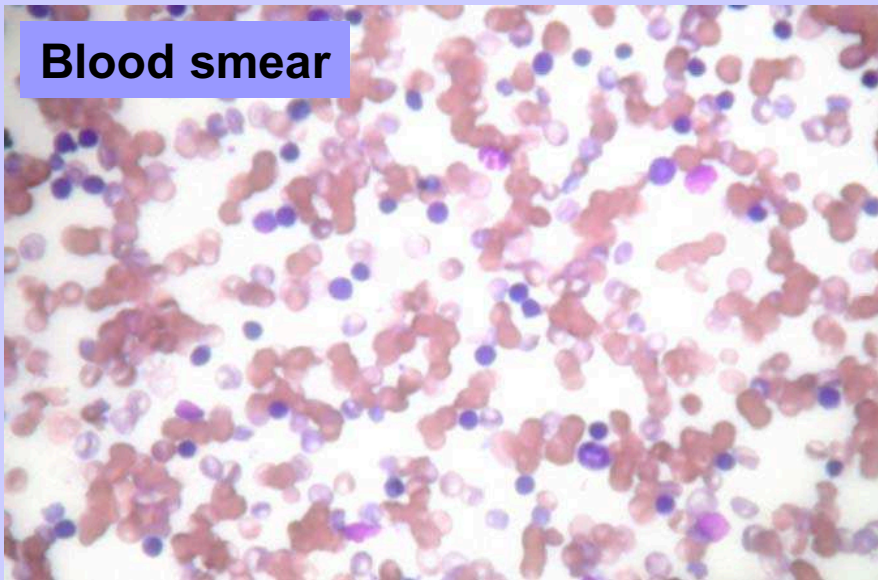
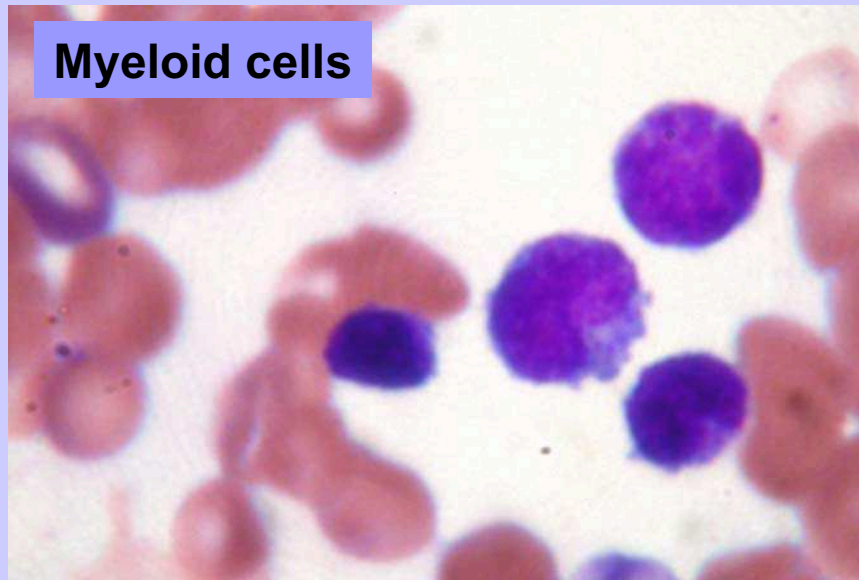


Chronic Lymphatic Leucemia

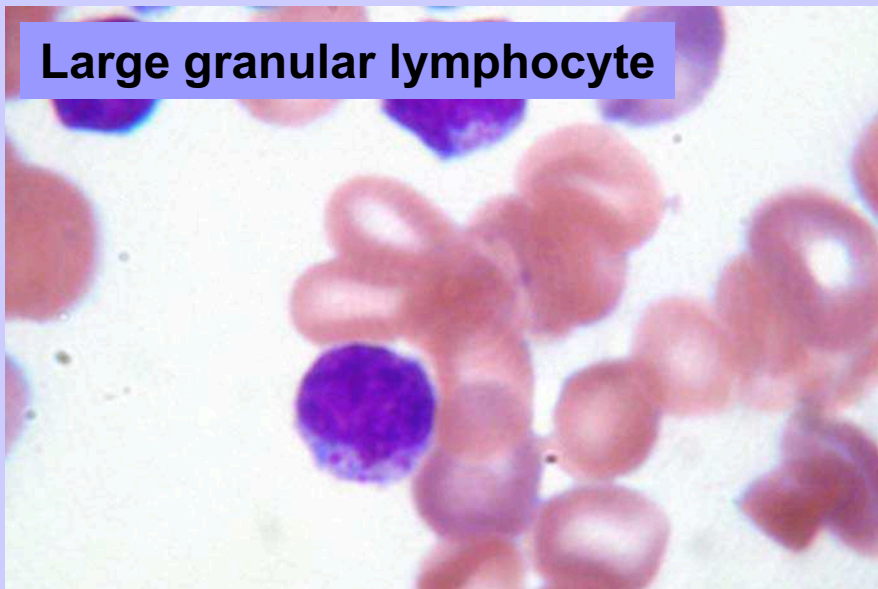
Blood smear



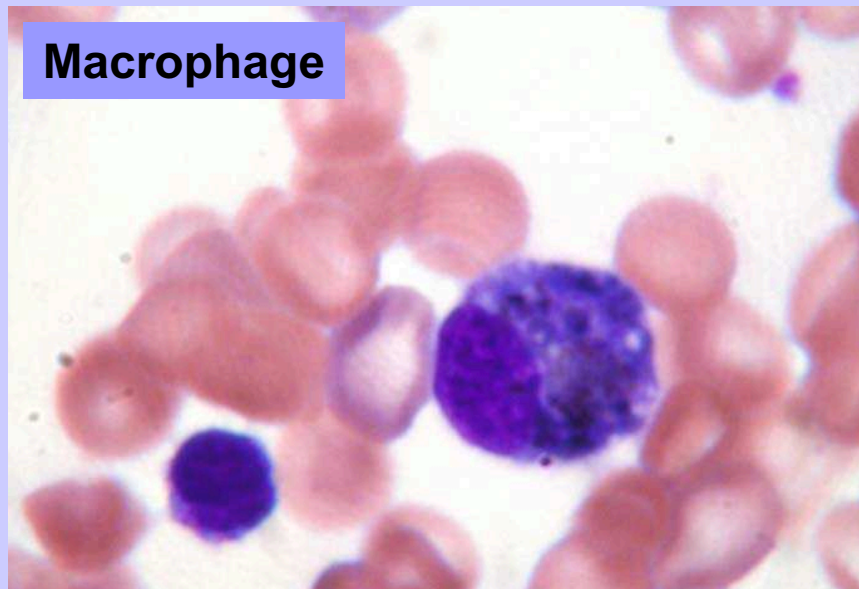
Myeloid cells



Large granular lymphocyte



Macrophage

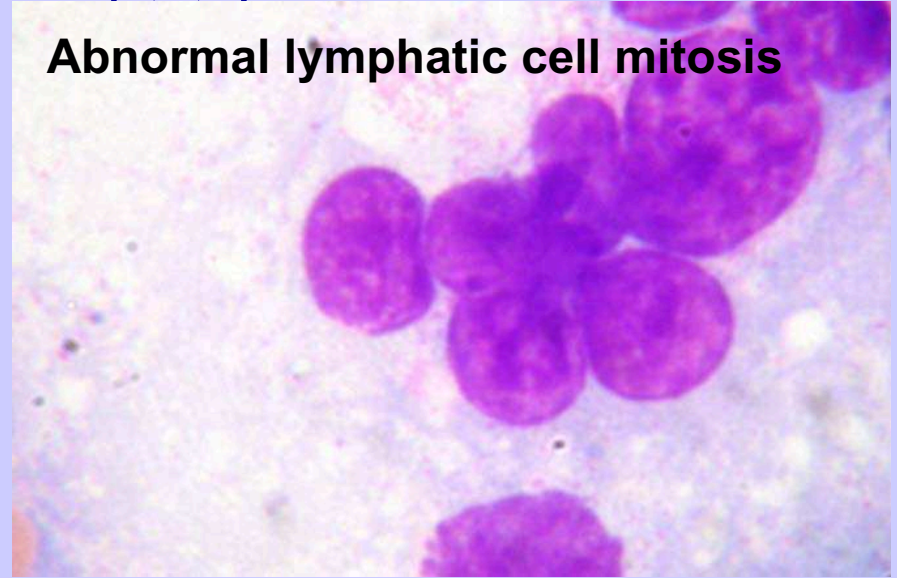


Chronic Lymphatic Leucemia

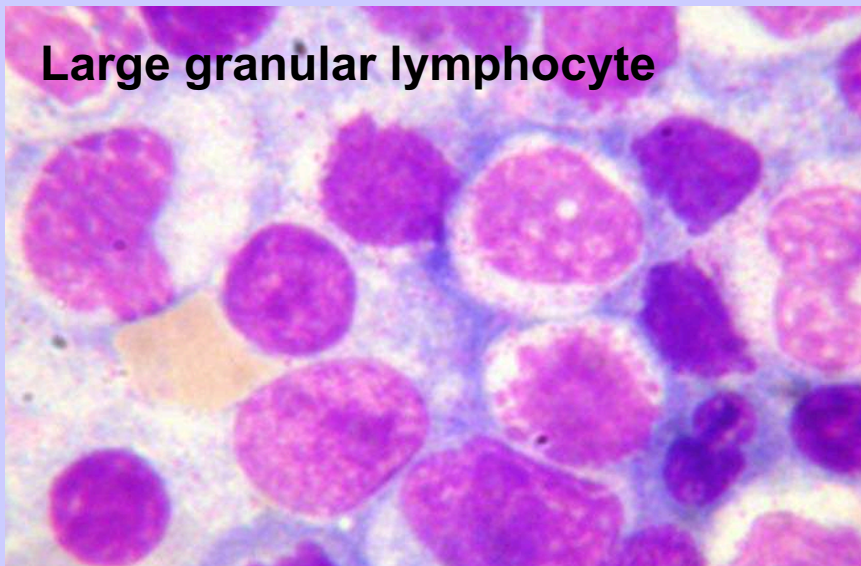
Immature and changed myeloid cells (1,2,3)



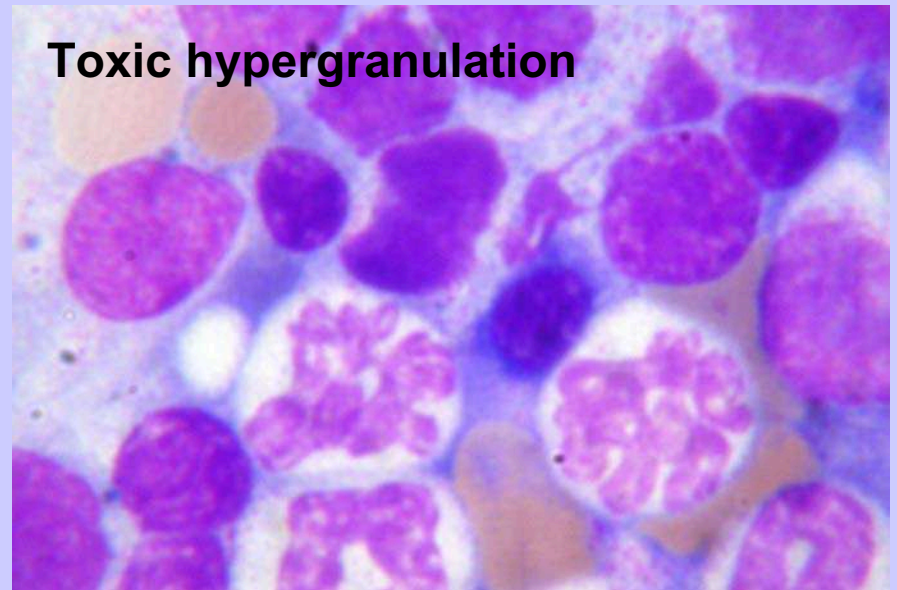
Abnormal lymphatic cell mitosis



Large granular lymphocyte



Toxic hypergranulation



Differentiation

- ✓ in minimum 500 cells
- ✓ never count damaged cells
- ✓ artifacts is due to drying or peripheral blood
- ✓ in case of doubt count to the left side



Linking science to progress

Bone Marrow Differentiation (Draft)				Page:
Test Item:				Project No.:
Test System:				Report No.:
Sponsor:				Date:
Group:				
Animal Number:		1	2	3
Rubriblasts		3	3	3
Prorubricytes		10	10	8
Basophilic Rubricytes		76	51	76
Polychromatophilic Rubricytes		88	95	124
Metarubricytes		26	42	29
Total Erythroid Cells:		203	201	240
Myeloblasts		11	9	6
Promyelocytes		14	7	9
Total Precursors:		25	16	15
Neutrophilic Myelocytes		43	28	27
Neutrophilic Metamyelocytes		59	50	54
Neutrophilic Band Cells		165	104	122
Neutrophilic Segmented Granulocytes		57	58	64
Total Neutrophils:		324	240	267
Eosinophilic Myelocytes		3	2	2
Eosinophilic Metamyelocytes		7	2	5
Eosinophilic Band Cells		0	4	0
Eosinophilic Segmented Granulocytes		0	2	0
Total Eosinophils:		10	10	7
Basophilic Myelocytes		0	1	0
Basophilic Metamyelocytes		0	0	1
Basophilic Band Cells		0	0	1
Basophilic Segmented Granulocytes		0	0	0
Total Basophils:		0	1	2
Total Granulocytic Cells:		359	267	291
M:E Ratio:		1.77	1.33	1.21
Monoblasts		0	0	0
Promonocytes		0	0	0
Monocytes		3	9	2
Macrophages		1	0	1
Total Monocytic Cells:		4	9	3
Prolymphocytes		1	1	1
Lymphocytes		12	24	10
Total Lymphocytes:		13	25	11
Total Plasma Cells:		9	3	10
Total Cells (Erythroid, Granulocytic, Monocytic, Lymphoid, Plasma Cells):		588	505	555
Megakaryoblasts		0	0	0
Promegakaryocytes		0	0	0
Megakaryocytes		0	1	1
Total Megakaryocytic Cells:		0	1	1
Mitoses		3	5	3
Osteoclasts		0	0	0
Osteoblasts		0	0	0
Stromal cells		0	3	1
Adipocytes		0	0	0
Mast cells		0	0	0
Total Other Cells:		3	9	5



Linking science to progress

Bone Marrow Differentiation (Draft)				Page:
				Project No.:
Test Item:				Report No.:
Test System:				Date:
Sponsor:				
Group:				
Animal Number:	1	2	3	
Rubriblasts	0.51	0.594	0.541	
Prorubricytes	1.701	1.98	1.441	
Basophilic Rubricytes	12.93	10.1	13.69	
Polychromatophilic Rubricytes	14.97	18.81	22.34	
Metarubricytes	4.422	8.317	5.225	
Total Erythroid Cells:	34.52	39.8	43.24	
Myeloblasts	1.871	1.782	1.081	
Promyelocytes	2.381	1.386	1.622	
Total Precursors:	4.252	3.168	2.703	
Neutrophilic Myelocytes	7.313	5.545	4.865	
Neutrophilic Metamyelocytes	10.03	9.901	9.73	
Neutrophilic Band Cells	28.06	20.59	21.98	
Neutrophilic Segmented Granulocytes	9.694	11.49	11.53	
Total Neutrophils:	55.1	47.52	48.11	
Eosinophilic Myelocytes	0.51	0.396	0.36	
Eosinophilic Metamyelocytes	1.19	0.396	0.901	
Eosinophilic Band Cells	0	0.792	0	
Eosinophilic Segmented Granulocytes	0	0.396	0	
Total Eosinophils:	1.701	1.98	1.261	
Basophilic Myelocytes	0	0.198	0	
Basophilic Metamyelocytes	0	0	0.18	
Basophilic Band Cells	0	0	0.18	
Basophilic Segmented Granulocytes	0	0	0	
Total Basophils:	0	0.198	0.36	
Total Granulocytic Cells:	61.05	52.87	52.43	
M:E Ratio:	1.77	1.33	1.21	
Monoblasts	0	0	0	
Promonocytes	0	0	0	
Monocytes	0.51	1.782	0.36	
Macrophages	0.17	0	0.18	
Total Monocytic Cells:	0.68	1.782	0.541	
Prolymphocytes	0.17	0.198	0.18	
Lymphocytes	2.041	4.752	1.802	
Total Lymphocytes:	2.211	4.95	1.982	
Total Plasma Cells:	1.531	0.594	1.802	
Total Cells (Erythroid, Granulocytic, Monocytic, Lymphoid, Plasma Cells):	100	100	100	
Megakaryoblasts	0	0	0	
Promegakaryocytes	0	0	0	
Megakaryocytes	0	11.11	20	
Total Megakaryocytic Cells:	0	11.11	20	
Mitoses	100	55.56	60	
Osteoclasts	0	0	0	
Osteoblasts	0	0	0	
Stromal cells	0	33.33	20	
Adipocytes	0	0	0	
Mast cells	0	0	0	
Total Other Cells:	100	100	100	



Linking science to progress

Statistics: Males, Group 1 and T-Test (Males: Group 1 and 4, two-tailed)

	Mean	Min	Max	T-Test
Rubriblasts	1.291757257	0.780	1.705	0.804654539
Prorubricytes	2.549341483	1.931	2.985	0.059212544
Basophilic Rubricytes	10.21400273	8.459	12.741	0.044957151
Polychromatophilic Rubricytes	13.37378998	9.942	18.097	0.308258575
Metarubricytes	3.768447738	1.754	5.985	0.058947637
Total Erythroid Cells:	31.19733919	25.731	37.066	0.035517216
Myeloblasts	4.222210371	3.899	4.887	0.016003815
Promyelocytes	4.259991506	3.195	5.224	0.311122323
Total Precursors:	8.482201877	7.797	9.328	0.059938915
Neutrophilic Myelocytes	18.48227791	16.216	25.564	0.092294235
Neutrophilic Metamyelocytes	7.859335792	5.970	10.136	0.120417902
Neutrophilic Band Cells	13.97929839	10.714	17.154	0.055048322
Neutrophilic Segmented Granulocytes	15.74057433	12.406	17.934	0.034022748
Total Neutrophils:	56.06148642	51.544	62.768	0.020931813
Eosinophilic Myelocytes	0.759991023	-	1.515	0.227635355
Eosinophilic Metamyelocytes	0.606824331	-	1.316	0.289611593
Eosinophilic Band Cells	0.725067121	0.373	1.136	0.864048329
Eosinophilic Segmented Granulocytes	0	-	-	0.373900966
Total Eosinophils:	2.091882476	0.560	3.598	0.828186293
Basophilic Myelocytes	0.193071575	-	0.390	0.525066774
Basophilic Metamyelocytes	0.037313433	-	0.187	
Basophilic Band Cells	0.715424926	-	2.820	0.976856325
Basophilic Segmented Granulocytes	0.07518797	-	0.376	0.373900966
Total Basophils:	1.020997903	0.187	3.195	0.847754891
Total Granulocytic Cells:	67.65656868	61.583	72.710	0.034525467
Monoblasts	0	-	-	
Promonocytes	0	-	-	
Monocytes	0.57515476	0.187	1.170	0.173527333
Macrophages	0	-	-	#DIV/0!
Total Monocytic Cells:	0.57515476	0.187	1.170	0.283132263
Prolymphocytes	0.077596393	-	0.195	0.416358515
Lymphocytes	0.152503811	-	0.195	0.009375667
Total Lymphocytes:	0.230100205	-	0.390	0.00950386
Plasma Cells	0.340837172	-	0.579	0.108725003
Plasma C.+ Russell Bodies	0	-	-	
Total Plasma Cells:	0.340837172	-	0.579	0.108725003

When Differentiation?

- ✓ Findings/Lesions in peripheral blood
- ✓ Suspicious compound
- ✓ Findings in bone marrow (sections)
- ✓ Findings leading to assessment of immunotoxicity

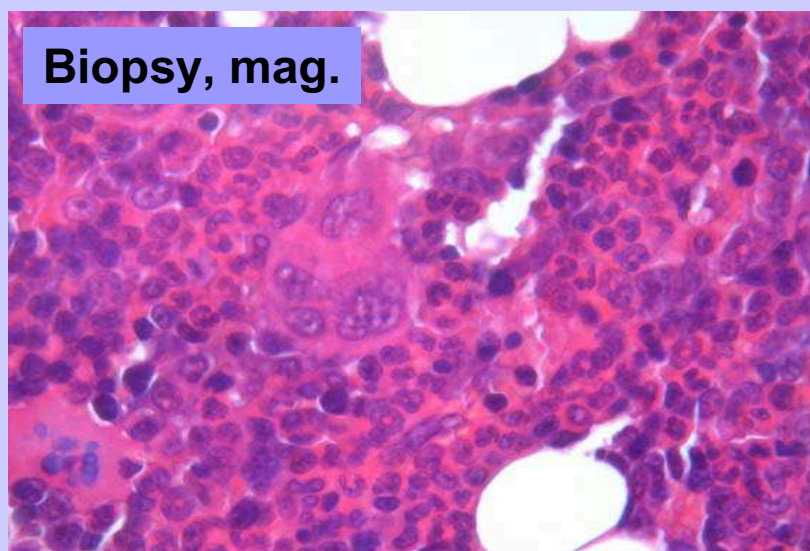
- ✓ Bone marrow differentiation need to be based on sections and hematology data

Different Techniques, Different Results?

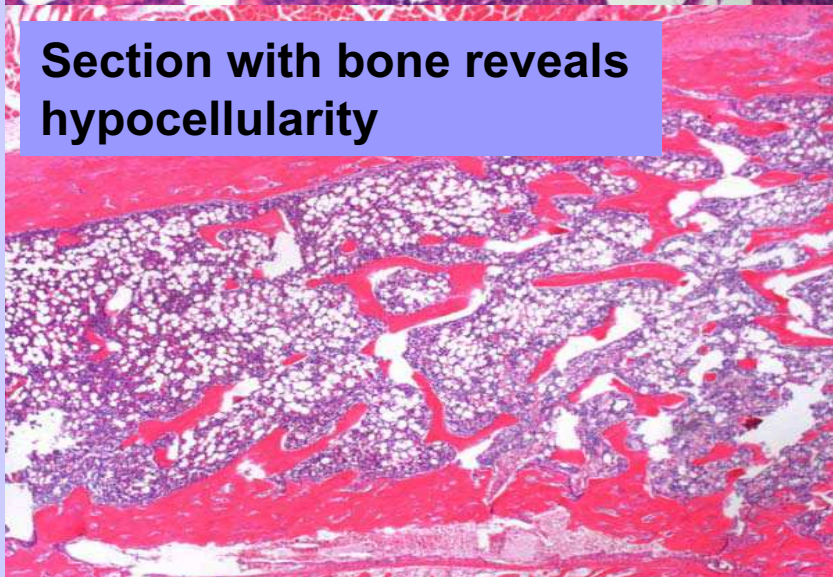
Biopsy reveals increased granulopoiesis



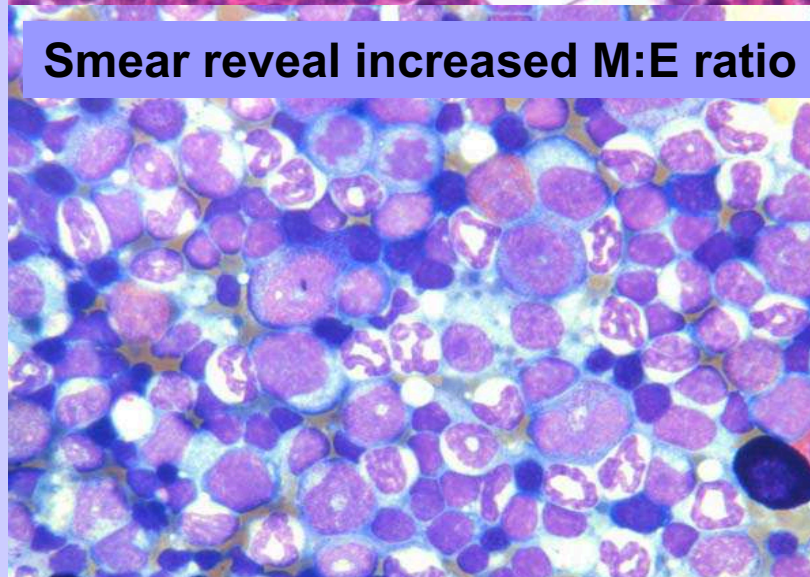
Biopsy, mag.



Section with bone reveals hypocellularity

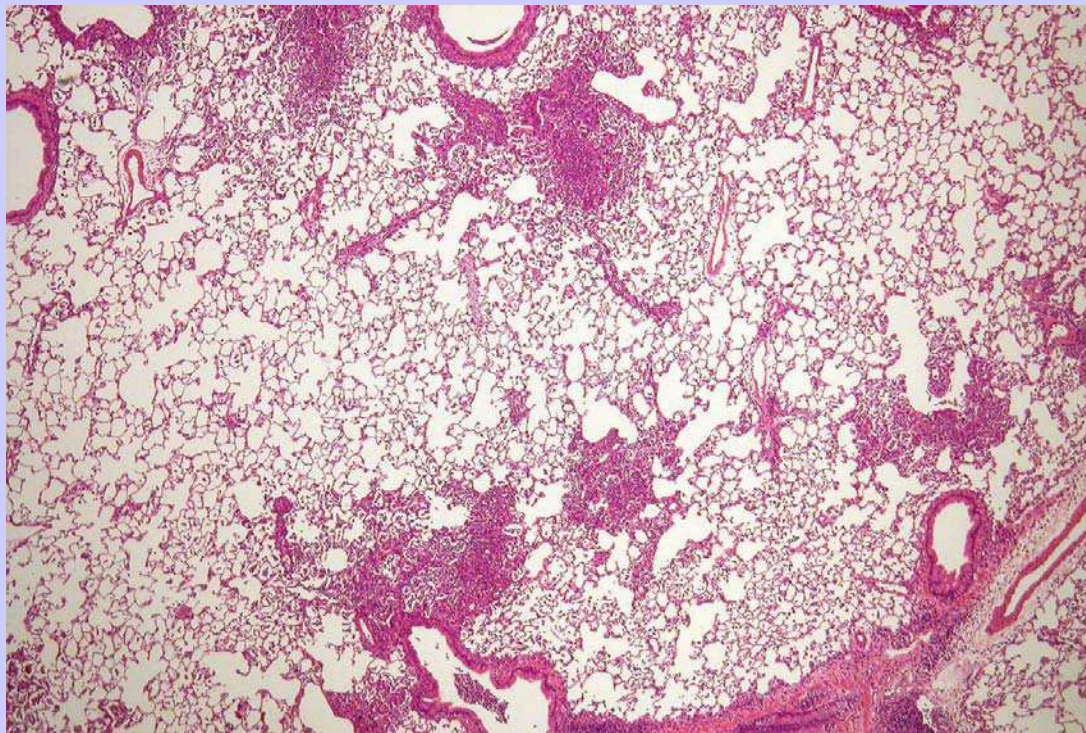


Smear reveal increased M:E ratio



Questions?

- ✓ **Age-Related Differences?**
- ✓ **Influence by Vehicle?**
 - e.g. Methyl cellulose vs water



Age-Related Differences: Dog, Males

Study Type	Minimum	Maximum	Mean	Stand.Dev.
4-W Gavage	1.308	1.576	1.468	0.115
4-W i.v., bolus	1.204	1.652	1.332	0.185
26-W Gavage	0.933	1.384	1.188	0.189
39-W Capsule	1.474	1.726	1.601	0.091
39-W Inhalation	0.975	1.333	1.165	0.140
52-W Capule	0.957	1.786	1.535	0.336

Age-Related Differences: Dog, Females

Study Type	Minimum	Maximum	Mean	Stand.Dev.
4-W Gavage	0.913	1.145	1.051	0.100
4-W i.v., bolus	1.204	1.652	1.332	0.185
26-W Gavage	1.033	1.593	1.226	0.260
39-W Capsule	1.447	1.574	1.521	0.055
39-W Inhalation	1.159	1.657	1.440	0.213
52-W Capule	0.865	1.380	1.199	0.197

Age-Related Differences: Rat, Males

Study Type	Minimum	Maximum	Mean	Stand.Dev
28-D Dermal	0.973	1.870	1.348	0.326
104-W, Gavage	0.870	1.790	1.284	0.293
Repro, Feeding	1.063	1.750	1.339	0.236

Age-Related Differences: Rat, Females

Study Type	Minimum	Maximum	Mean	Stand.Dev
28-D Dermal	0.937	2.087	1.426	0.350
104-W, Gavage	0.973	1.350	1.112	0.126
Repro, Feeding	0.734	1.657	1.201	0.317
Repro, Gavage	0.902	2.000	1.220	0.308

Differences from Reprotoxicity: Erythropoiesis in Females

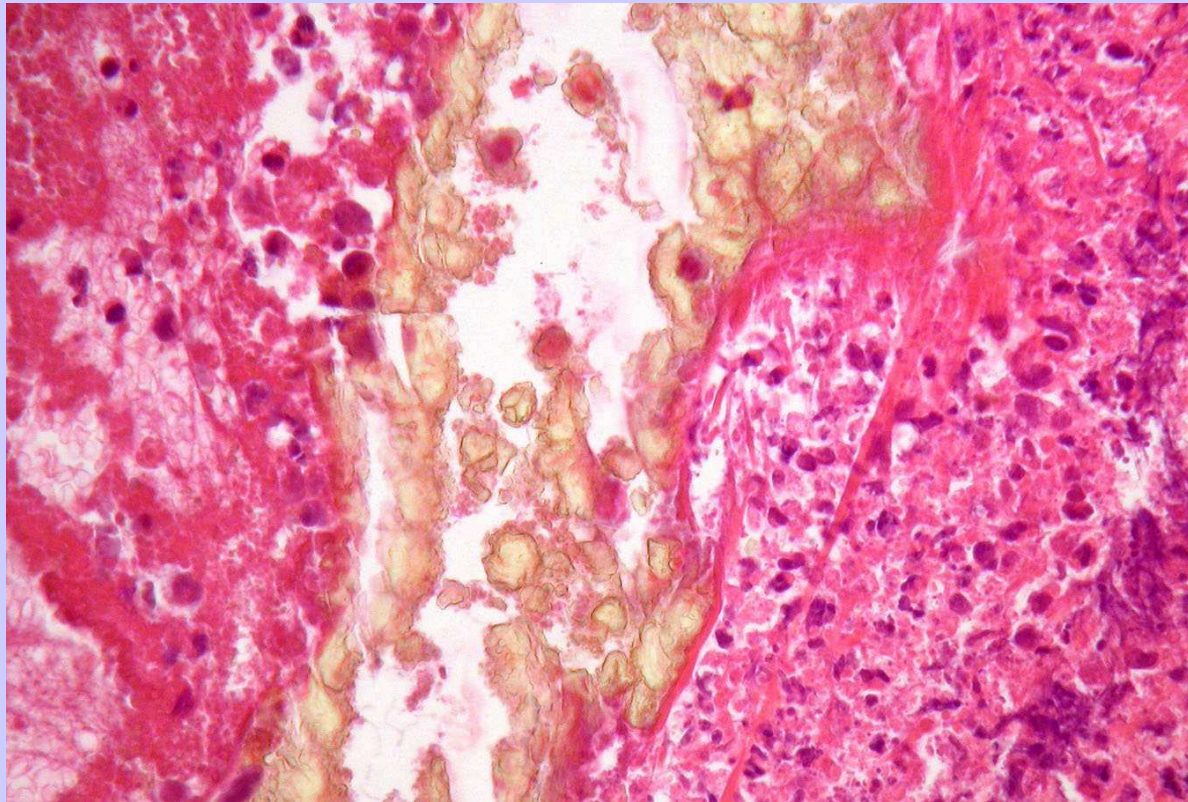
Study Type	Minimum	Maximum	Mean	Stand.Dev
28-D Dermal	30.164	45.932	38.106	5.067
104-W, Gavage	39.527	46.741	44.077	2.364
Repro, Feeding	35.484	55.200	43.587	6.721
Repro, Gavage	32.333	49.812	42.768	5.221

Differences from Reprotoxicity: Lymphocytes in Females

Study Type	Minimum	Maximum	Mean	Stand.Dev
28-D Dermal	4.748	13.531	7.313	2.575
104-W, Gavage	2.508	7.619	4.502	1.443
Repro, Feeding	2.521	6.607	4.233	1.075

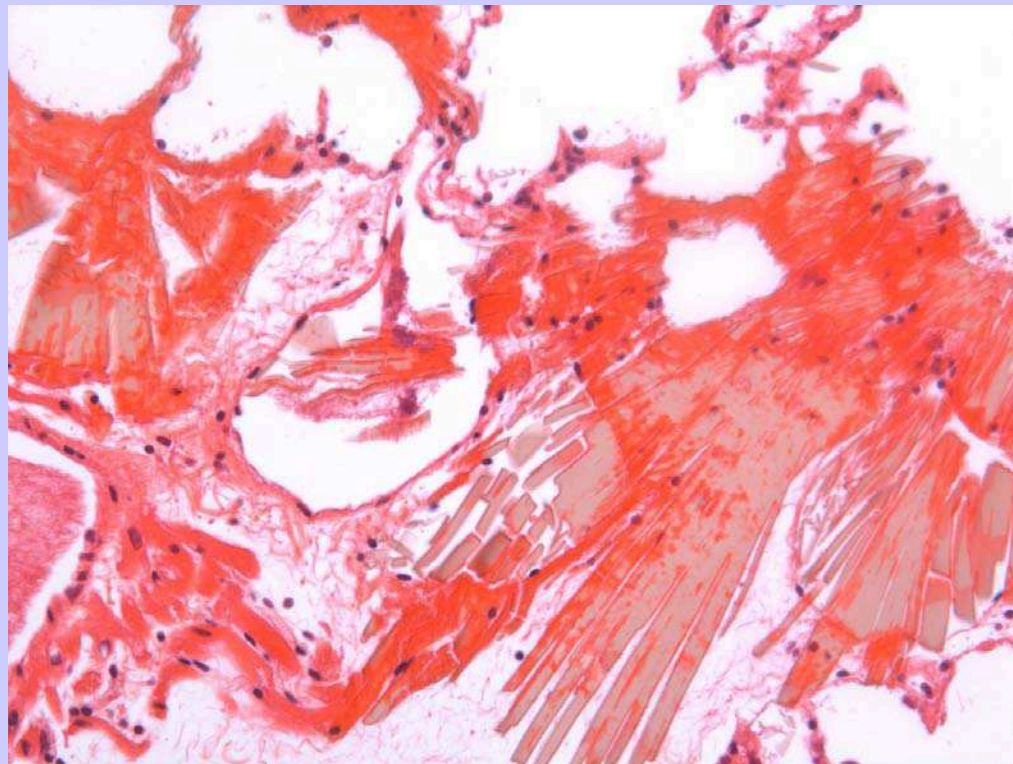
Questions?

- ✓ **Influence by Route of Administration?**
- e.g. Oral vs Continuous Infusion



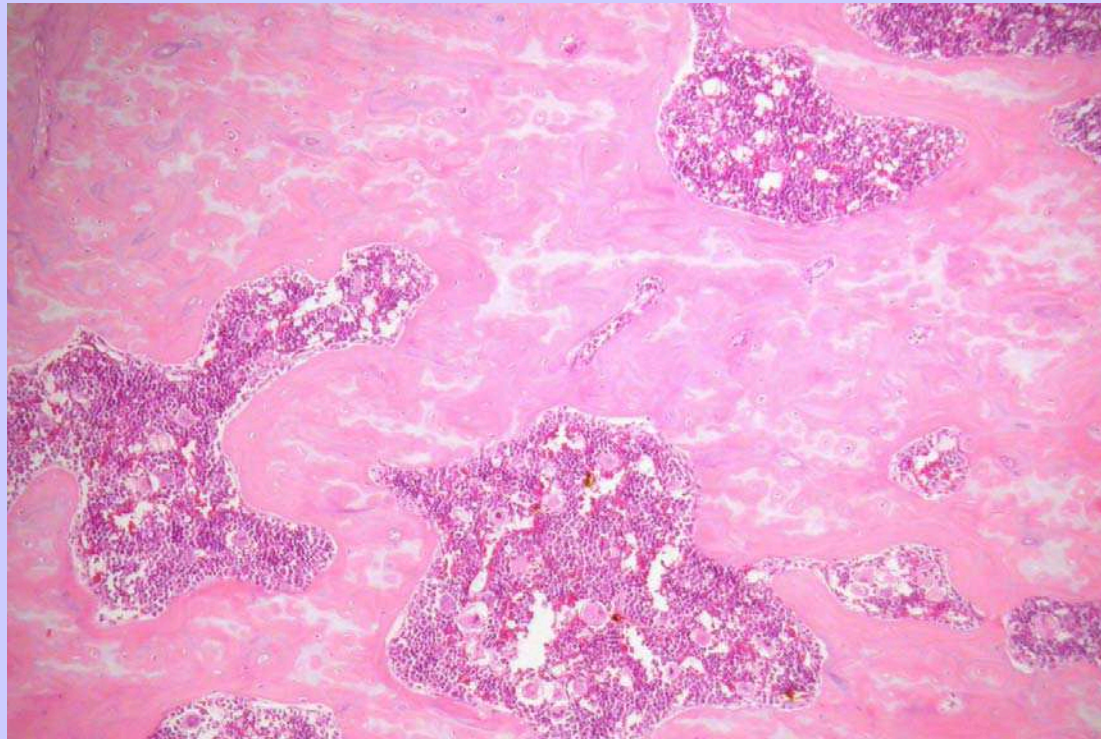
Questions?

- ✓ **Influence by Test Item Formulation?**
e.g. liposomes



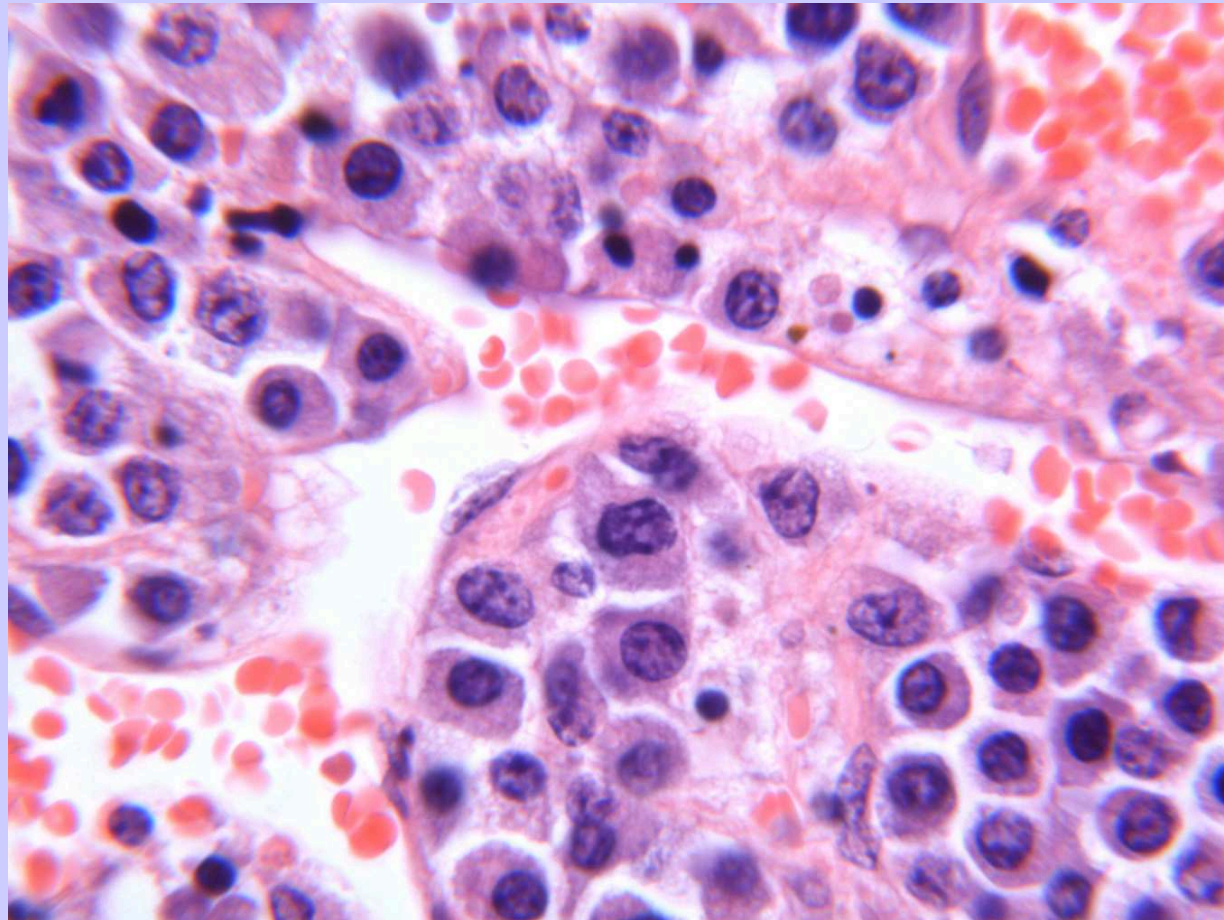
Questions?

- ✓ **Influence by Primary Test Item-Related Lesions?**
 - e.g. phytoestrogens



Questions?

- ✓ **Species-Specific Lesions?**
e.g. hamster and plasmacytoma



Questions: Not perfect species.....

✓ **Mouse:**

React always first with granulopoiesis!

✓ **Rabbit:**

Heterophiles!

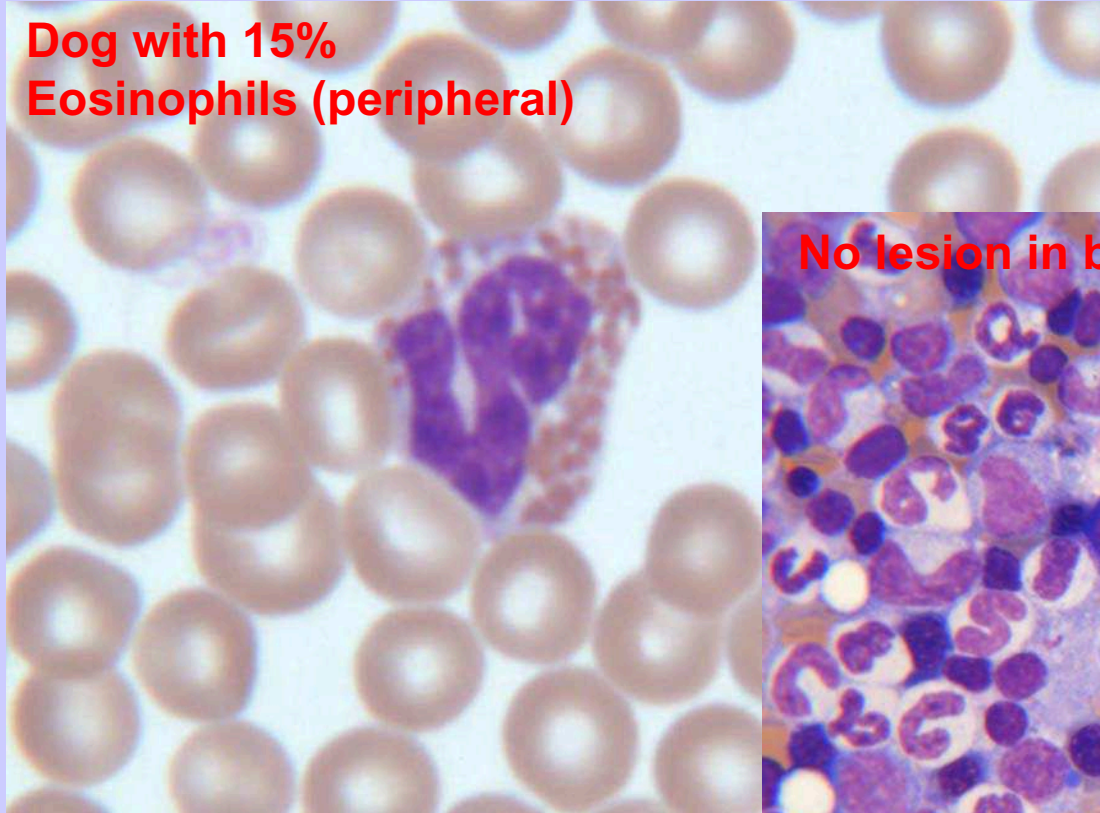
Pelger-Huet's anomaly of nuclei

Peripheral Blood Changes and Bone Marrow

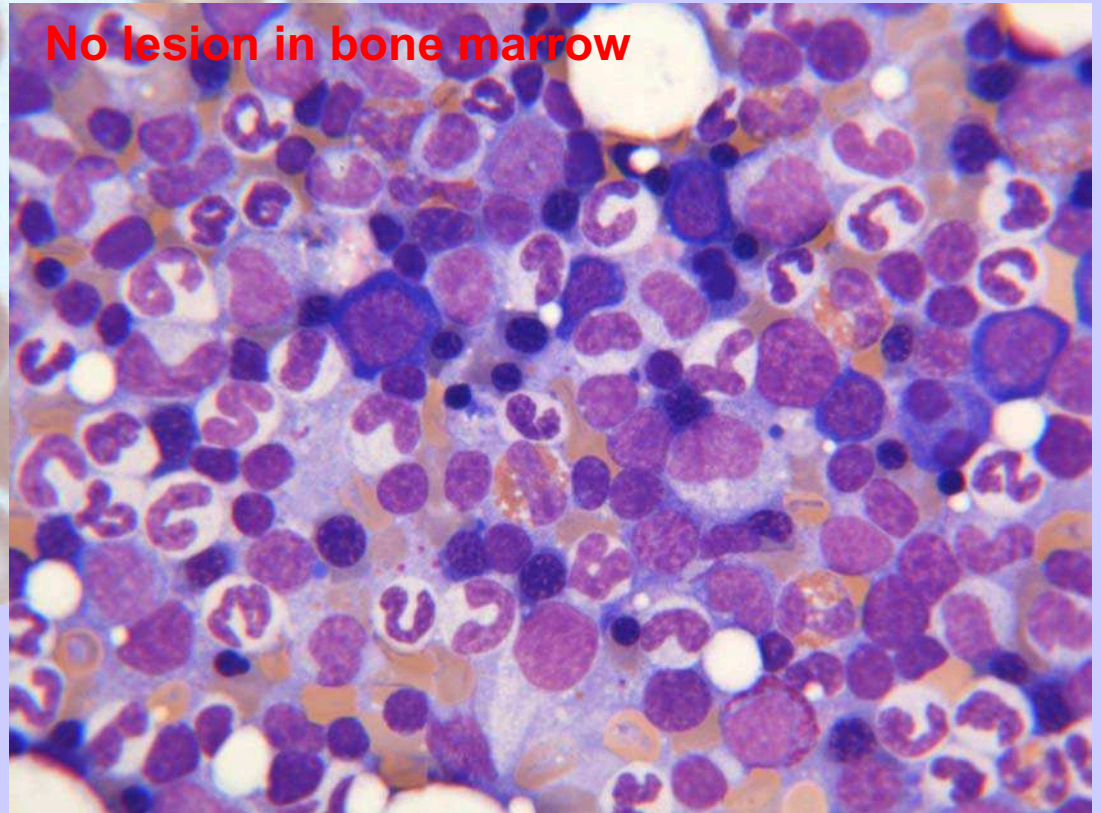
- ✓ **Changes in peripheral blood parameters are not necessarily by changes in the bone marrow**
- ✓ **In most cases of anemia no changes in bone marrow recorded**
- ✓ **If changes are obvious in bone marrow slides, than changes in smears: do not differ obvious doses**
- ✓ **In evidence of changed single cell populations, not necessarily changes in bone marrow**

Example: Peripheral Eosinophilia

**Dog with 15%
Eosinophils (peripheral)**



No lesion in bone marrow



Example: Corticosteroids, Rat

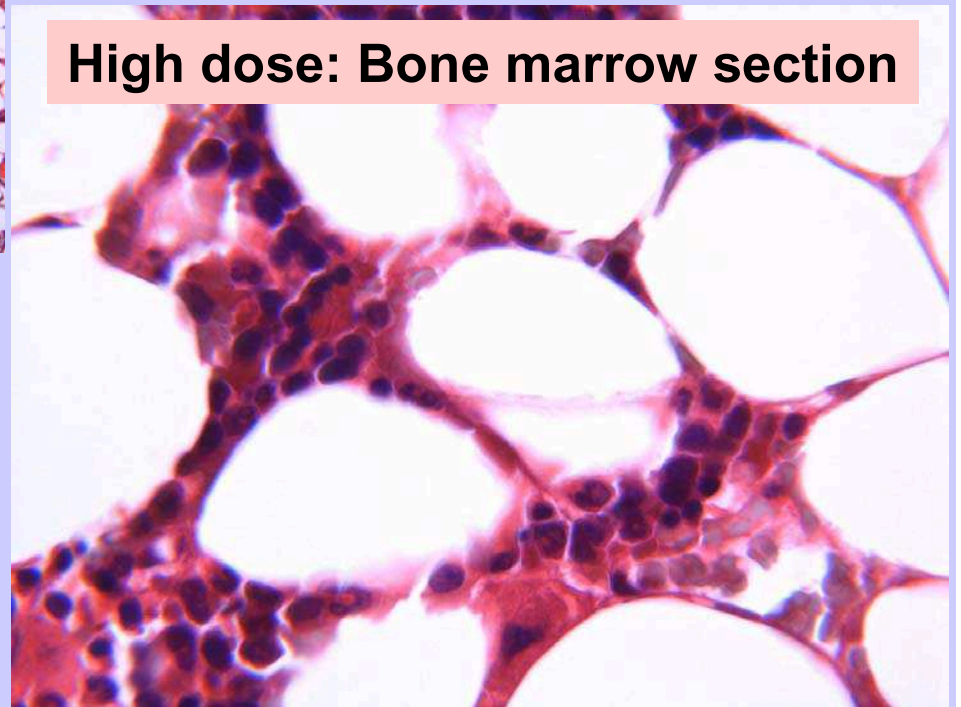
High dose: Rat, femur



- ✓ Femoral section reveals fatty replacement
- ✓ M:E ratios were normal

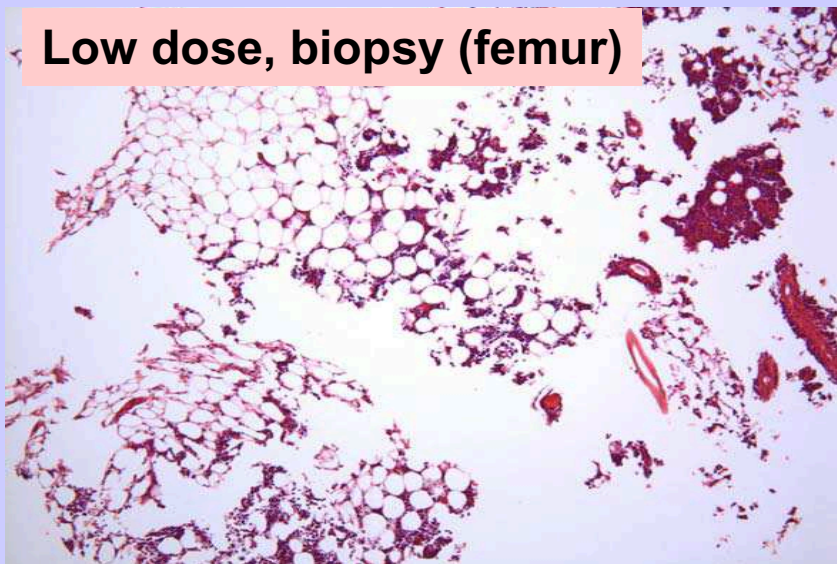
- ✓ Section of biopsy reveals hypocellularity, fatty replacement, hypocellularity in both granulopoiesis and erythropoiesis

High dose: Bone marrow section



Example: PPAR-agonist, Dog

Low dose, biopsy (femur)

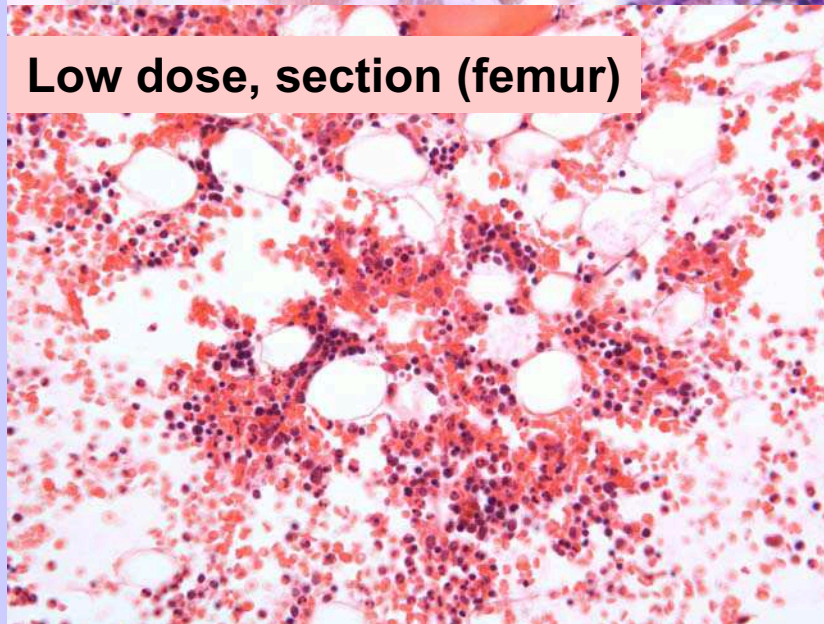


Low dose, smear



- ✓ Biopsy from femur with tremendous hypocellularity
- ✓ Bone marrow smear with little marrow cells but peripheral blood due to hyperpemia

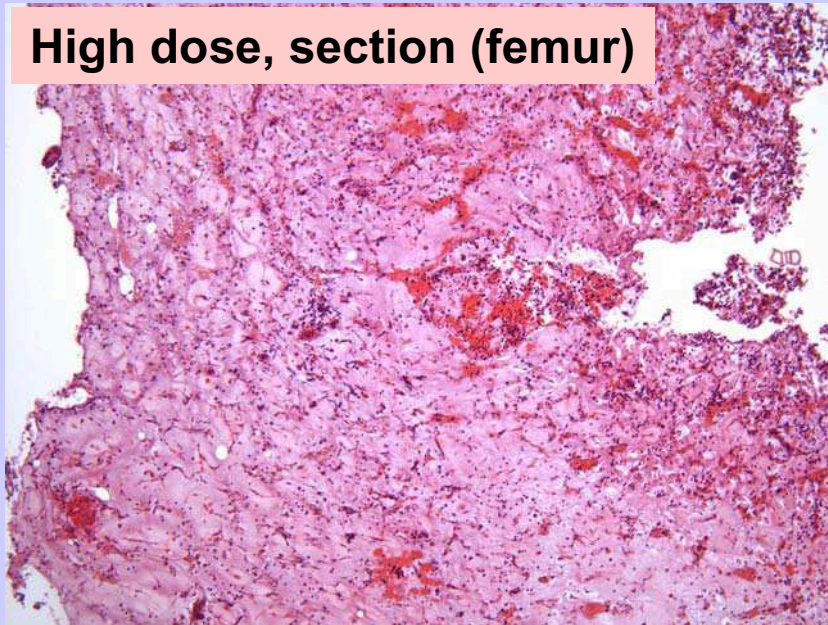
Low dose, section (femur)



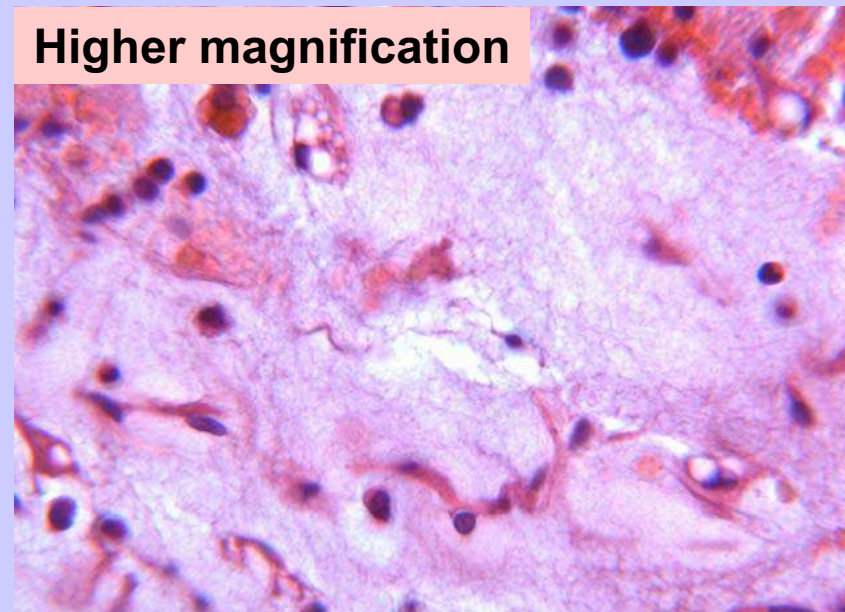
Example: PPAR-agonist, Dog

- ✓ high dose animals with gelatinous degeneration of bone marrow
- ✓ finally additional groups treated with less than 1 mg/kg bw revealed no differences in bone marrow differentiation compared to controls

High dose, section (femur)



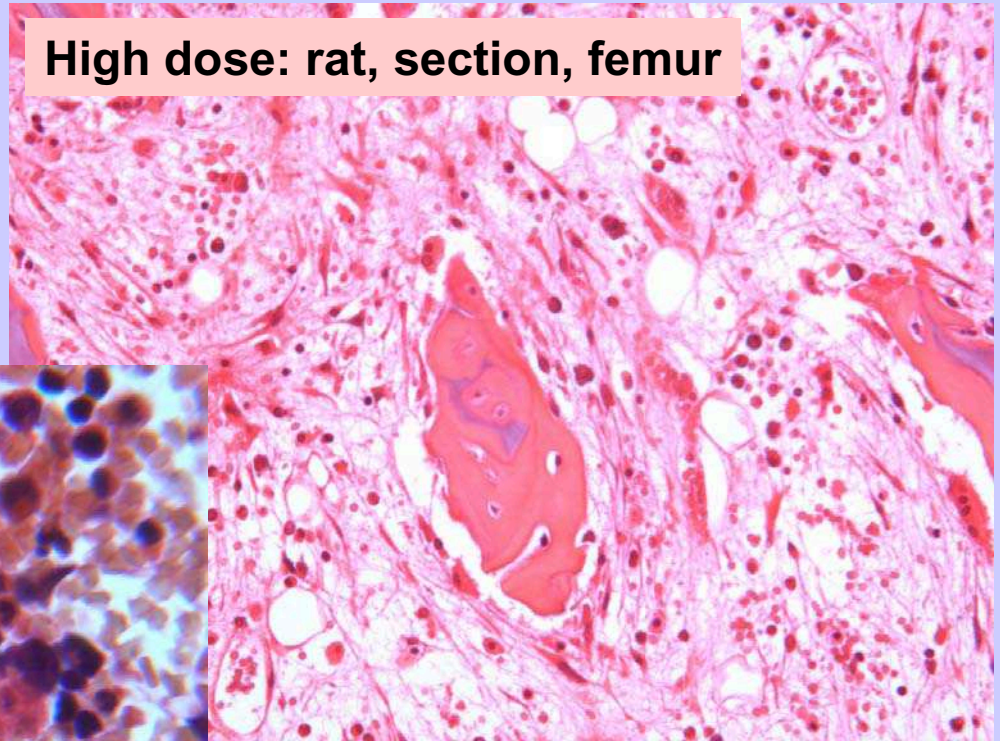
Higher magnification



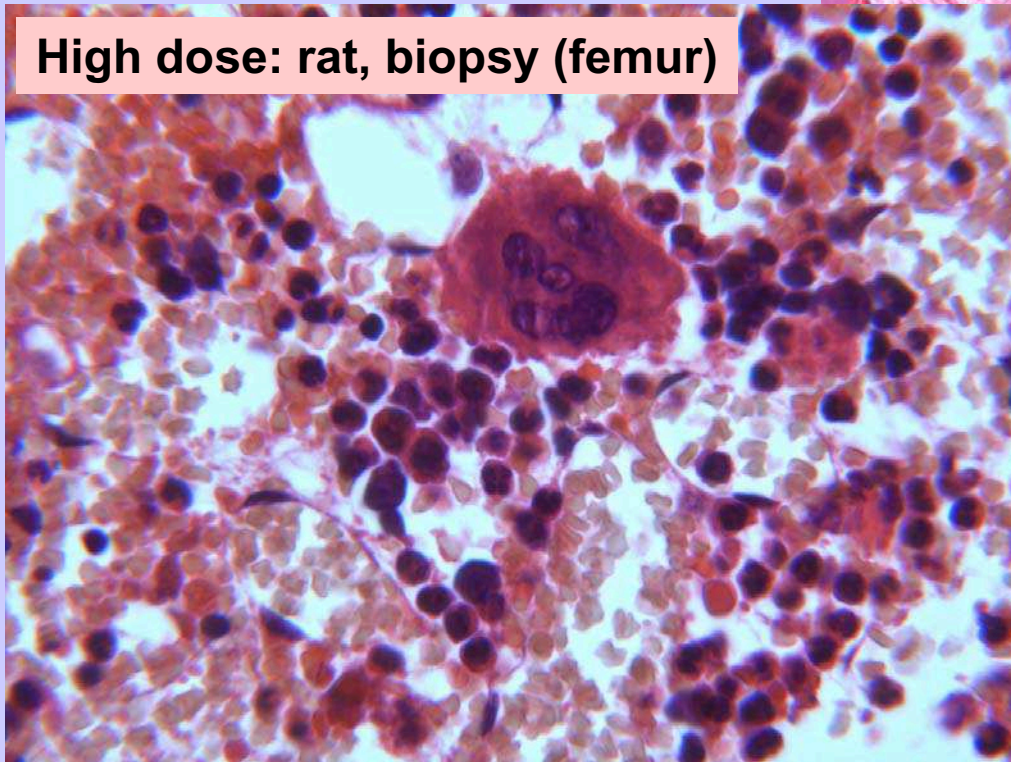
Example: Doxorubicin, Rat

- ✓ Section reveals full degeneration

High dose: rat, section, femur



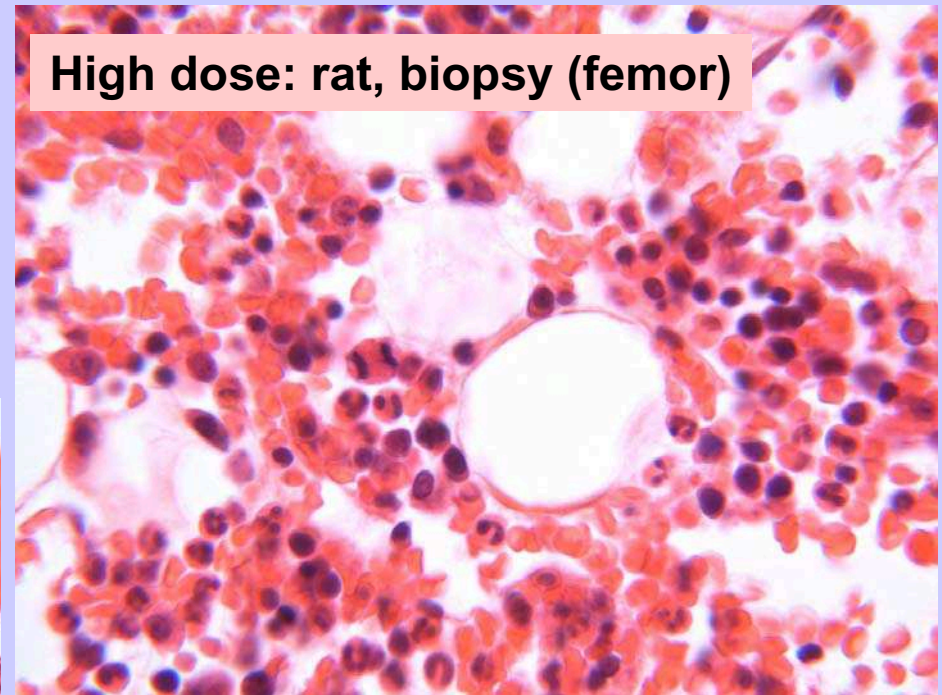
High dose: rat, biopsy (femur)



- ✓ Still granulopoiesis is going on in biopsy sample

Example: Platinum Compound, Rat

- ✓ **Section reveals hypocellularity and hyperemia**



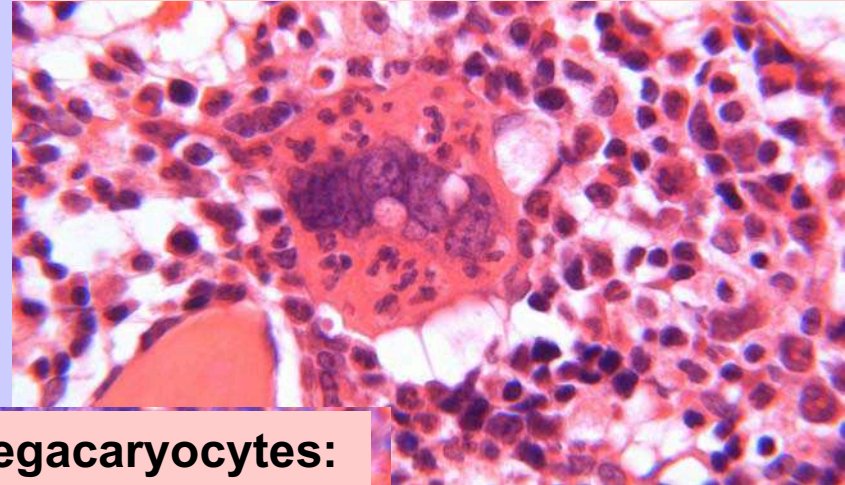
- ✓ **biopsy reveals hypocellularity and hyperemia, but granulopoiesis and erythropoiesis are still ongoing**

Example: LPS

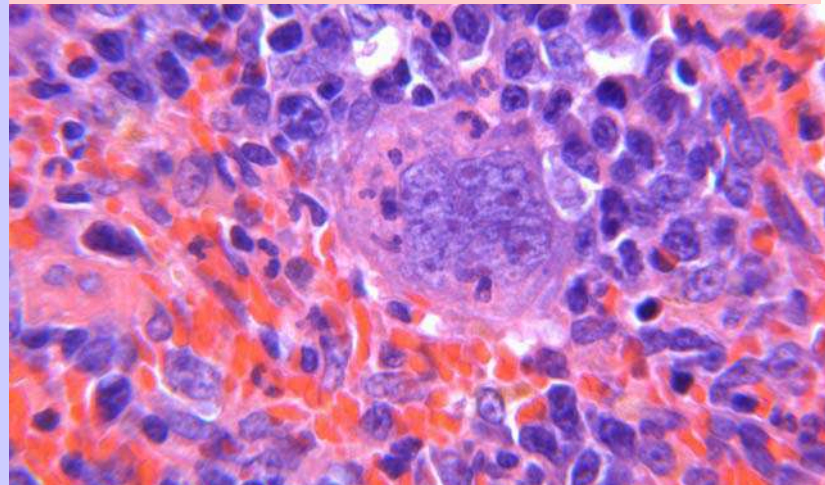
Bone marrow: femur



**Emperipolesis is Megacaryocytes
(Bone marrow: femur)**



**Emperipolesis Megacaryocytes:
Spleen**



In Oncogenicity Studies?

- ✓ **Not to recommend!**

- ✓ **Age-related lesions**
Mouse: Amyloidosis
Rat: CPN

- ✓ **Systemic Tumors:**
 - Mouse: lymphoma and histiocytic sarcoma
 - Hamster: lymphoma and plasmacytoma

Conclusions

- ✓ **Bone marrow differentiation is not specifically asked by guidelines**
- ✓ **is an interesting tool for tresholf effects (e.g. PPAR agonists)**
- ✓ **is expensive and time-consuming**
- ✓ **needs to be well discussed before**