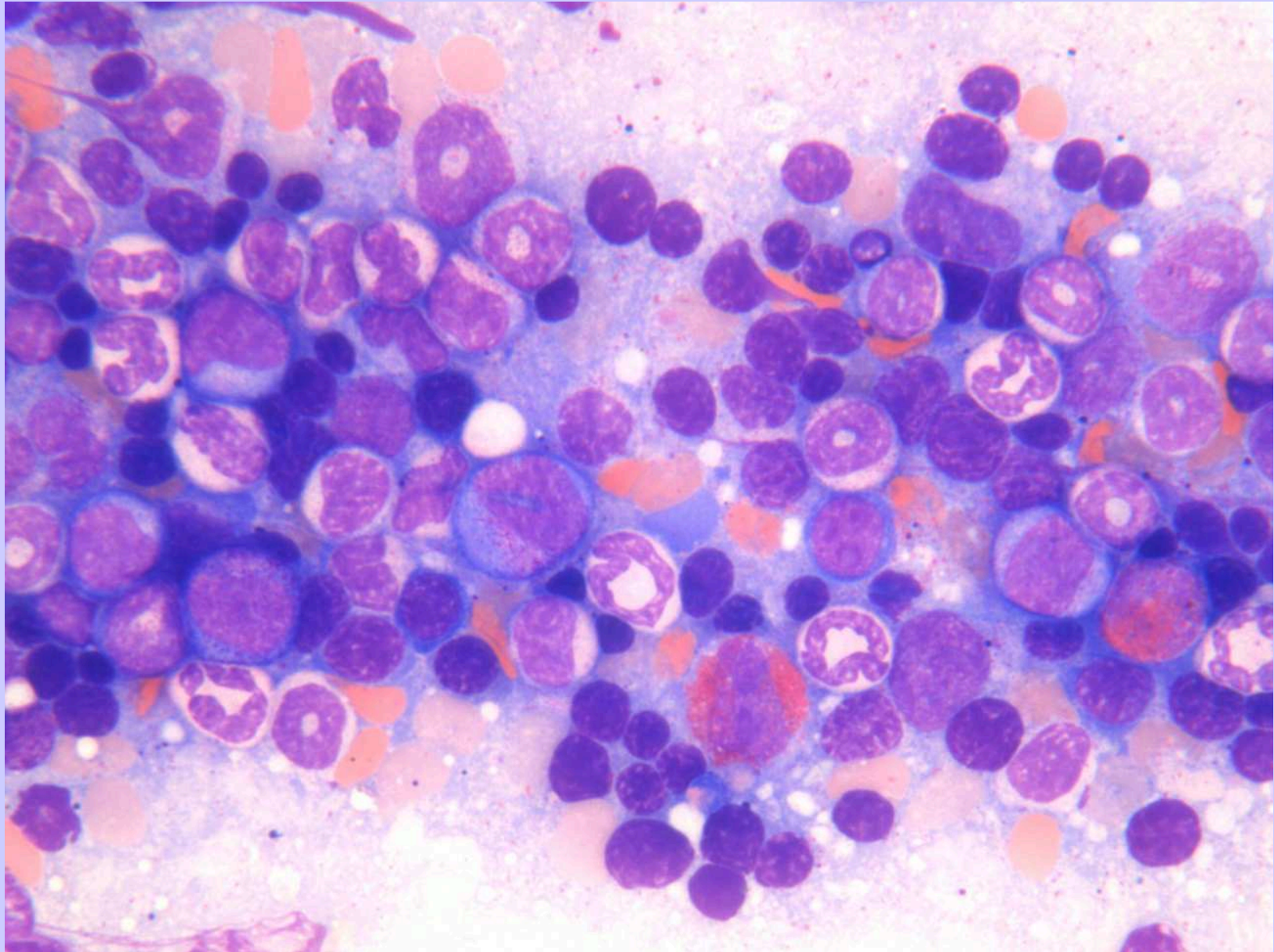
Parameter	Specific Component
Hematology	Total and absolute differential leukocyte counts
Clinical Chemistry	Globulin levels ¹ and A/G ratios
Gross pathology	Lymphoid organs / tissues
Organ weights	Thymus, spleen (optional: lymph nodes)
Histology	Thymus, spleen, draining lymph node and at least one additional lymph node, bone marrow ² , Peyer's patch ³ , BALT ⁴ , NALT ⁴

Bone marrow

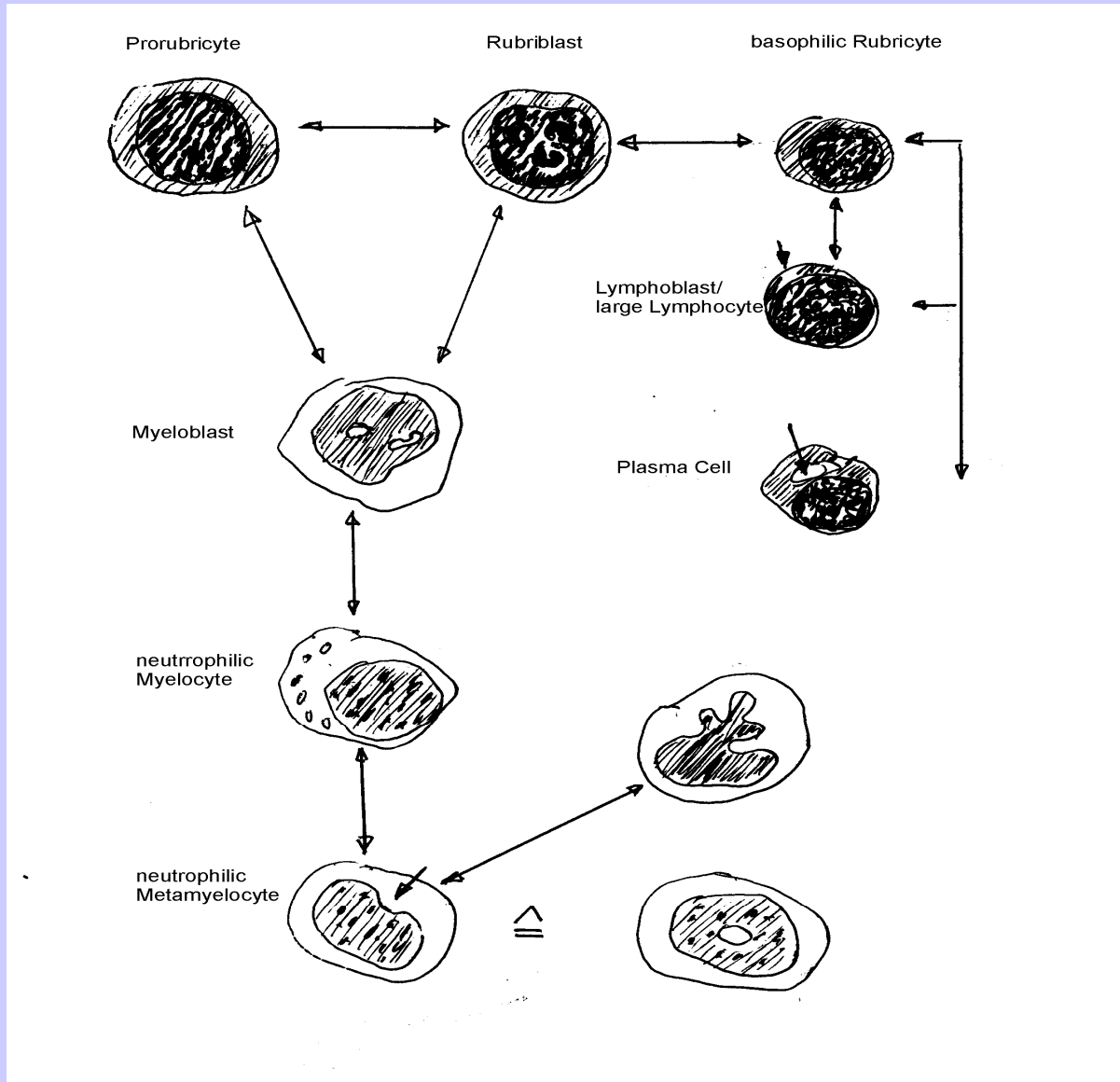


Peripheral blood

Bone Marrow: Overview



Differentiation Problems



How to learn differentiation?

Die Blutmorphologie der Laboratoriumstiere

VON
DR. MED. VET. DR. MED. VET. H. C.
SIEGMUND SCHERMER
em. O. O. PROFESSOR AN DER UNIVERSITÄT GÖTTINGEN

2., VERBESSERTE AUFLAGE.

MIT 54 z. T. FARBIGEN
ABBILDUNGEN IM TEXT

Institut für Zoologie
Reaktorzentrum Salzburg
Österreich



1 9 5 8

JOHANN AMBROSIOUS BARTH / VERLAG / LEIPZIG

How to learn differentiation?

ATLAS DER KLINISCHEN HÄMATOLOGIE UND CYTOLOGIE

IN DEUTSCHER, ENGLISCHER, FRANZÖSISCHER UND SPANISCHER SPRACHE

VON
LUDWIG HEILMEYER UND HERBERT BEGEMANN

MIT BETRÄGEN VON
W. MOHR UND W. LANGREDER

BILDBAND

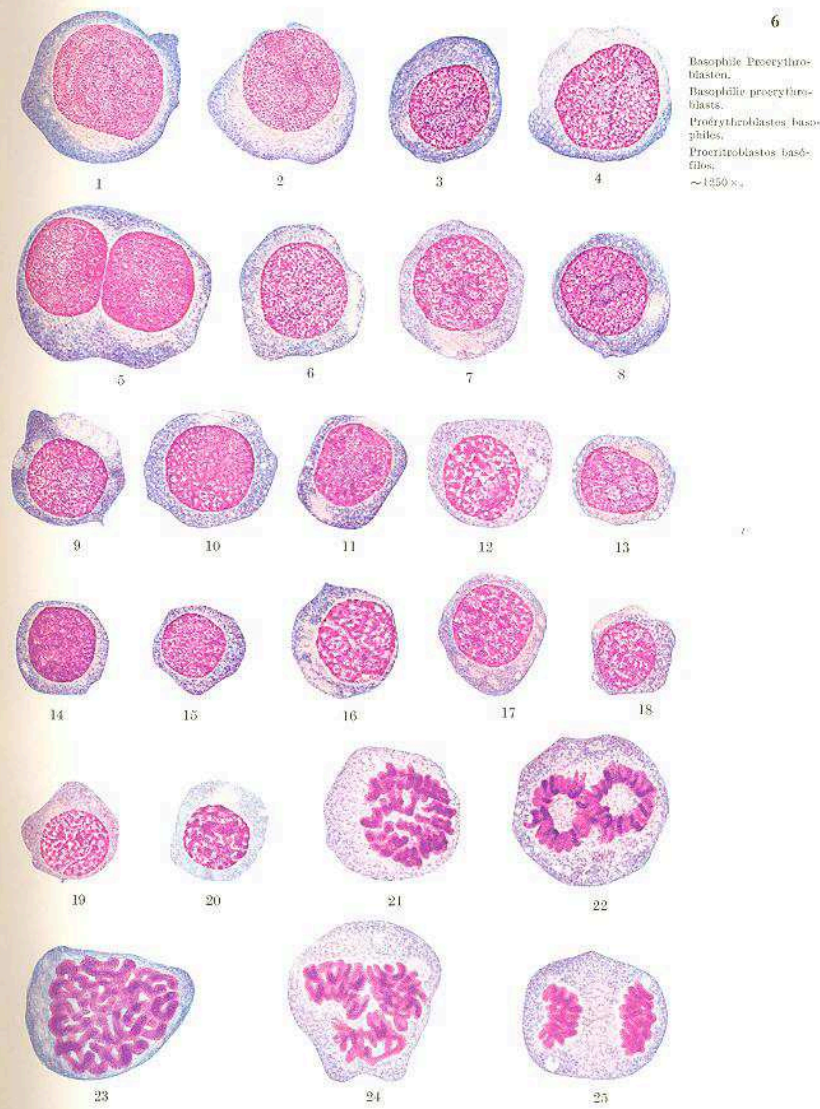
MIT 257 FARBIGEN UND 4 EINFARBIGEN ABBILDUNGEN

GEZEICHNET VON
HANS DETTELBACHER UND THEA BARNER-DETTLEBACHER



SPRINGER-VERLAG
BERLIN · GÖTTINGEN · HEIDELBERG

1955

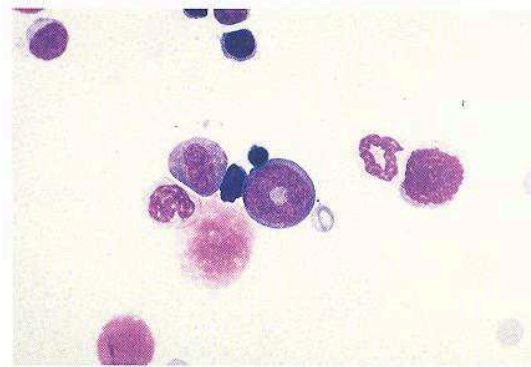
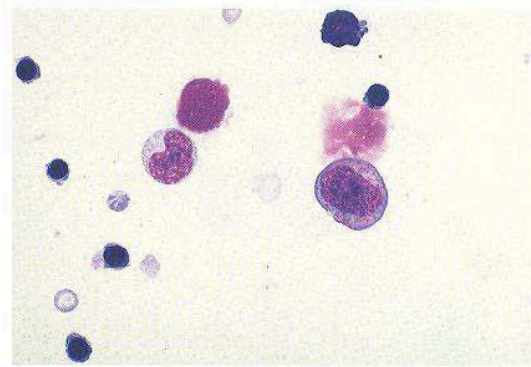


How to learn differentiation?

ATLAS OF THE HEMATOLOGY OF THE LABORATORY RAT

Edited by
A. HASEGAWA
K. FURUHAMA

Photo. 82D (x 850)



69

Photo. 82E (x 850)

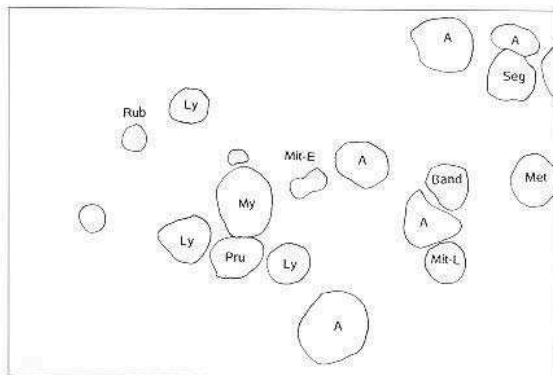
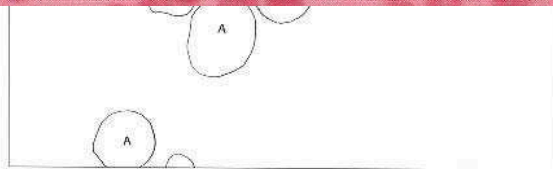
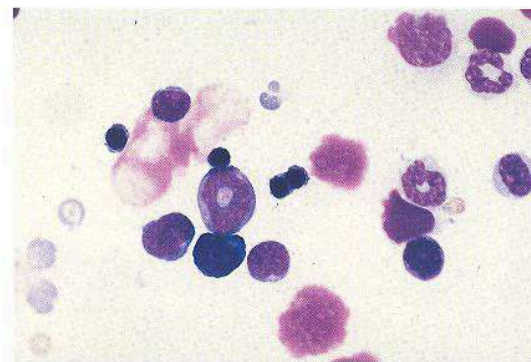


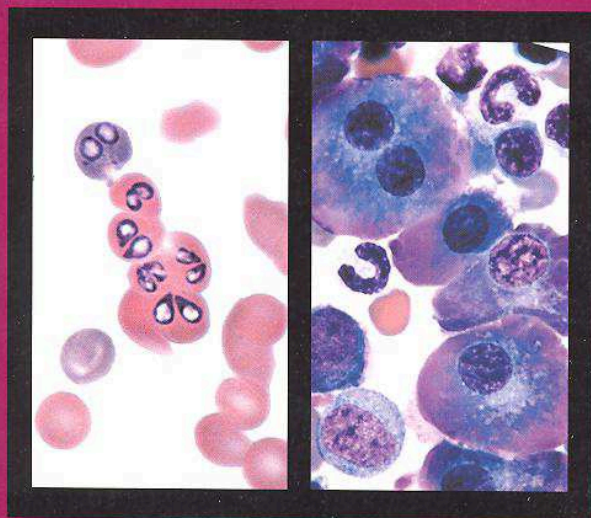
Photo. 82F (x 850)



How to learn differentiation?

ATLAS OF VETERINARY HEMATOLOGY

Blood and Bone Marrow
of Domestic Animals



John W. Harvey

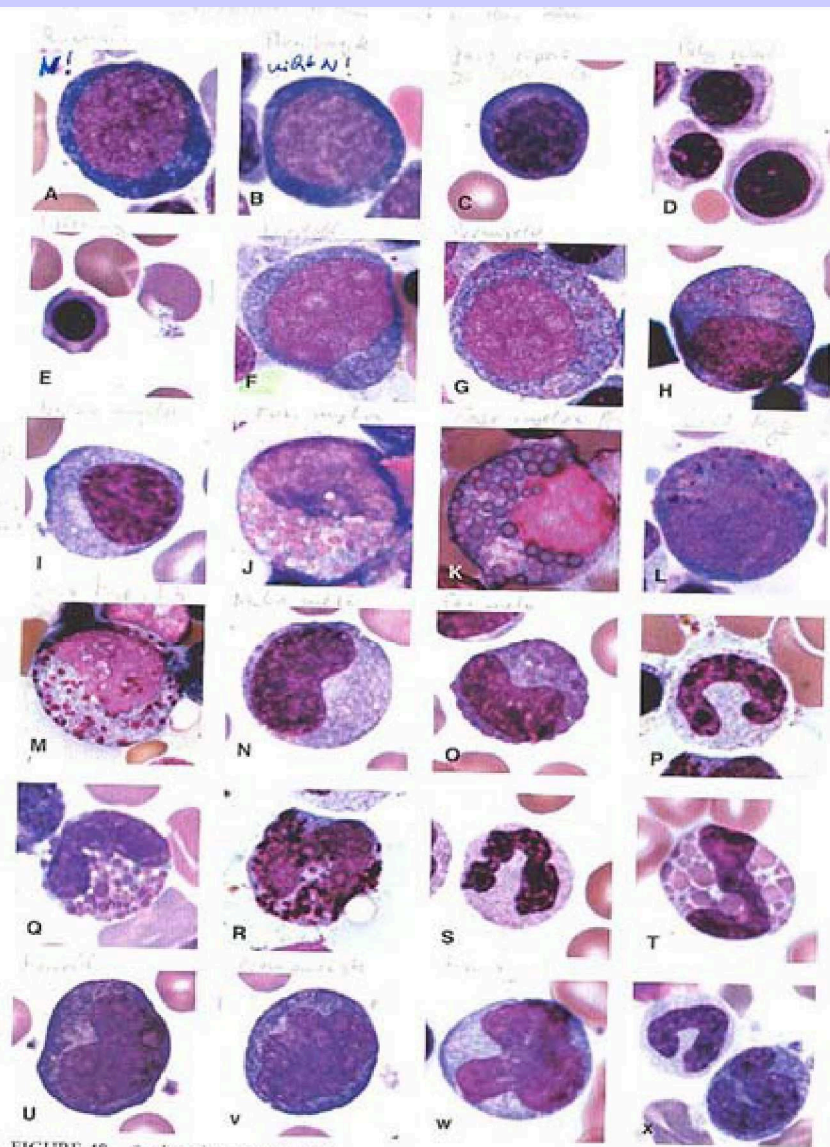
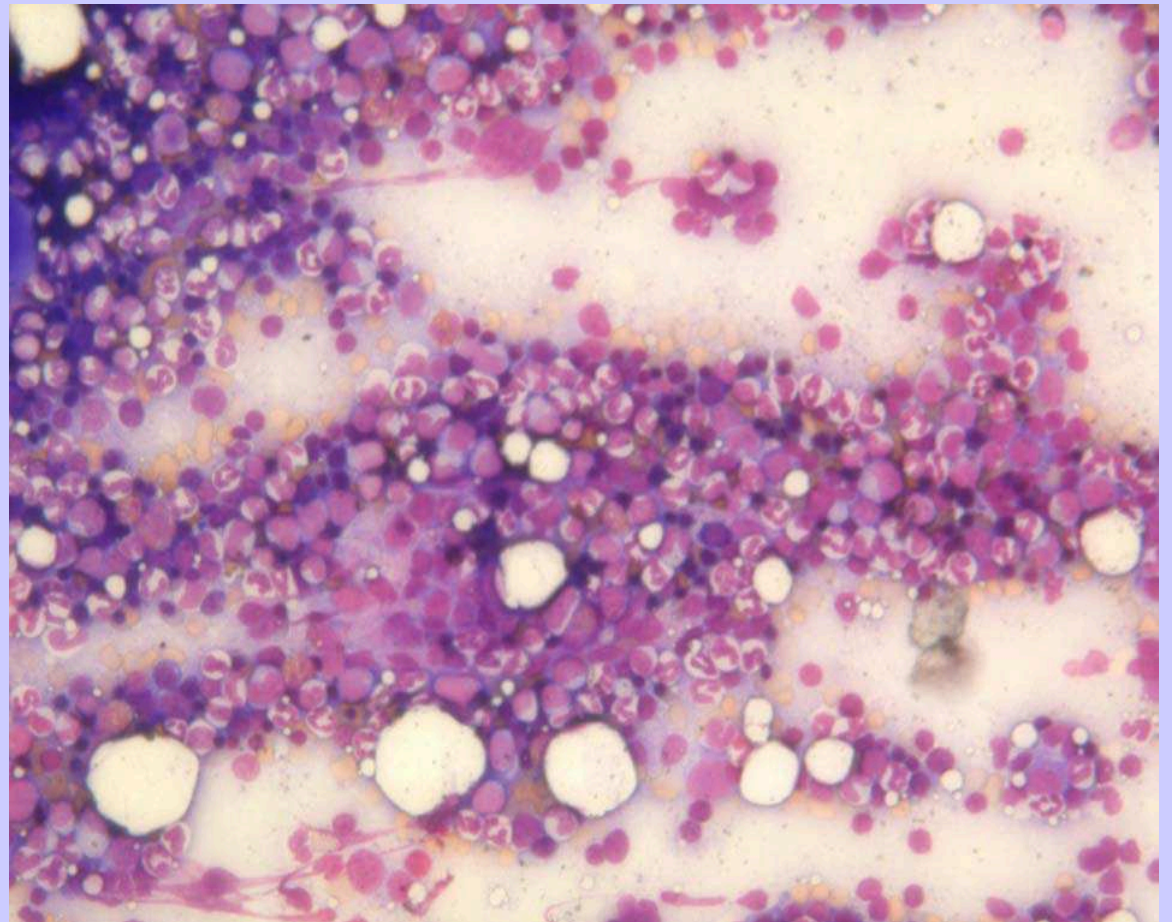


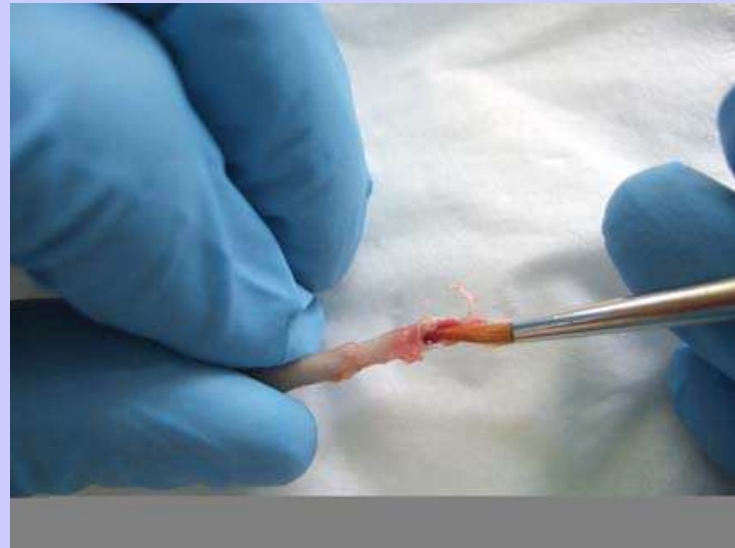
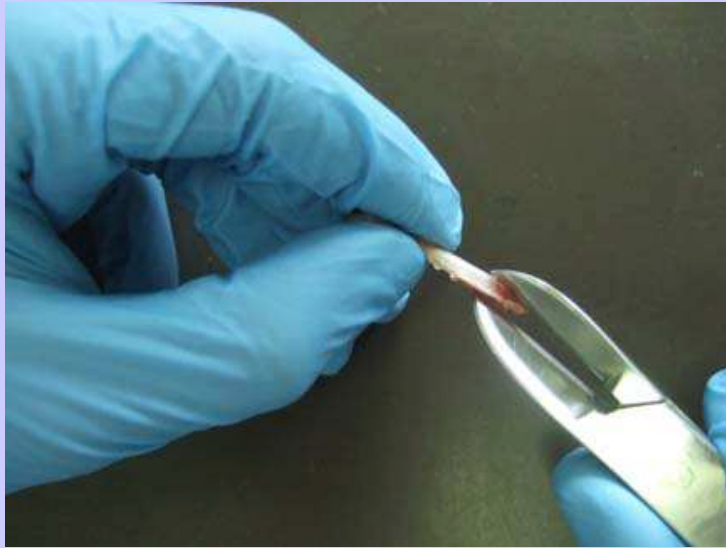
FIGURE 40. See legend on opposite page.

Bone Marrow: Techniques

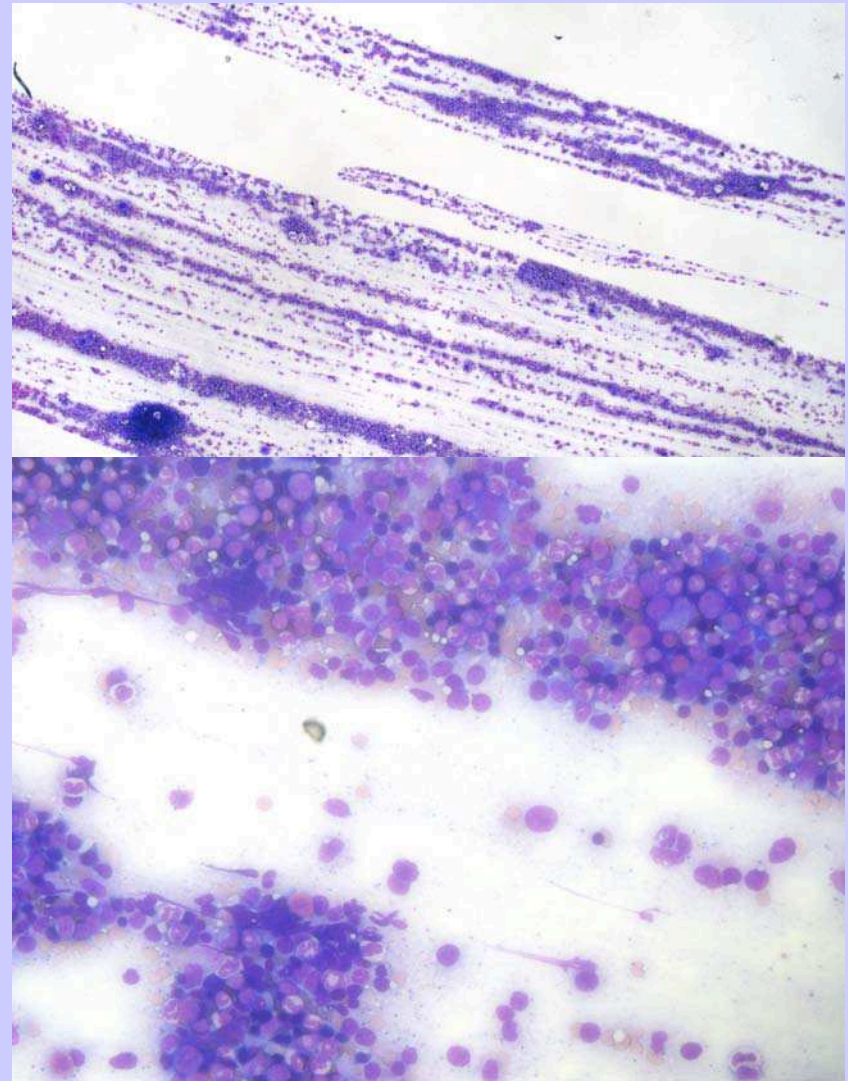
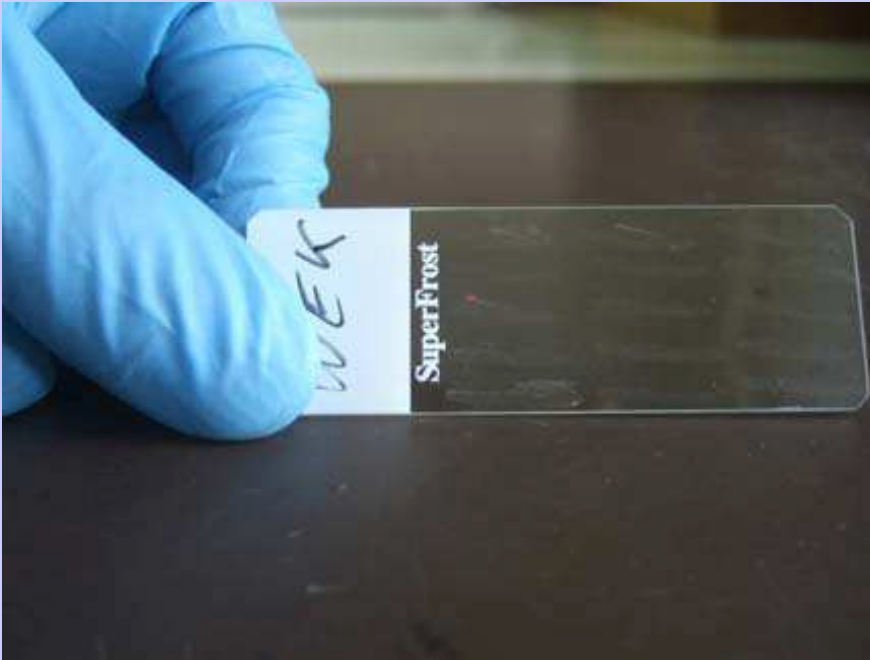
- ✓ Smear vs Brush
- ✓ Cytospin
- ✓ Plastic Embedding
- ✓ Paraffin Blocks



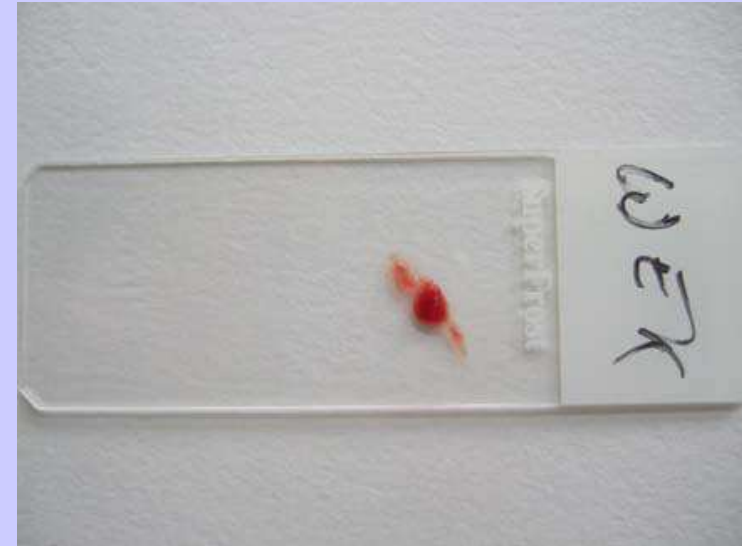
Techniques: Brush



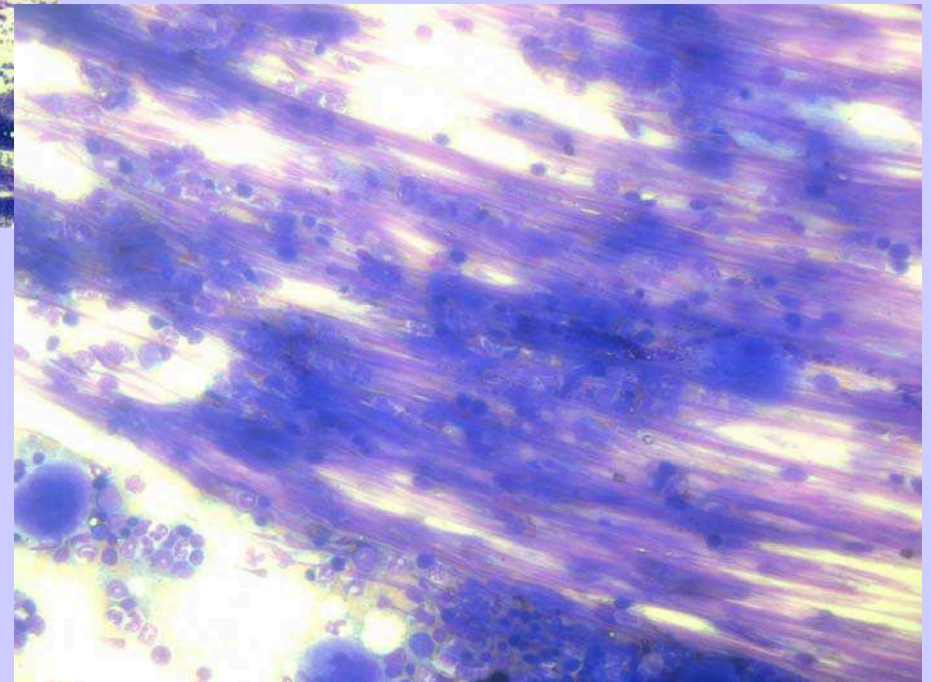
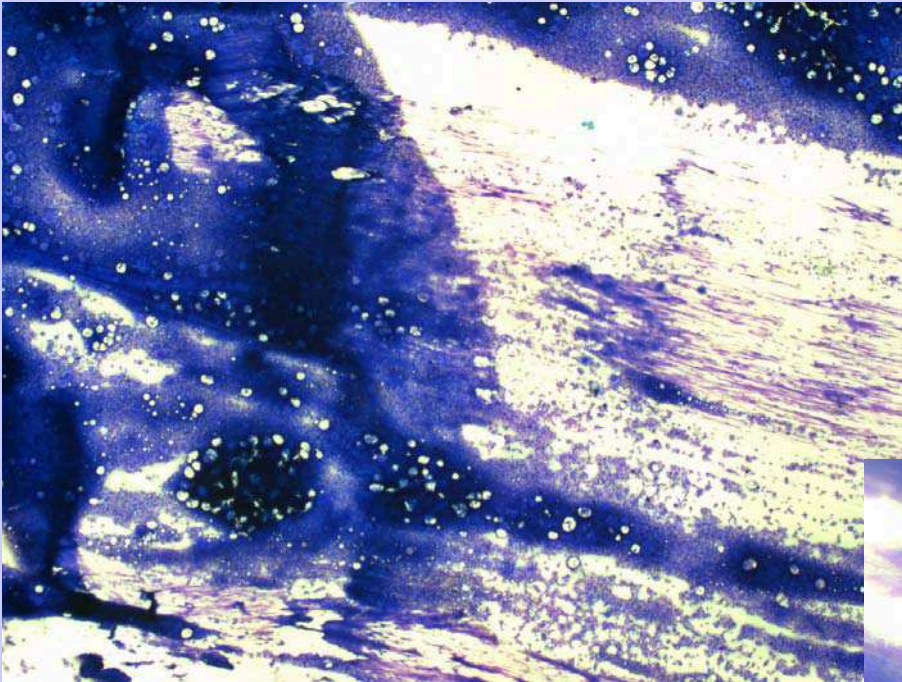
Techniques: Brush



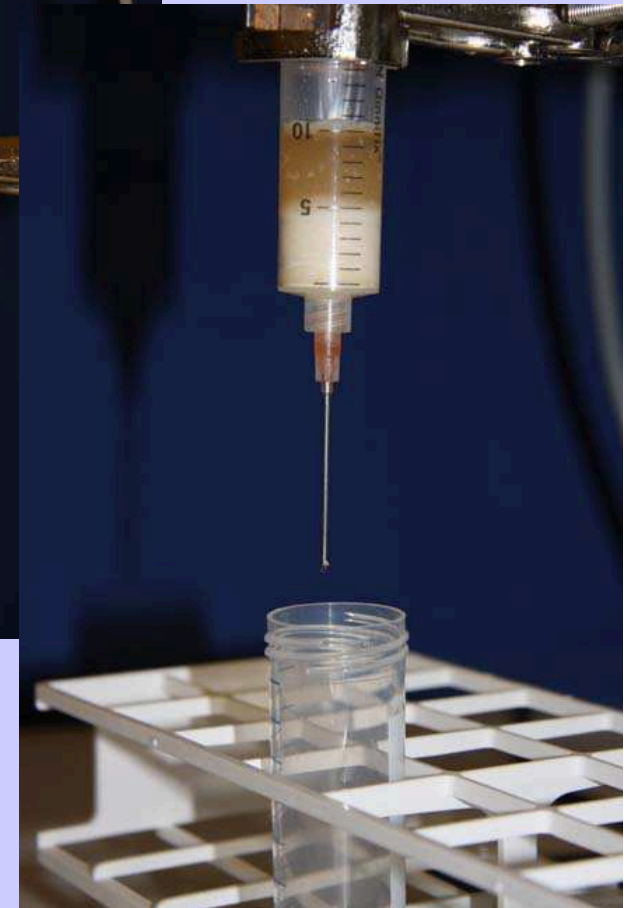
Techniques: Smear



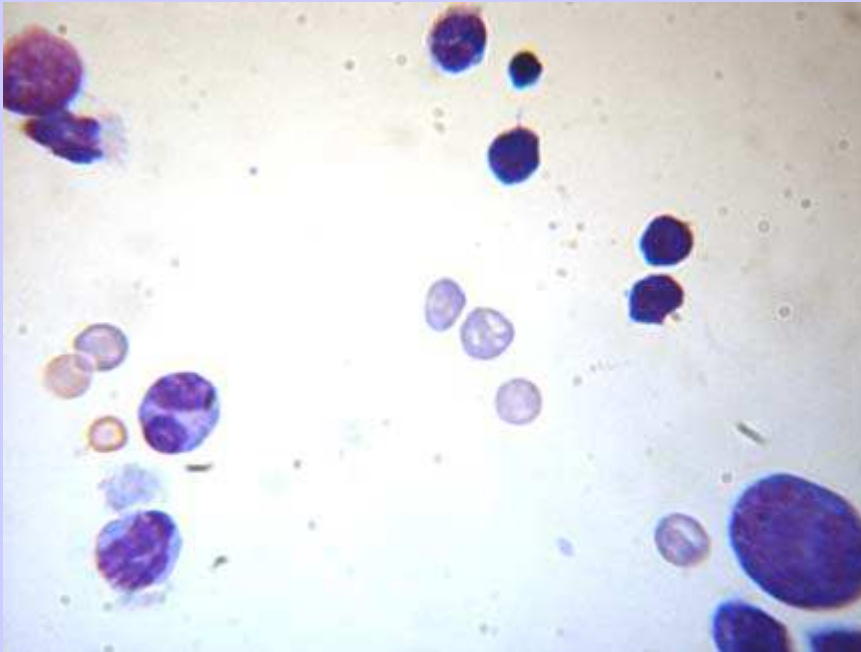
Techniques: Smear



Techniques: Cytospin

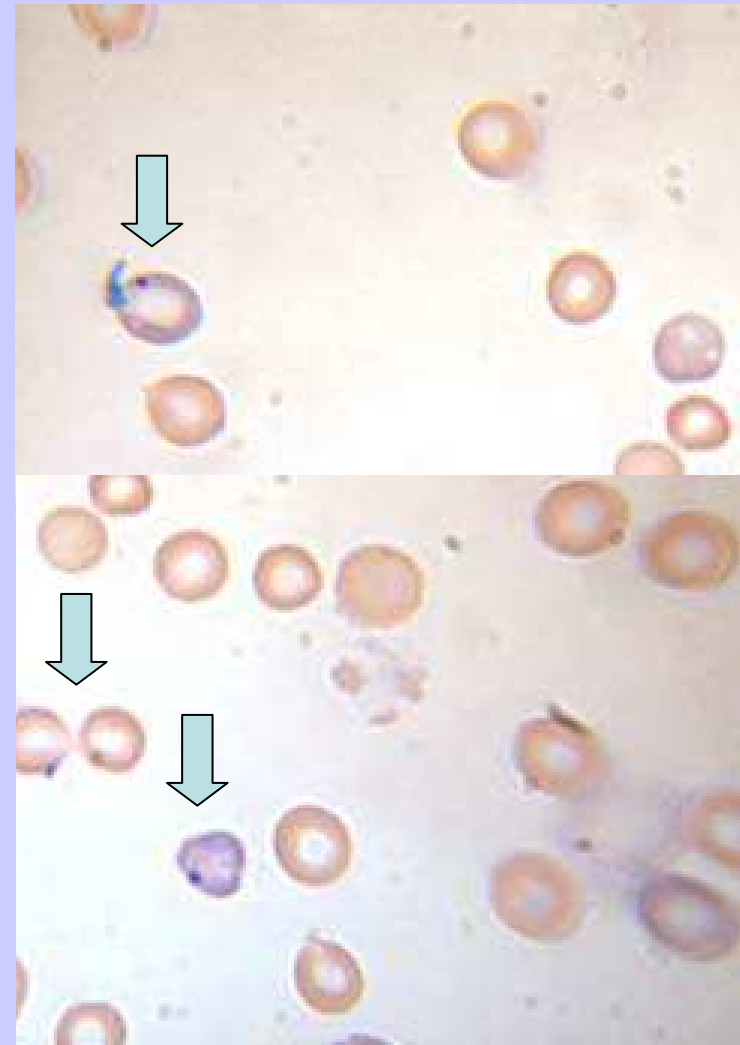


Techniques: Cytospin



✓ Bone marrow smear, mouse

Bone marrow smear, mouse
Micronuclei



Techniques: Plastic Embedding

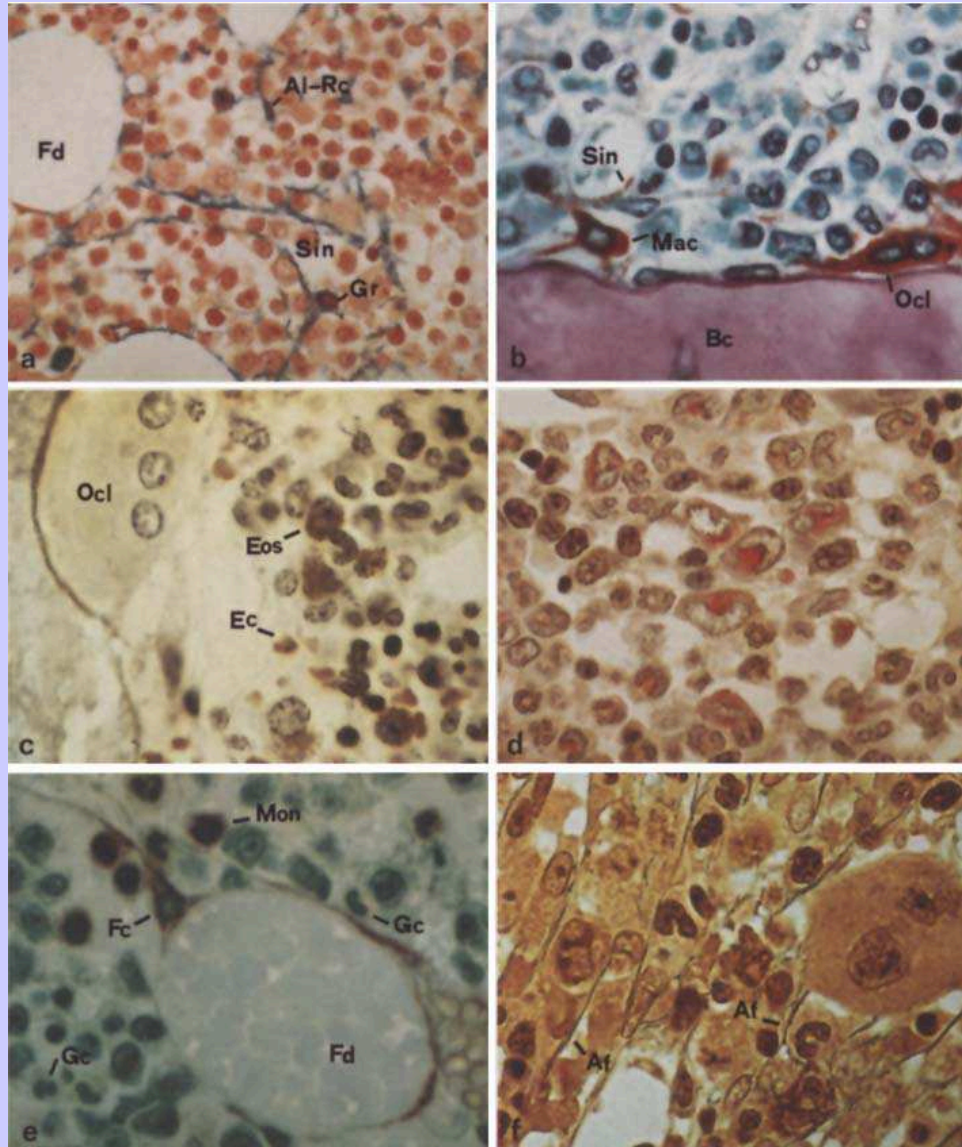
H. Westen, K.-F. Mück, and L. Post

Enzyme Histochemistry on Bone Marrow Sections after Embedding in Methacrylate at Low Temperature

Histochemistry 70, 95 - 105 (1981)

Summary. This paper presents a method for the application of light microscopy to enzyme histochemistry on semi-thin sections of non-decalcified bone marrow cylinders (4 x 15 mm), entire rat femurs and larger soft-tissue specimens (4 x 30 mm²) after embedding in a methacrylate mixture which is then polymerized at

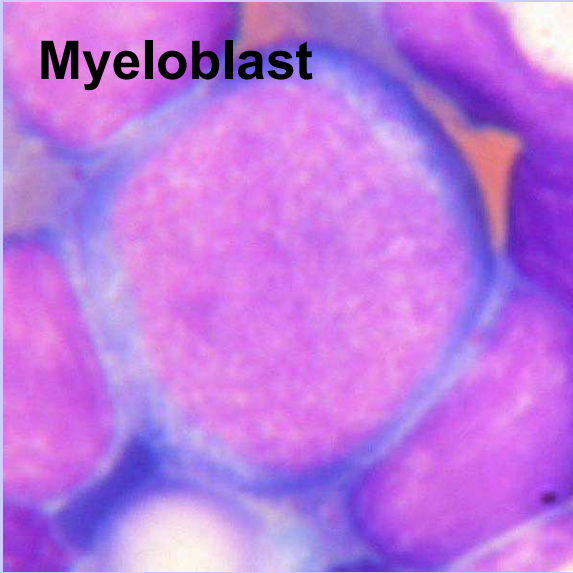
Techniques: Plastic Embedding



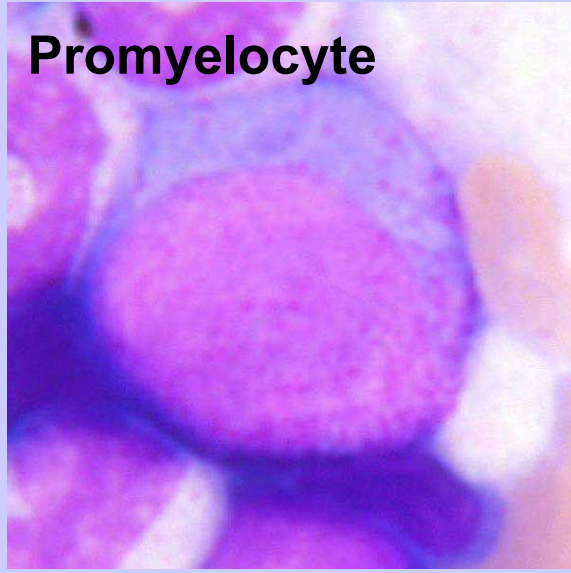
- ✓ A) Alkaline Phosphatase
- ✓ B) Acid Phosphatase
- ✓ C) Peroxidase
- ✓ D) Chloroacetate esterase
- ✓ E) Butyrate esterase
- ✓ F) Gömori

Cell Populations: Rat

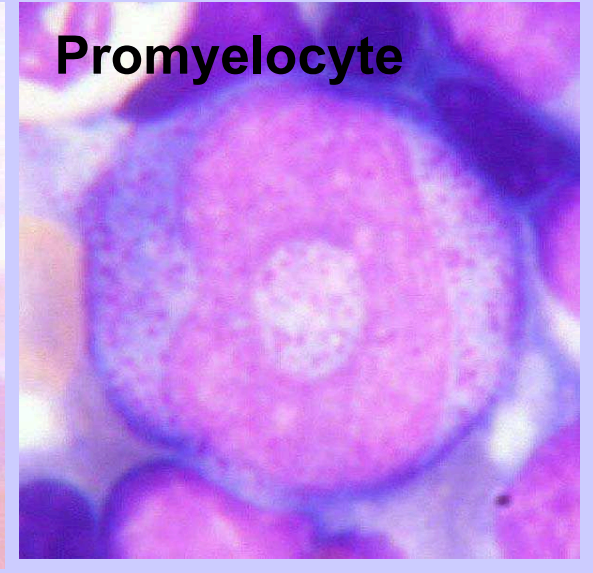
Myeloblast



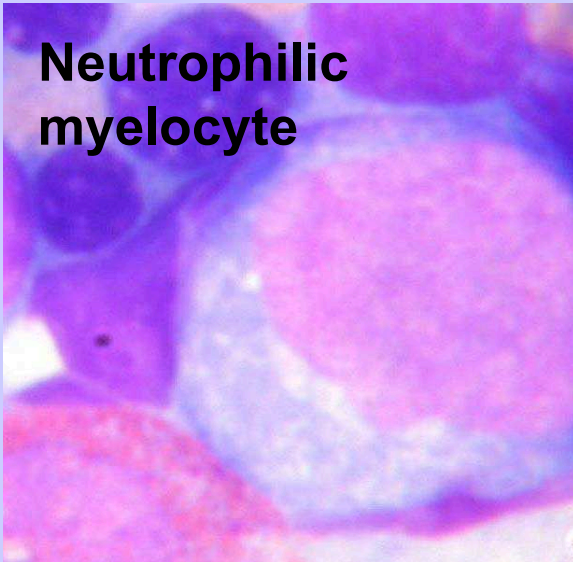
Promyelocyte



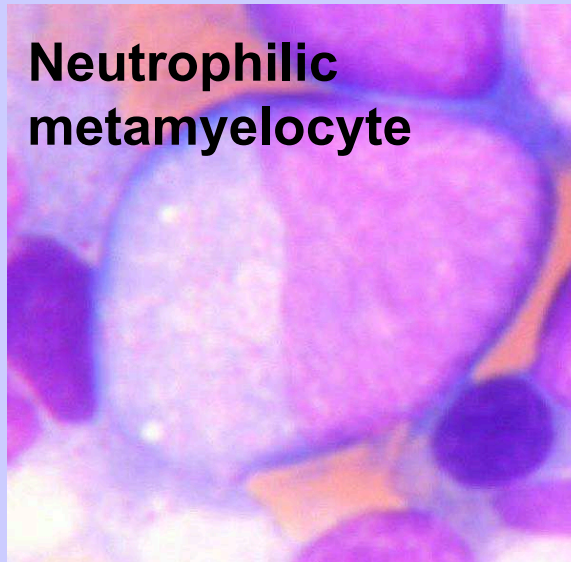
Promyelocyte



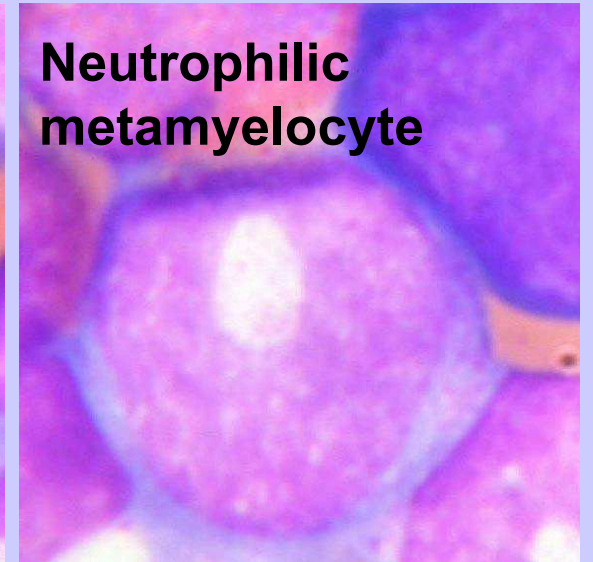
**Neutrophilic
myelocyte**



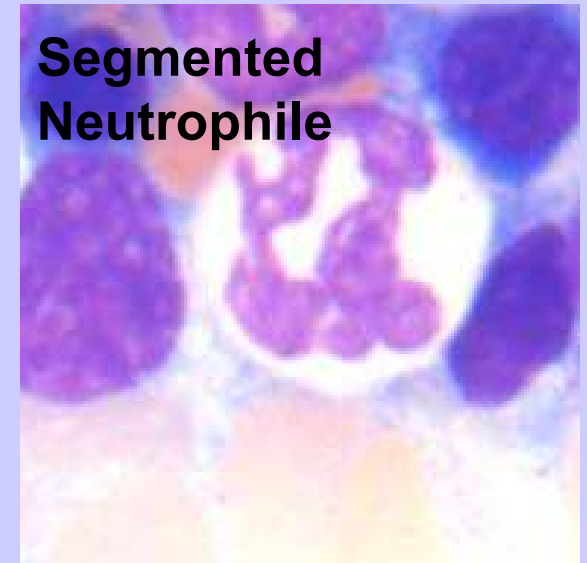
**Neutrophilic
metamyelocyte**



**Neutrophilic
metamyelocyte**



Cell Populations: Rat

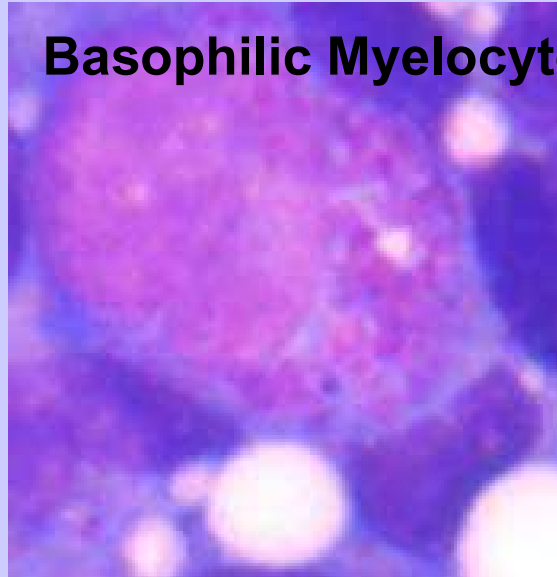


Cell Populations: Rat

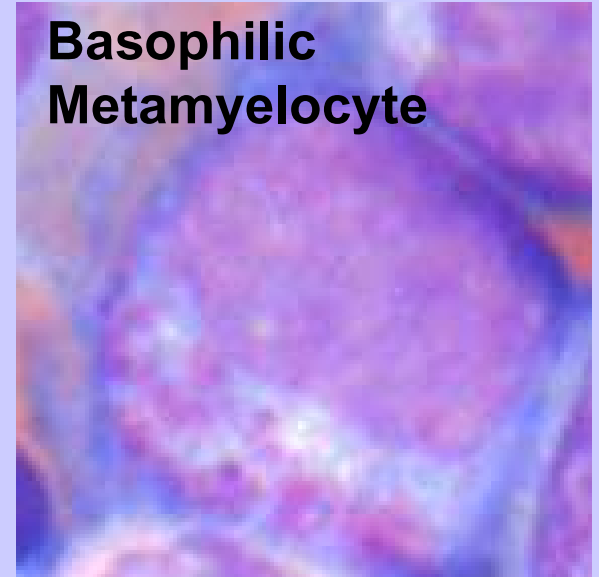
Basophilic Myelocyte



Basophilic Myelocyte



**Basophilic
Metamyelocyte**



Mastcell



Monoblast



Monocyte



Cell Populations: Rat

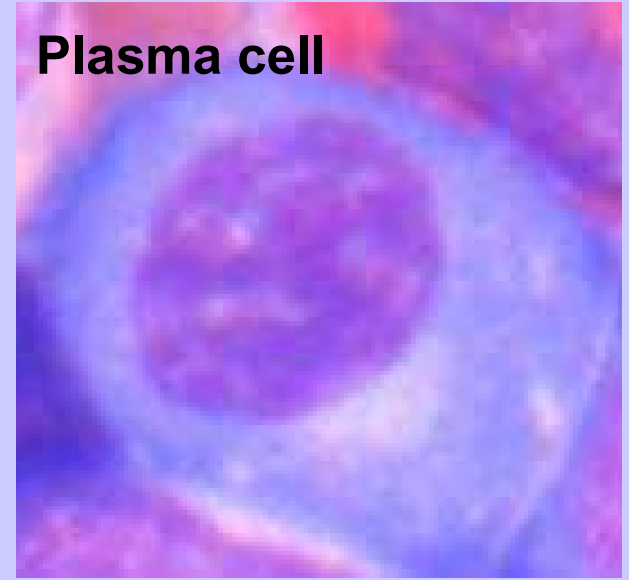
Lymphoblast



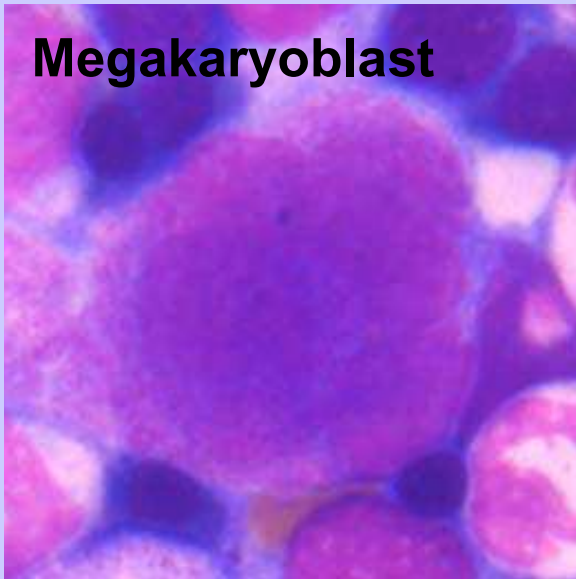
Large Lymphocyte



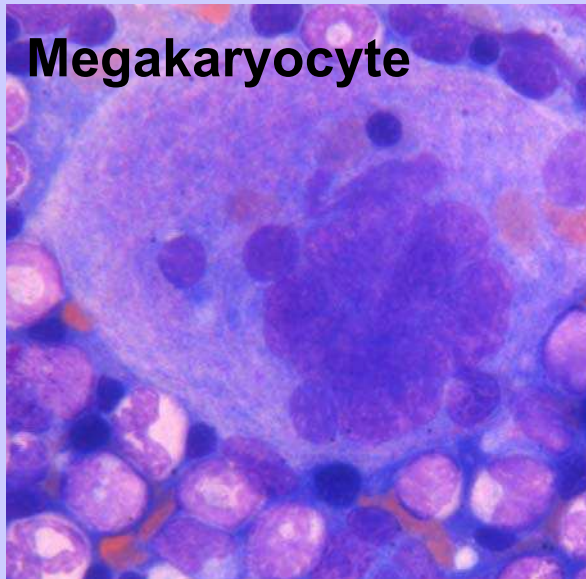
Plasma cell



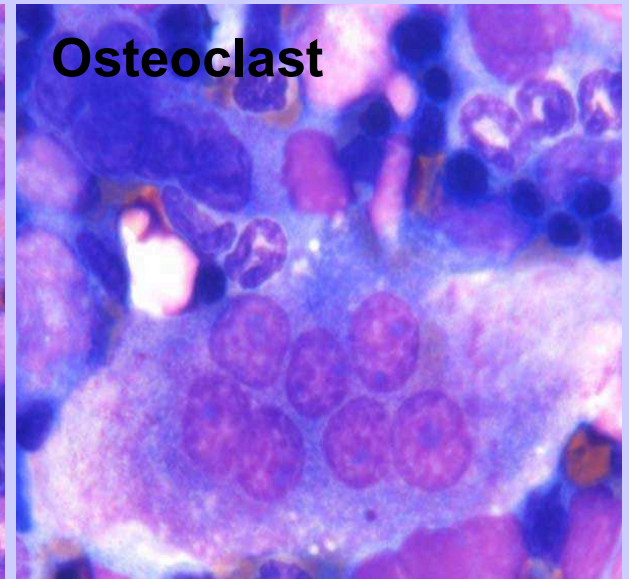
Megakaryoblast



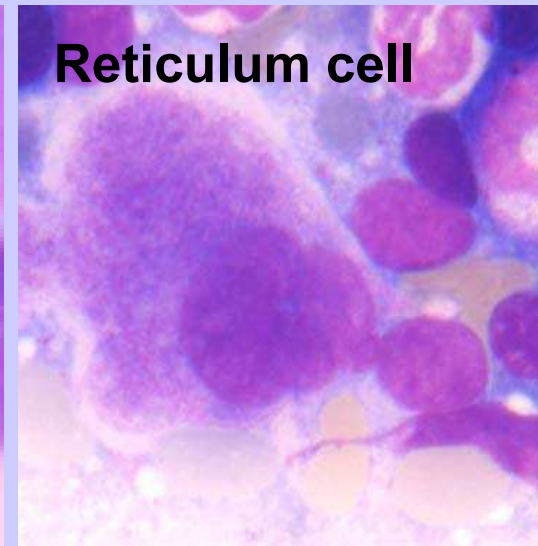
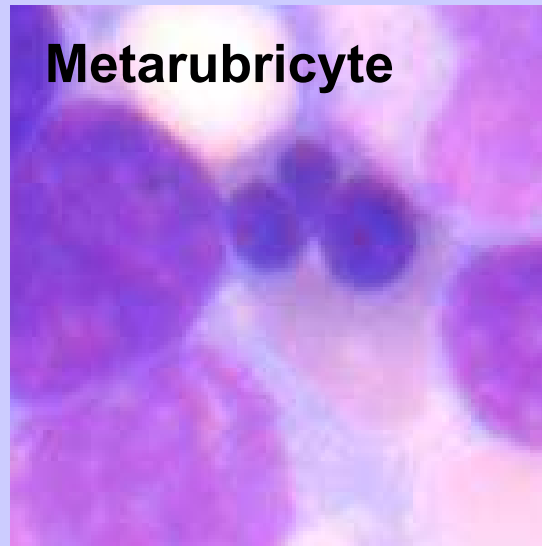
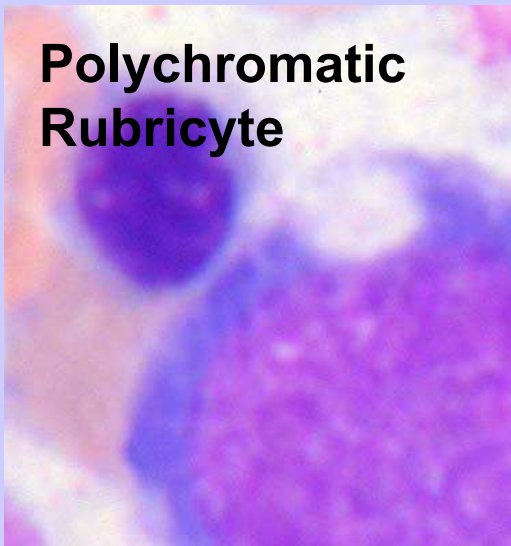
Megakaryocyte



Osteoclast

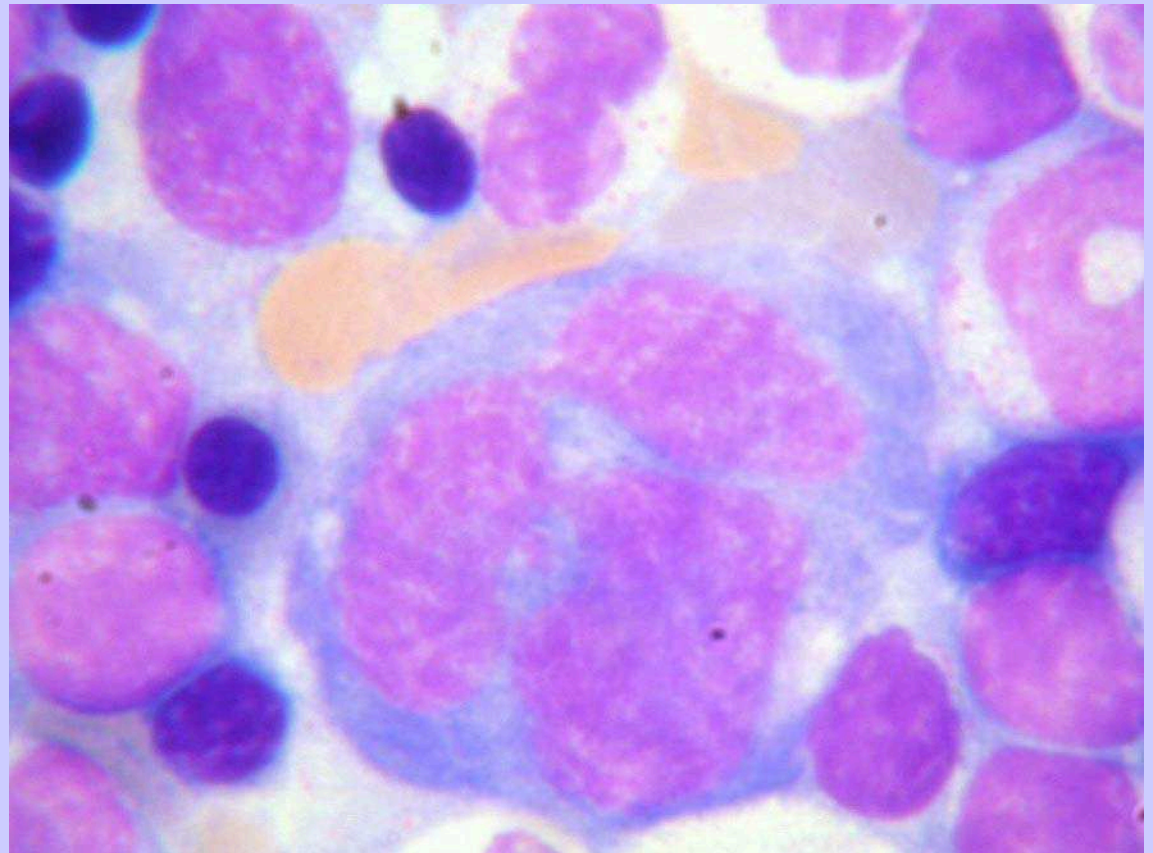
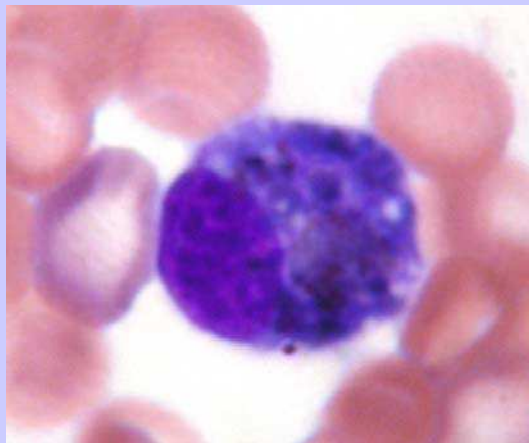
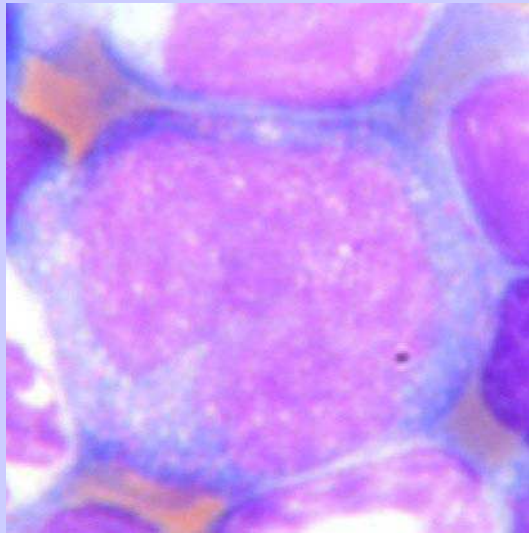


Cell Populations: Rat



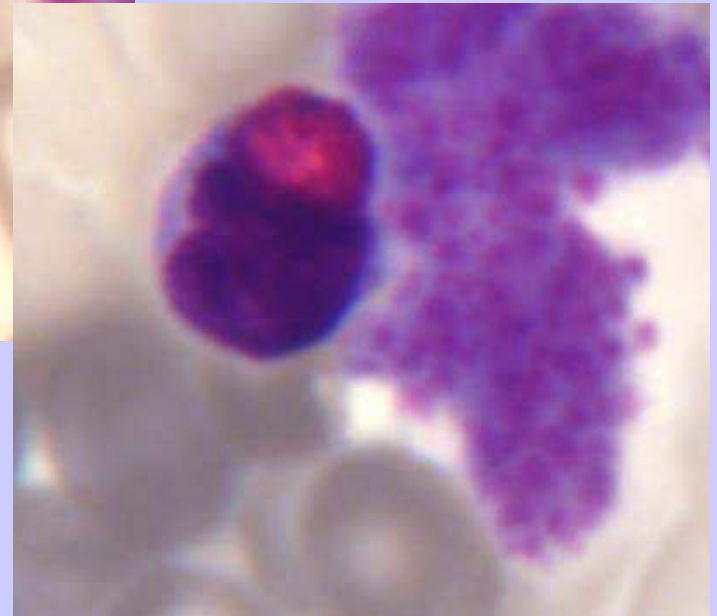
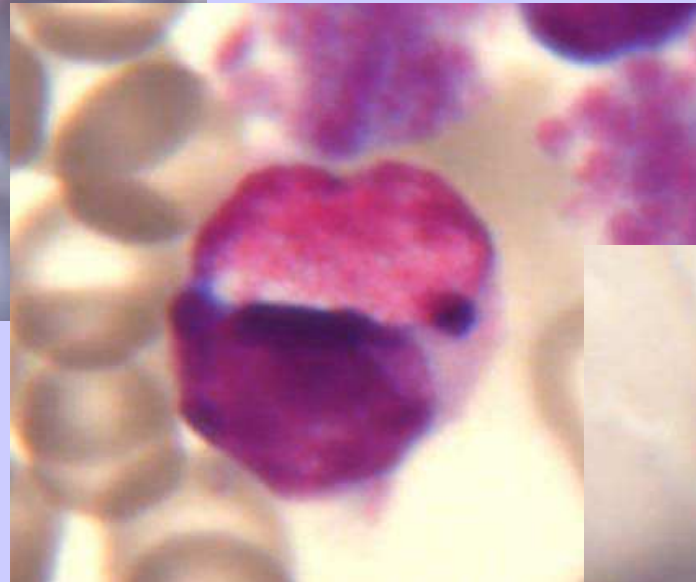
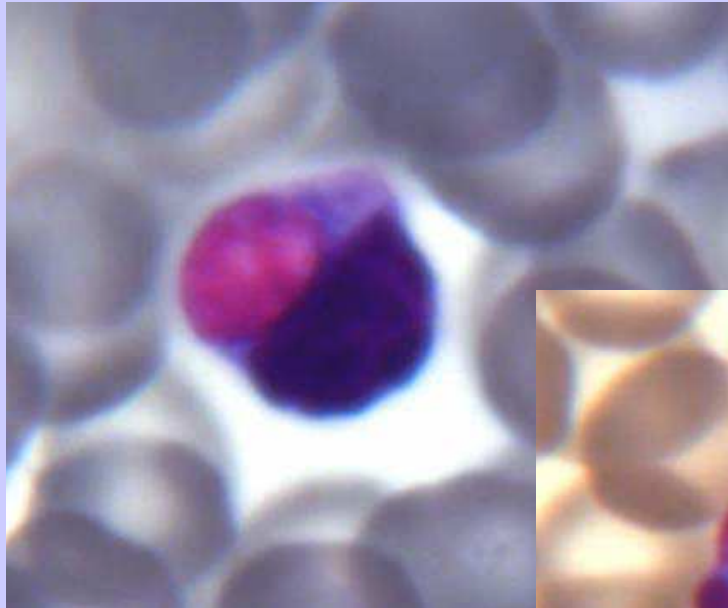
Cell Populations: Rat

Monocyte (top) vs. Macrophage (down)



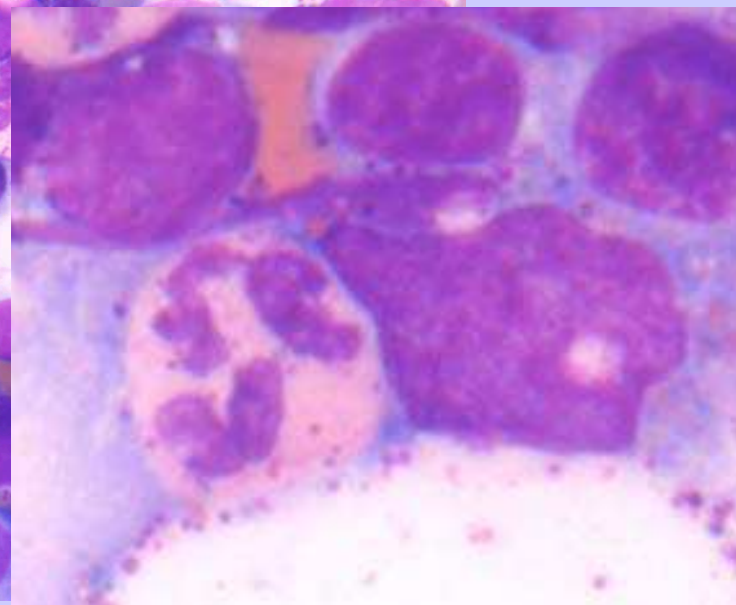
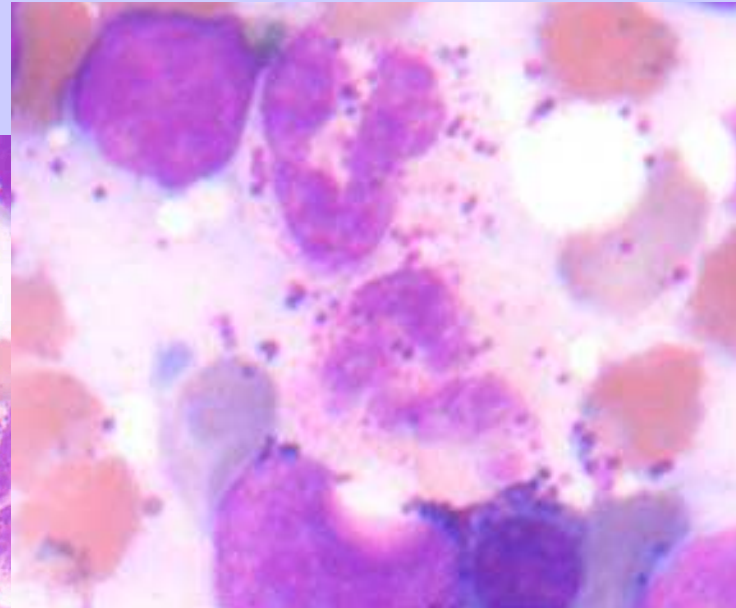
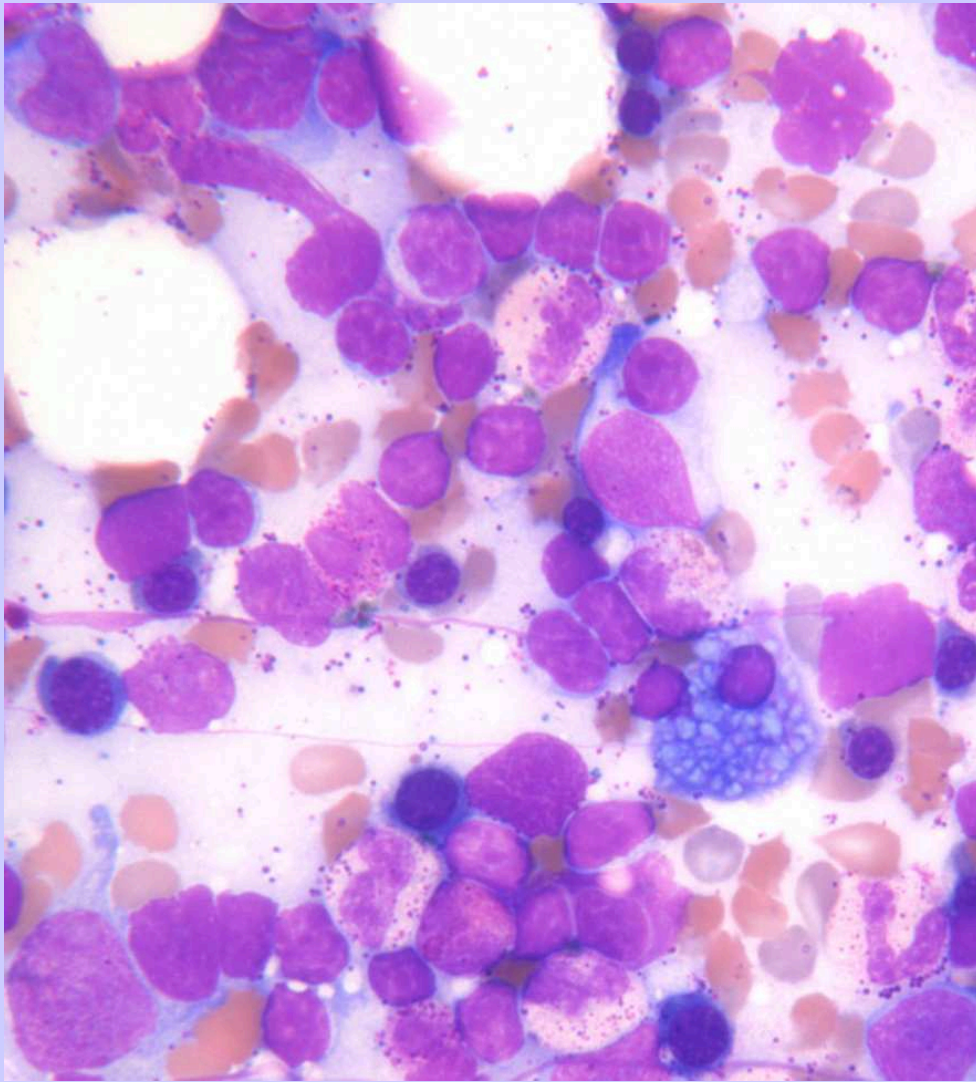
Special Cell Populations: Guinea Pig

✓ Foa-Kurloff



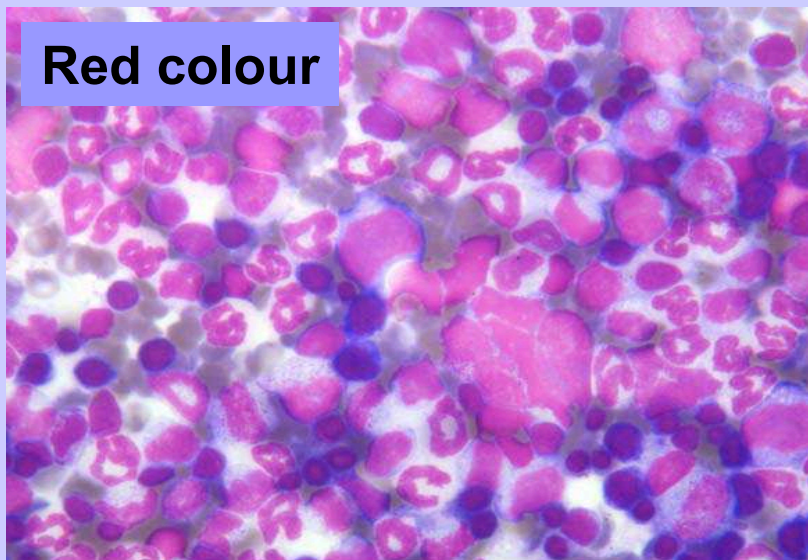
Special Features: Rabbit

✓ Pelger's disease

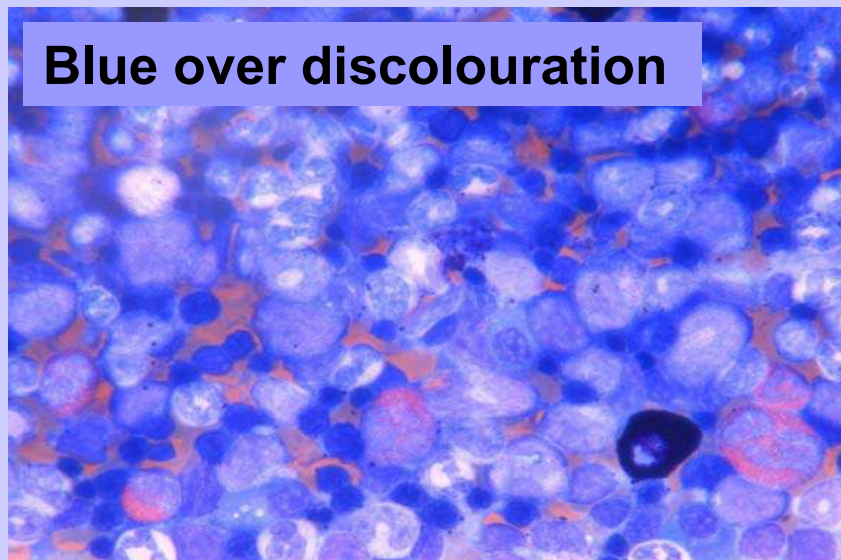


Artifacts

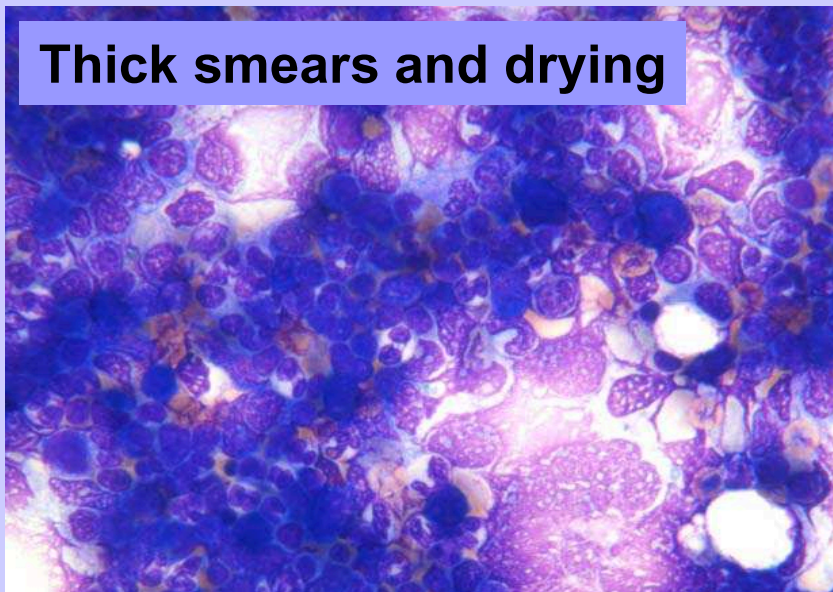
Red colour



Blue over discoloration



Thick smears and drying



Little cell amount

