

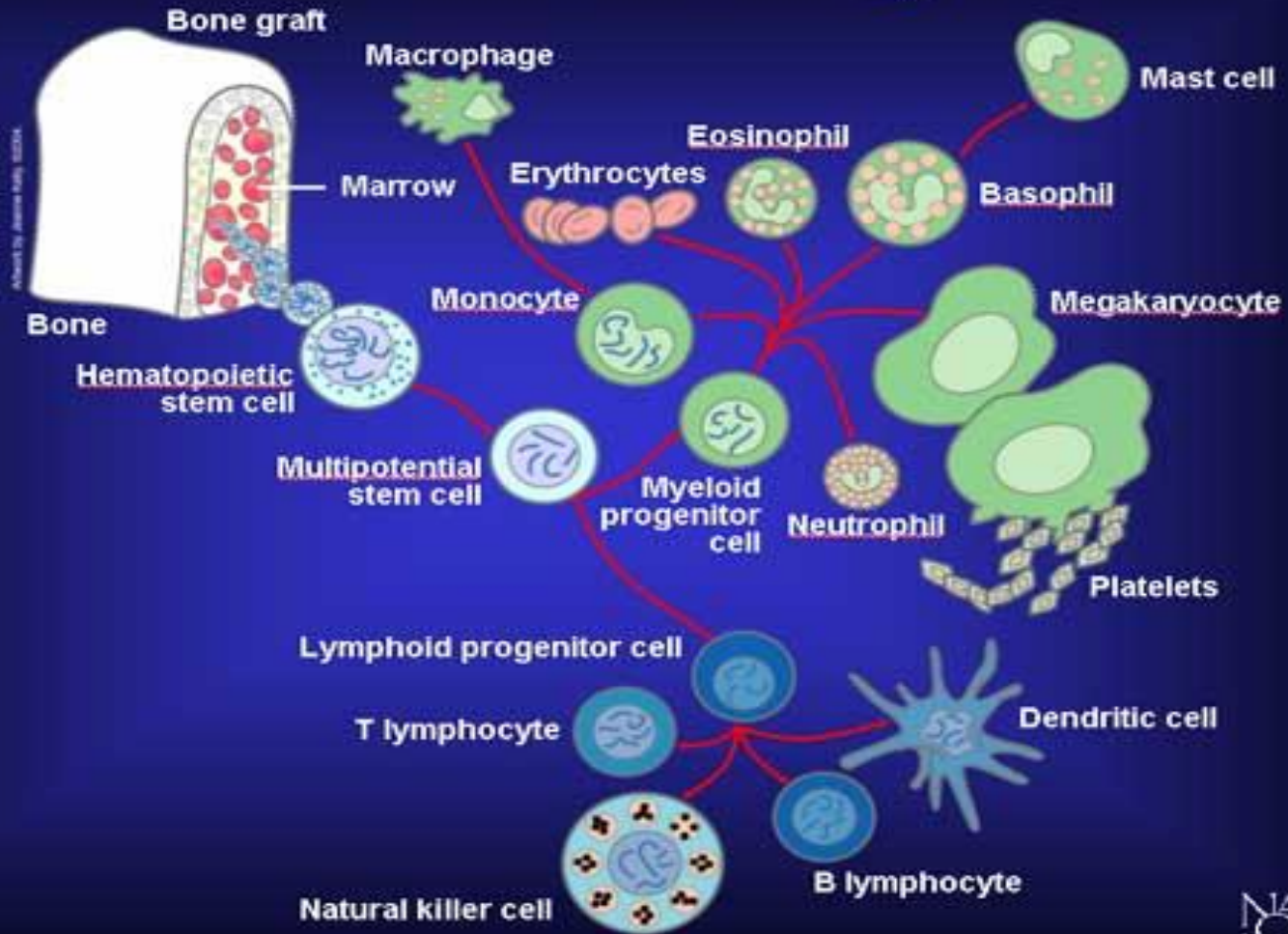
Toxicologic Pathology – Immune System of Laboratory Animals

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In Cooperation with
BSL Scientific Laboratories GmbH,
Planegg, Germany

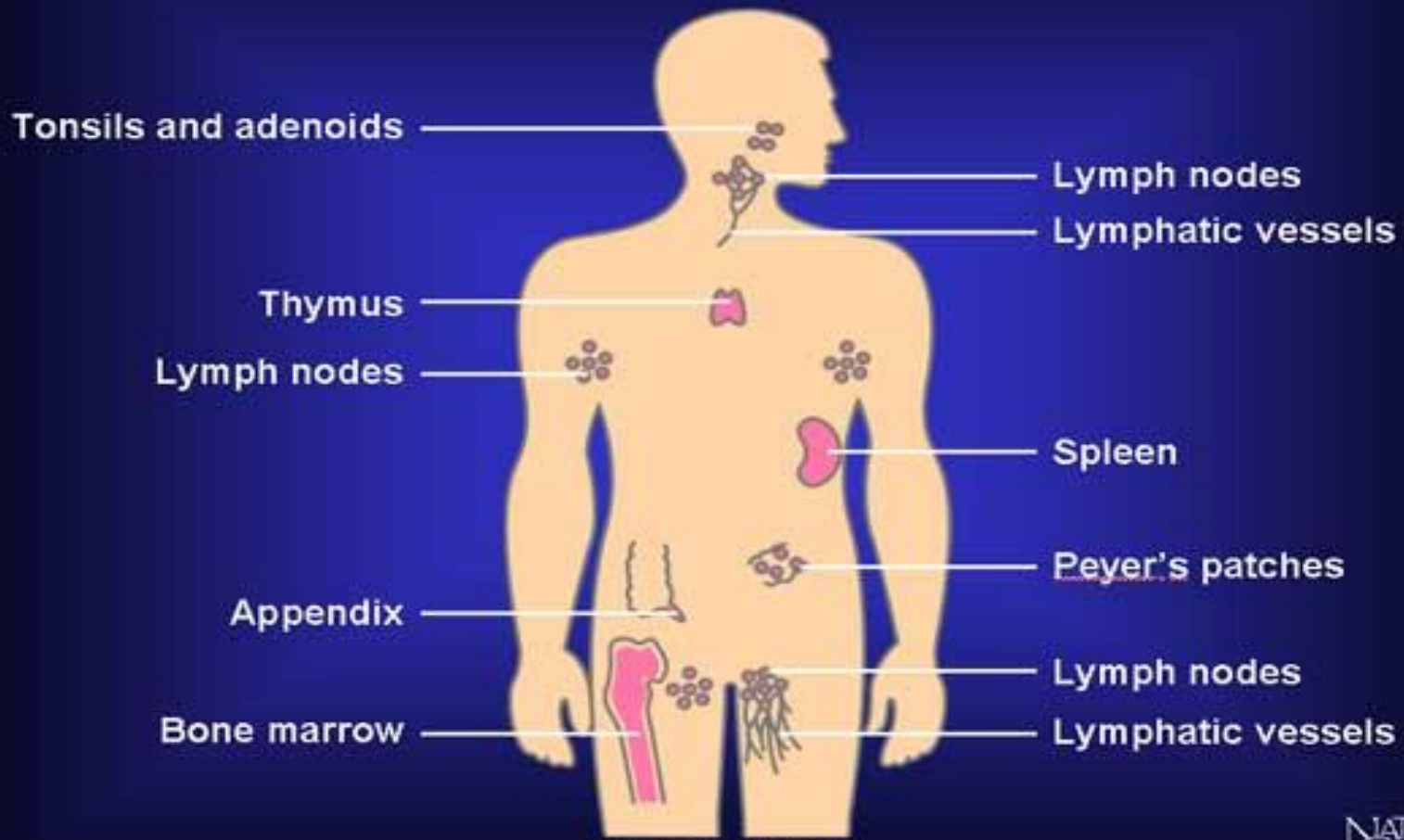
Immune System: What it is?

Cells of the Immune System



Compartmentation

Organs of the Immune System



Artwork by Jeanne Kelly, ©2004.

Guidelines?



May 2006
CHMP/167235/2004

**ICH Topic S 8
Immunotoxicity Studies for Human Pharmaceuticals**

Step 5

**NOTE FOR GUIDANCE ON IMMUNOTOXICITY STUDIES FOR HUMAN
PHARMACEUTICALS
(CHMP/167235/2004)**

- Detailed strategy
- All lymphoid tissues to be examined (incl. Peyer's patches)
- Immunohistochemistry superior to Facscan
- Interpretation of stress-related effects are necessary

Compartmentation

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

¹Unexplained alterations in globulin levels could call for measurements of immunoglobulins

²Unexplained alterations in peripheral blood cell lines or histopathological findings might suggest that cytologic evaluation of the bone marrow would be appropriate

³Oral administration only

⁴For inhalation or nasal route only



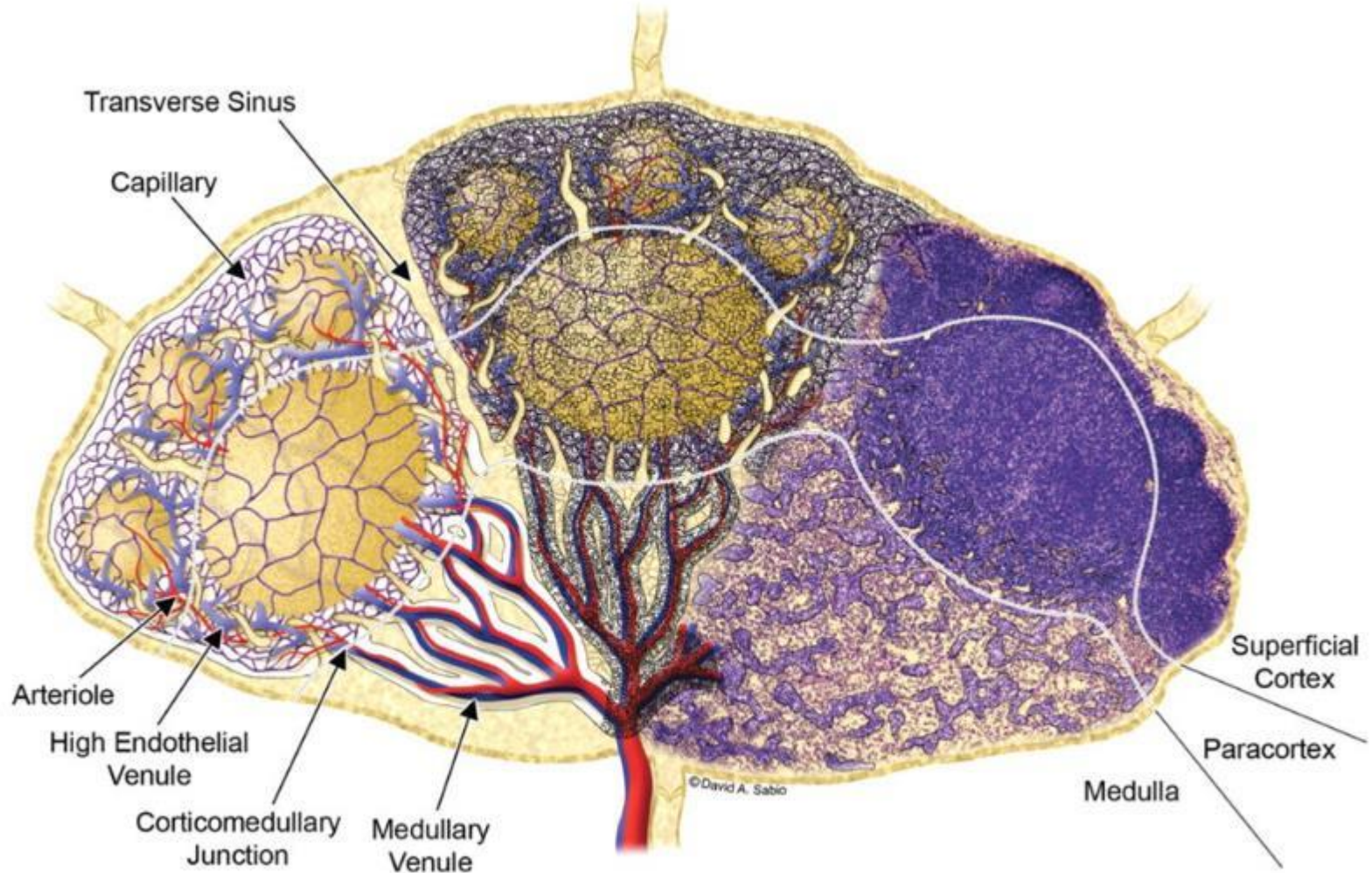
Lymph Nodes

AmmaPath

Functional Structure

- **Lymphocytes of the whole body turns over 10 to 48x/24 hrs**
- **DC - Dendritic cells as APC's (loosing ability to bind antigens during travel to lymph nodes but gaining ability to present) – presenting to T-cells with subsequent proliferation after 1-2 days**
- **FDC - Follicular dendritic cells: APC's that present to B-cells**
- **Germinal centers formed by B-cells where they are in contact with FDC's after 3-4 days with centrocytes after 7 days**
- **Reticular meshwork (fibroblastic reticular cells, FRC)**
- **Medullary and paracortical cords (peripheral deep cortical unit (DCU))**
- **Paracortical cords more numerous than medullary cords due to multiplication in tandem with blood vessels**
- **HEV located only in interfollicular cortex and peripheral DCU (when loosing high endothelium, HEV turn into regular meshwork venules but under stimulation, the height of endothelia will be increased)**
- **Paracortical sinuses ends blind into interfollicular cortex (under deactivation of Sphingosin 1-phosphate receptors, lymphocytes from cords fill the sinuses)**

Lymph node: An idealized midsagittal section of a small lymph node contains three lymphoid lobules.

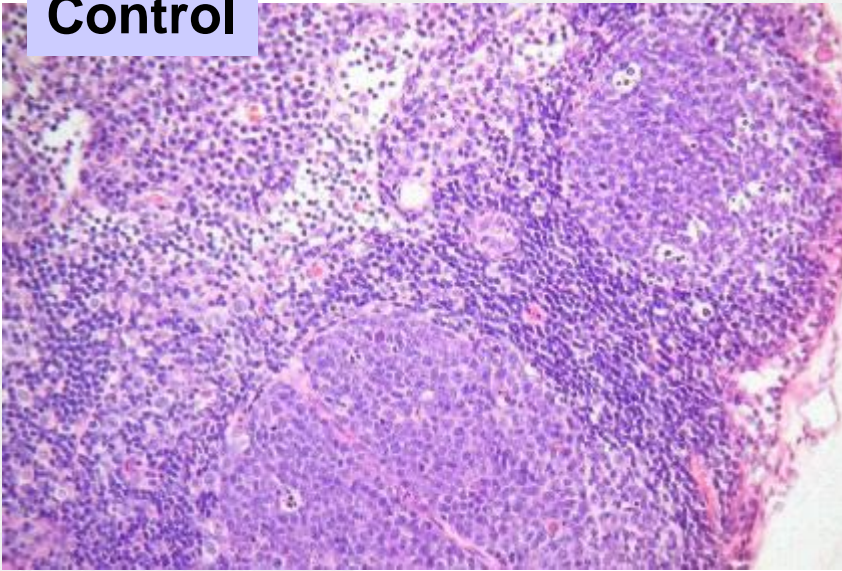


Willard-Mack C L Toxicol Pathol 2006;34:409-424

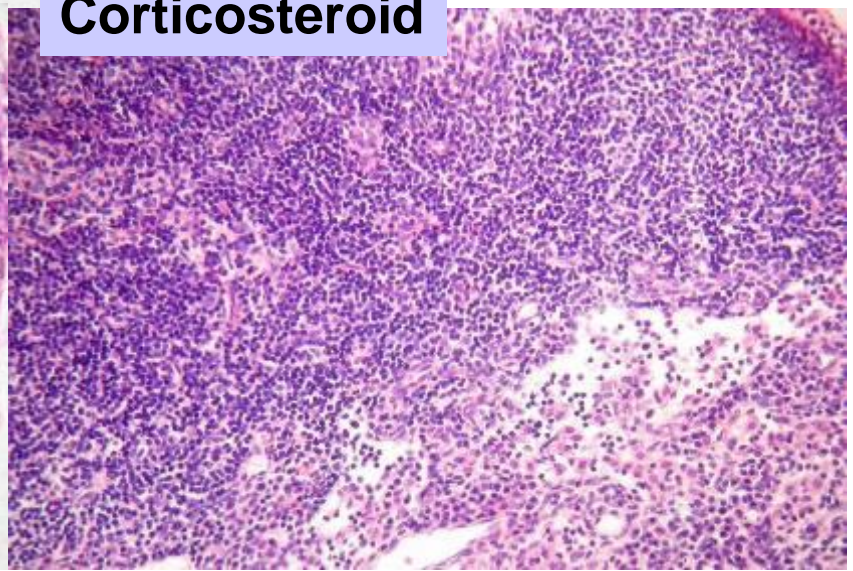
TOXICOLOGIC
PATHOLOGY

Lymph Node: Lymphoid depletion (Rat)

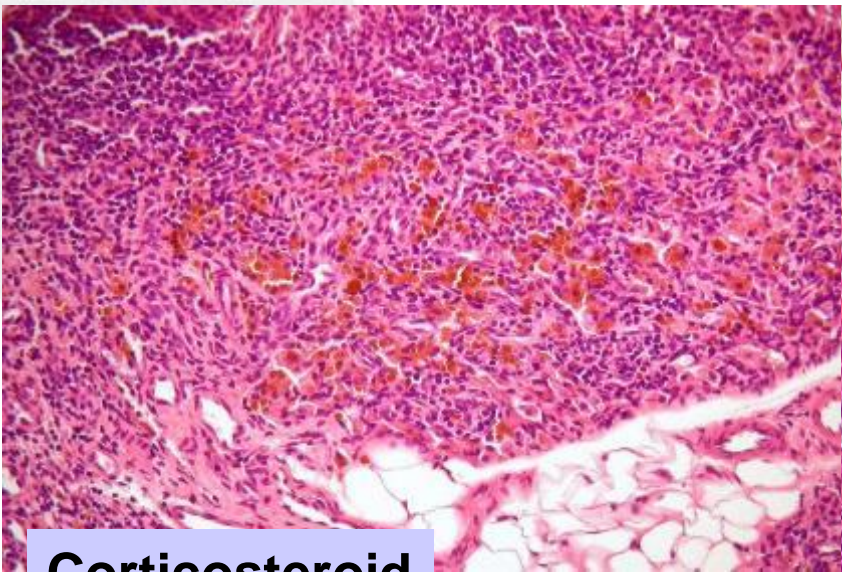
Control



Corticosteroid



Corticosteroid

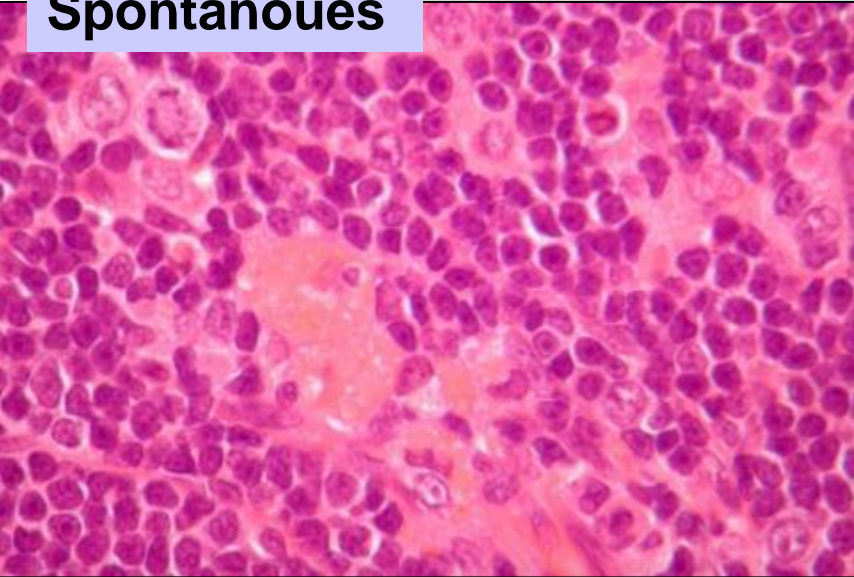


Ca-Channel Blocker

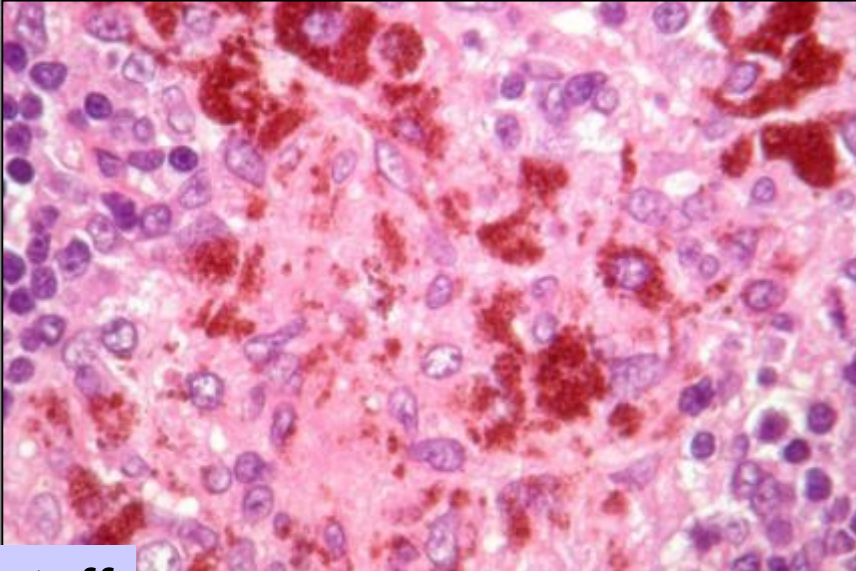
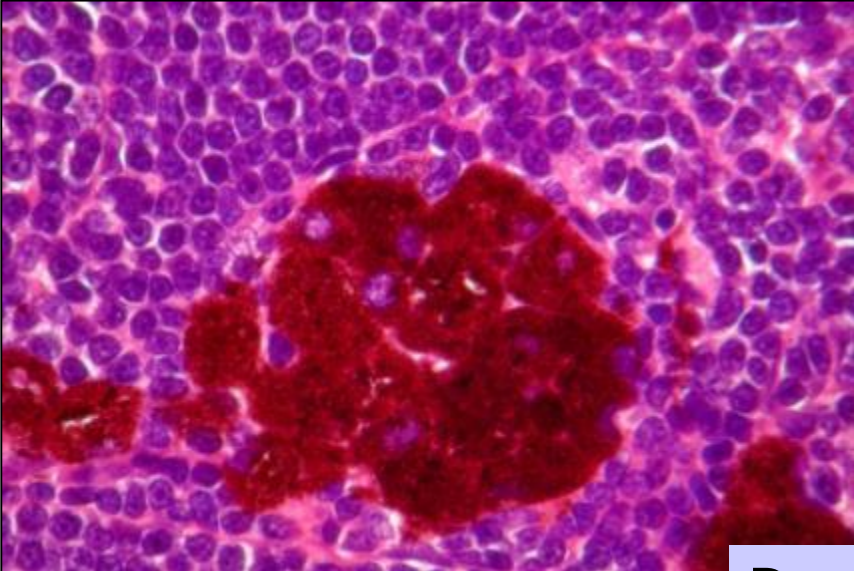


Lymph Node: Pigmentation (Rat)

Spontanoues



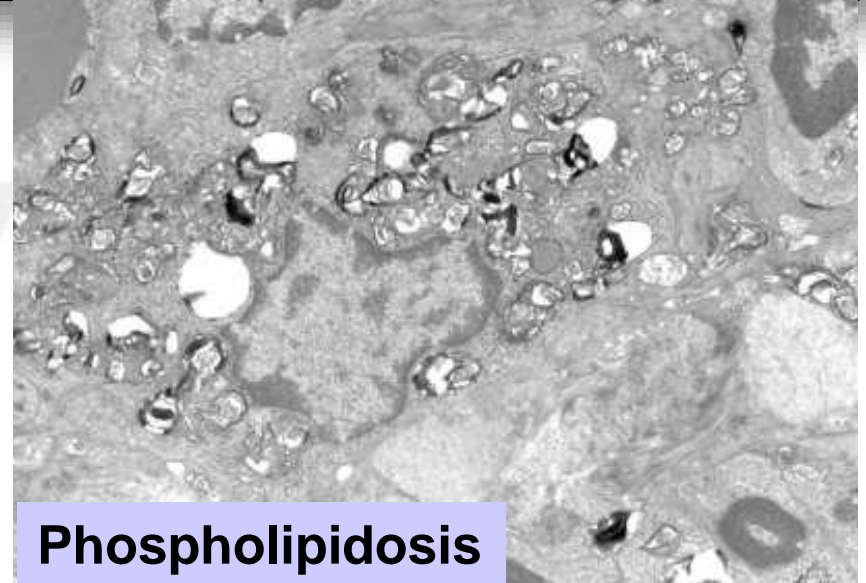
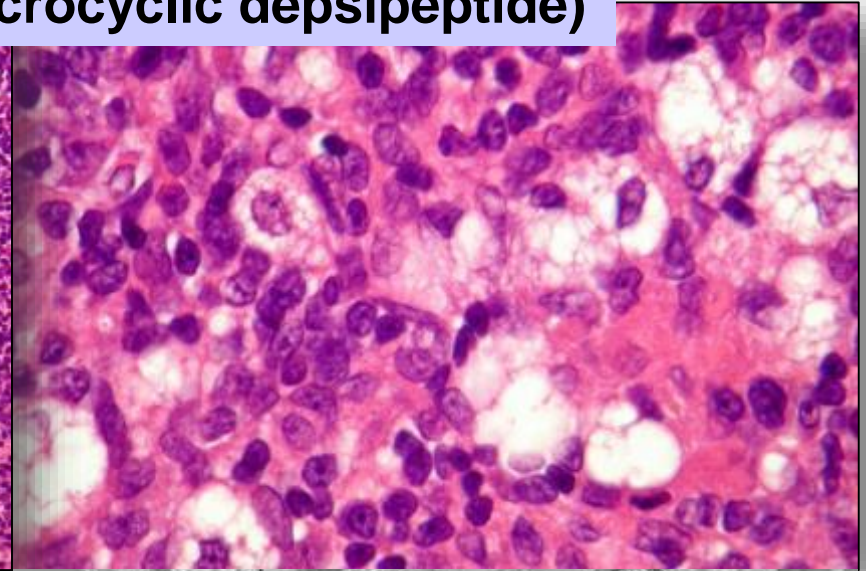
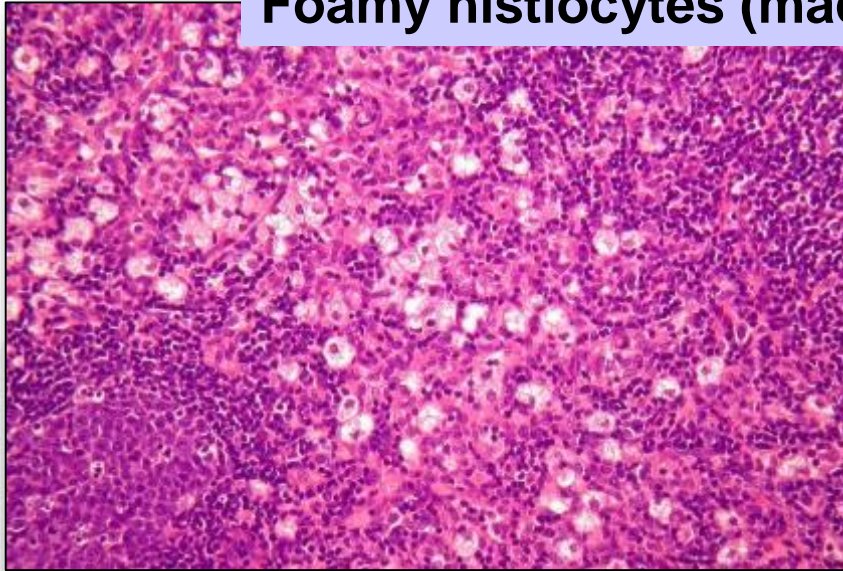
Dye stuff



Dye stuff

Lymph Node: Histiocytosis (foam, cell) (Rat)

Foamy histiocytes (macrocytic depsiptide)



Phospholipidosis

- Many compounds causing vacuolation
- Attention: diagnose PLP only by electron microscopy



Spleen

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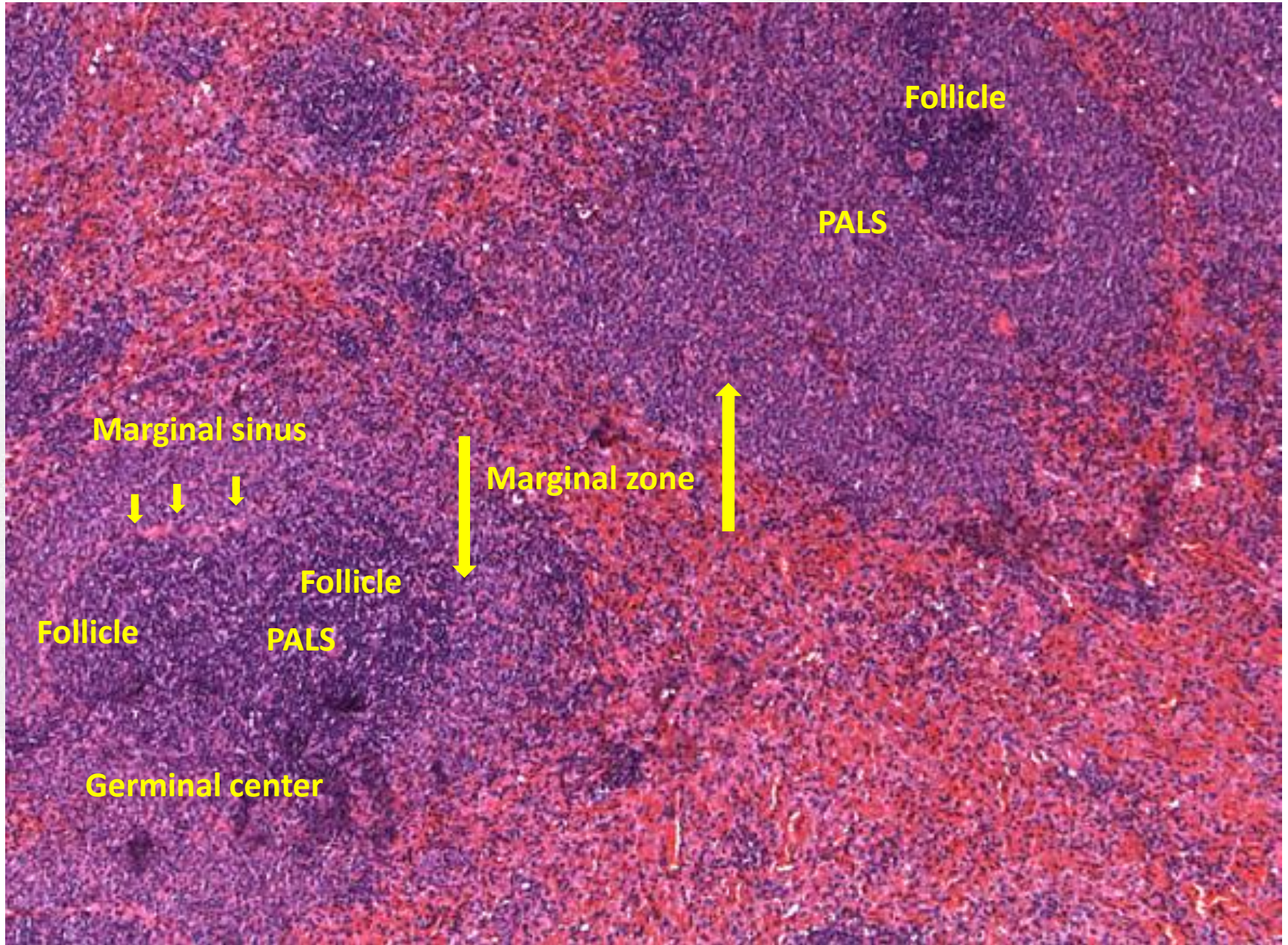
Functional Structure

- **Splenic artery divides into trabecular arteries from which branches small arteries that enter the red pulp forming the central arterioles**
- **Splenic cords: fibers and macrophages**
- **Red pulp: meshwork of splenic cords and venous sinuses containing siderites and extramedullary hemopoietic cells**
- **White pulp consisting of PALS, follicle and marginal zone**
- **Inner PALS (T-dependent Zone) with smaller lymphocytes (darker), mostly CD8+ (lesser CD4+) and occasionally DC's, B-cells,**
- **Outer PALS (T- and B-cells), mid-sized lymphocytes and occasionally macrophages, plasma cells**

Functional Structure

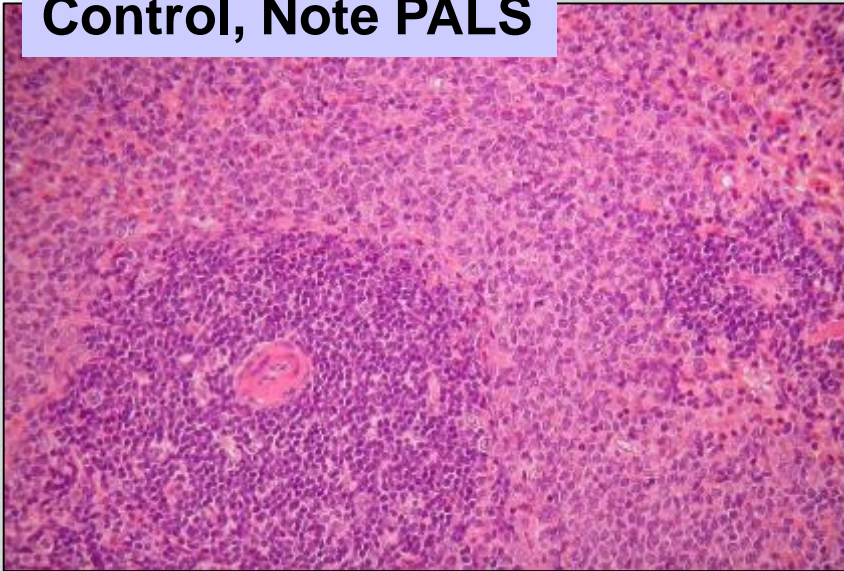
- **Follicles: continuous with PALS and most often at bifurcation of central arterioles, containing mainly B-cells, less FDC, CD4+ but NOT CD8+**
- **Marginal zone: interface to red pulp**
 - **band of marginal zone metallophilic macrophages (anti-MOMA-1)**
 - **peripheral to these macrophages: marginal sinuses lined by MADCAM1+ sinus endothelia and are in contact with vessels feeding PALS capillaries**
 - **peripheral to sinus: outer ring of marginal zone with marginal zone macrophages (ERTR-9+) with TLR's**

Schematic Overview of Splenic Structures

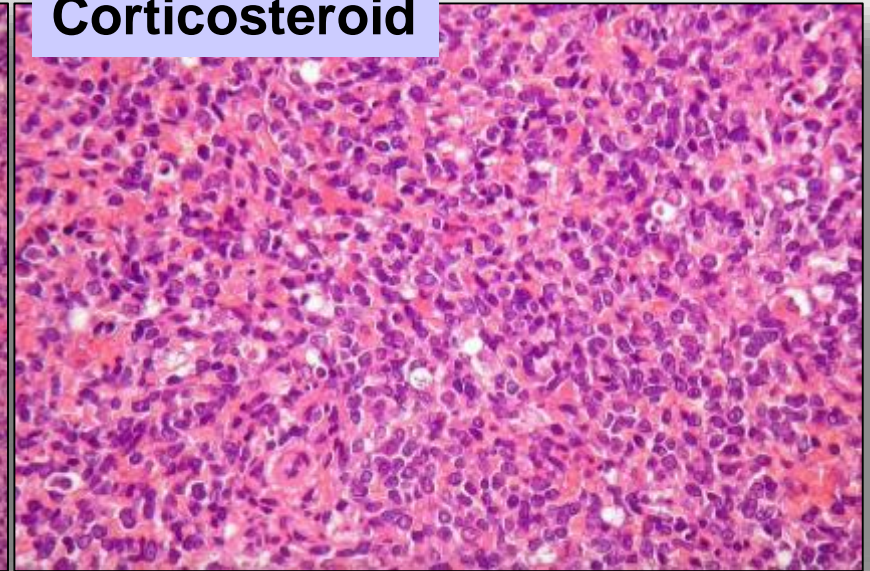


Spleen: Lymphoid depletion (Rat)

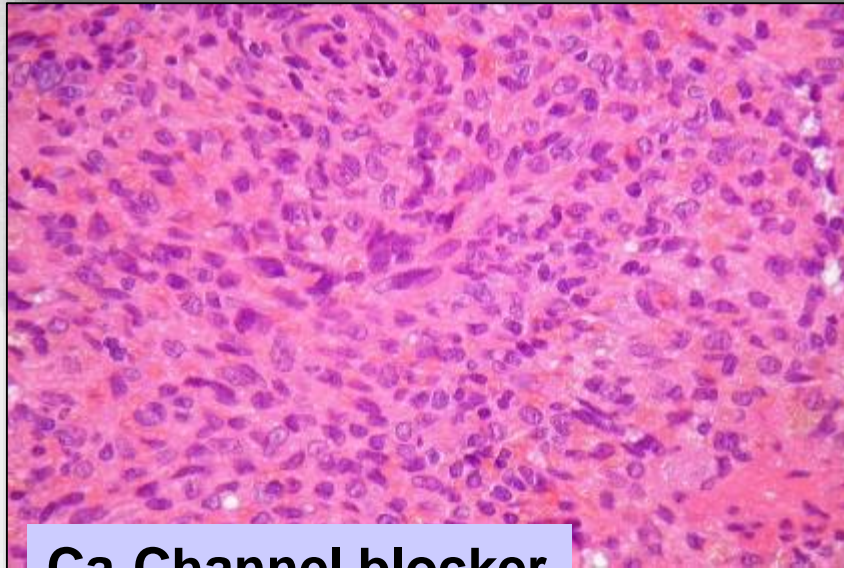
Control, Note PALS



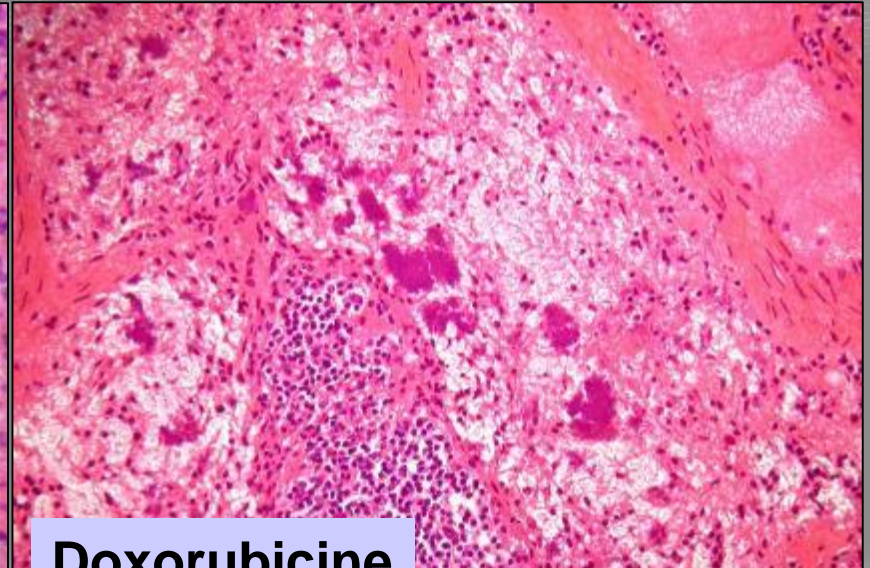
Corticosteroid



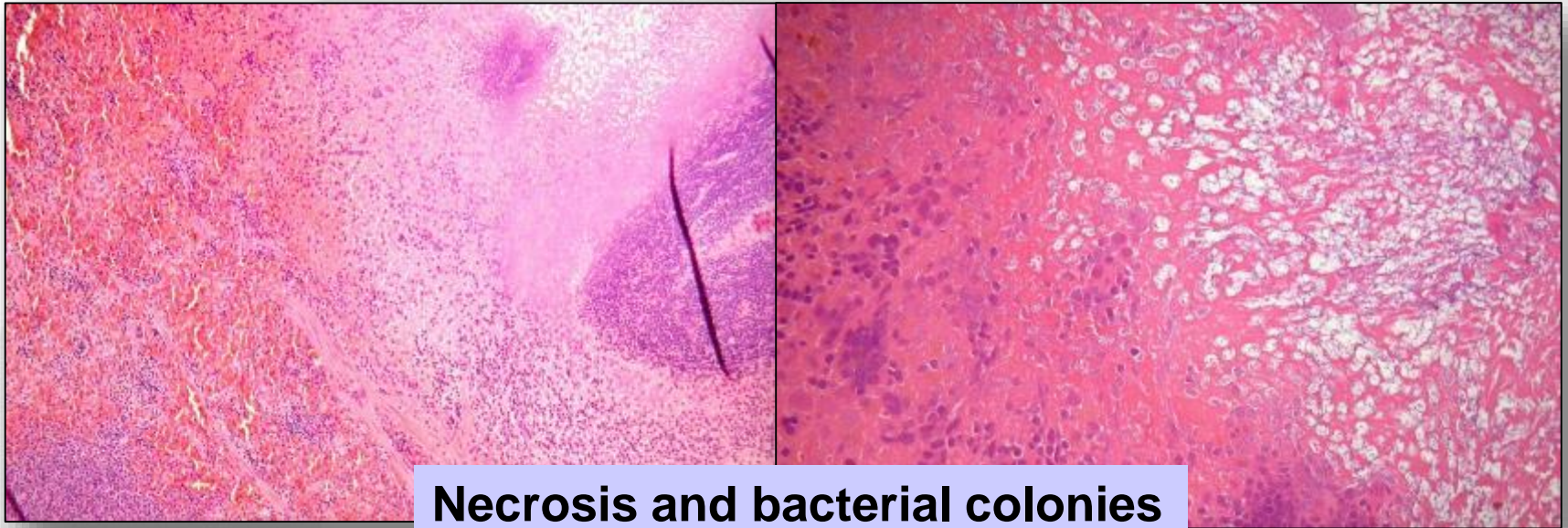
Ca-Channel blocker



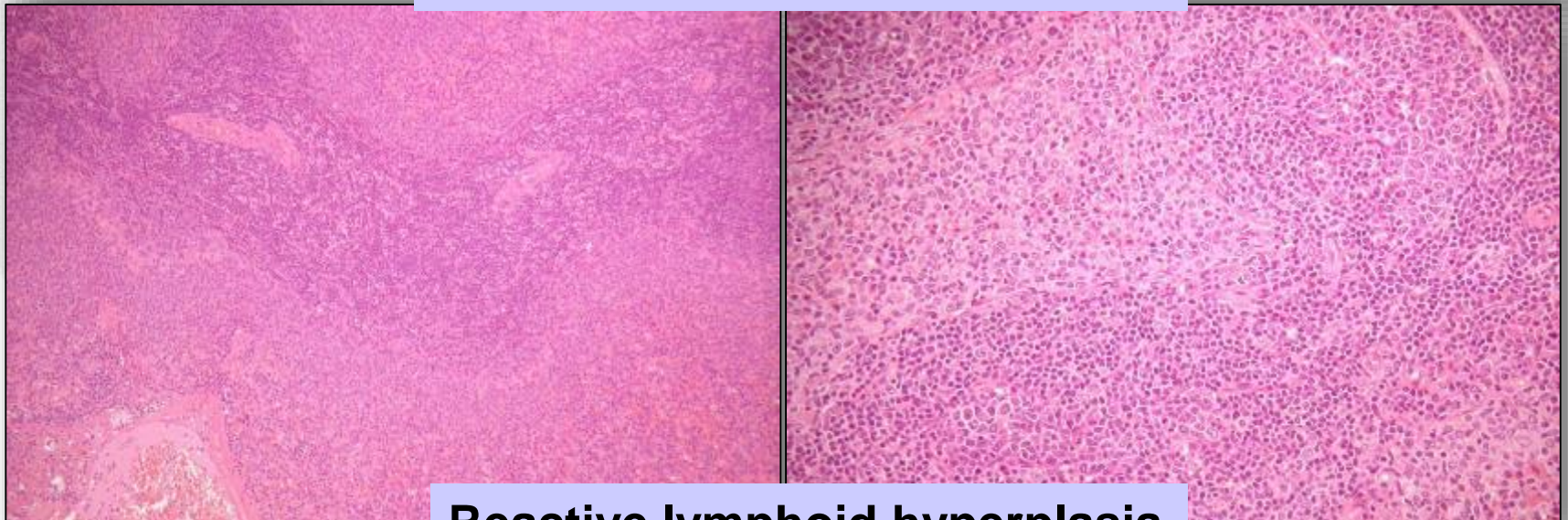
Doxorubicine



Spleen: Necrosis, Lymphoid Hyperplasia (Rat)

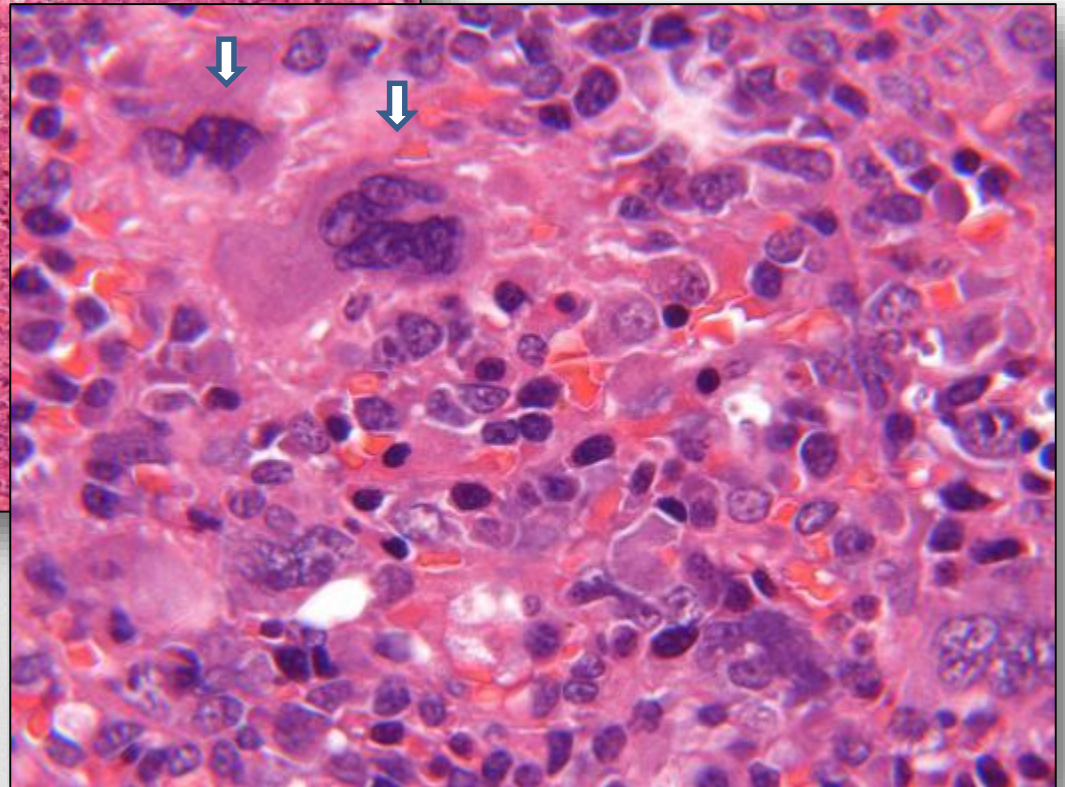
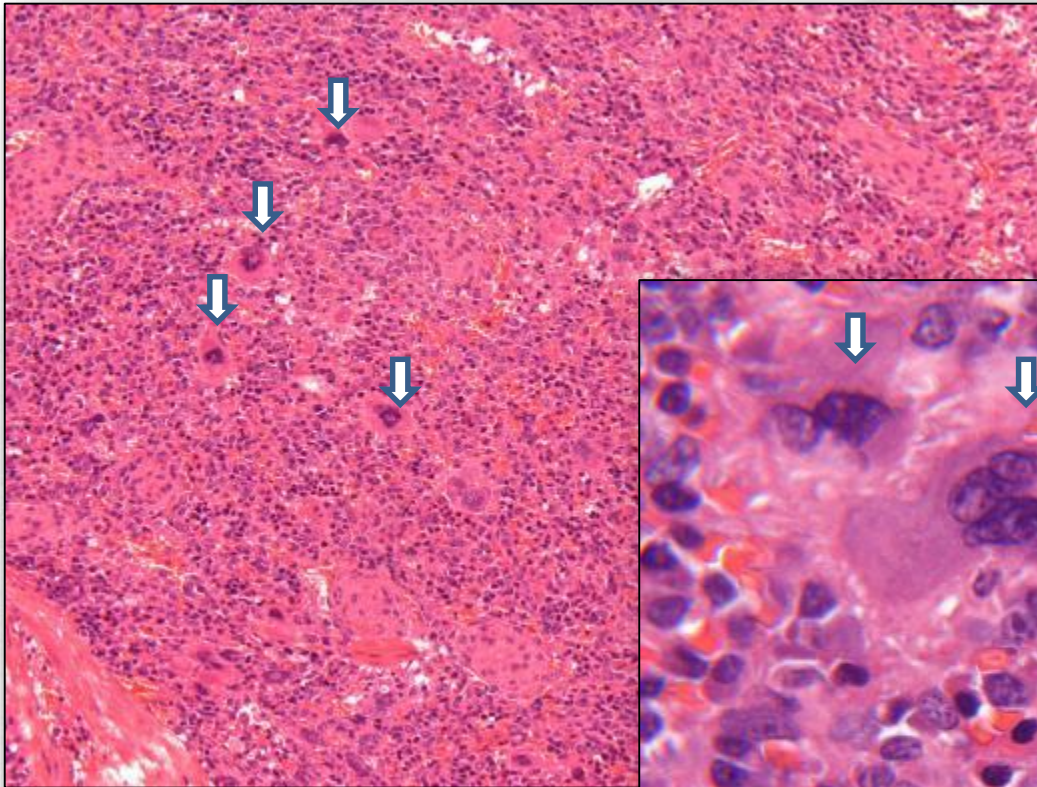


Necrosis and bacterial colonies



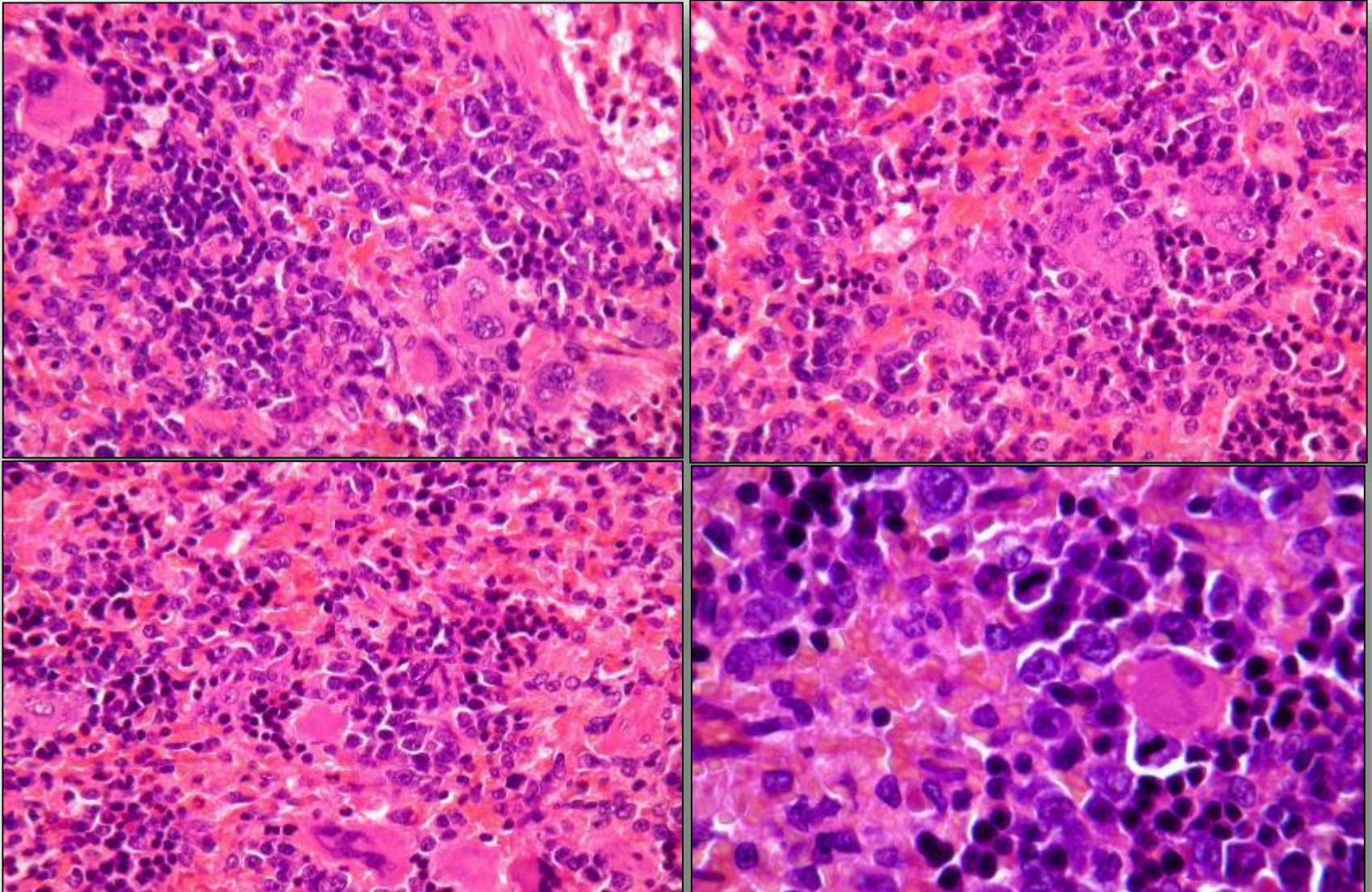
Reactive lymphoid hyperplasia

Spleen: Thrombocytopenia (PPAR agonist, Dog)



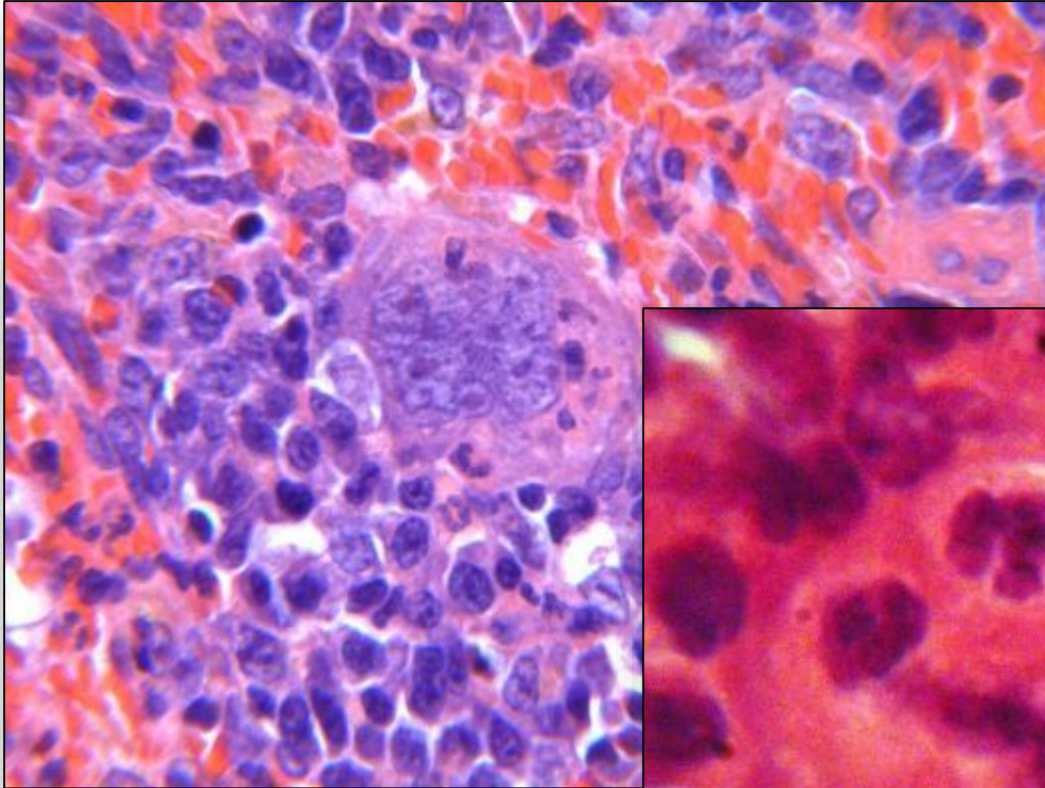
Dysthrombopoiesis
Note abnormal mega-
Karyocytes.

Spleen: Changes in Cell Populations (Rat)

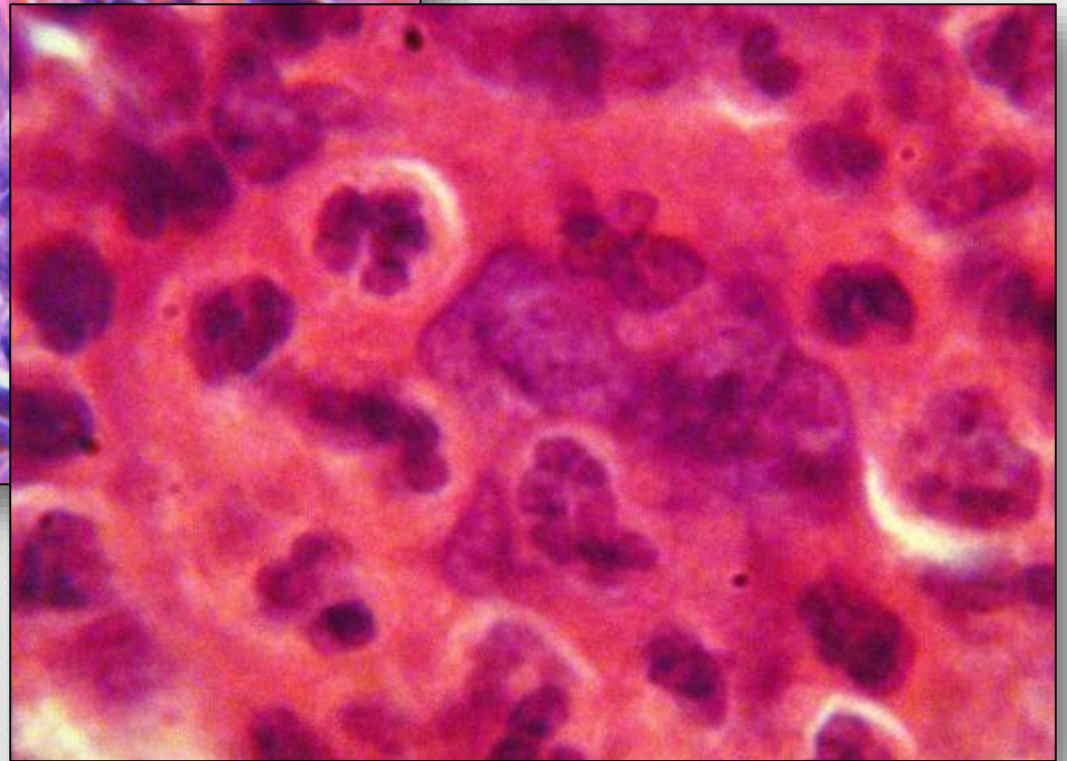


Increased megakaryoctosis and apoptosis (macrocyclic depsipeptide)

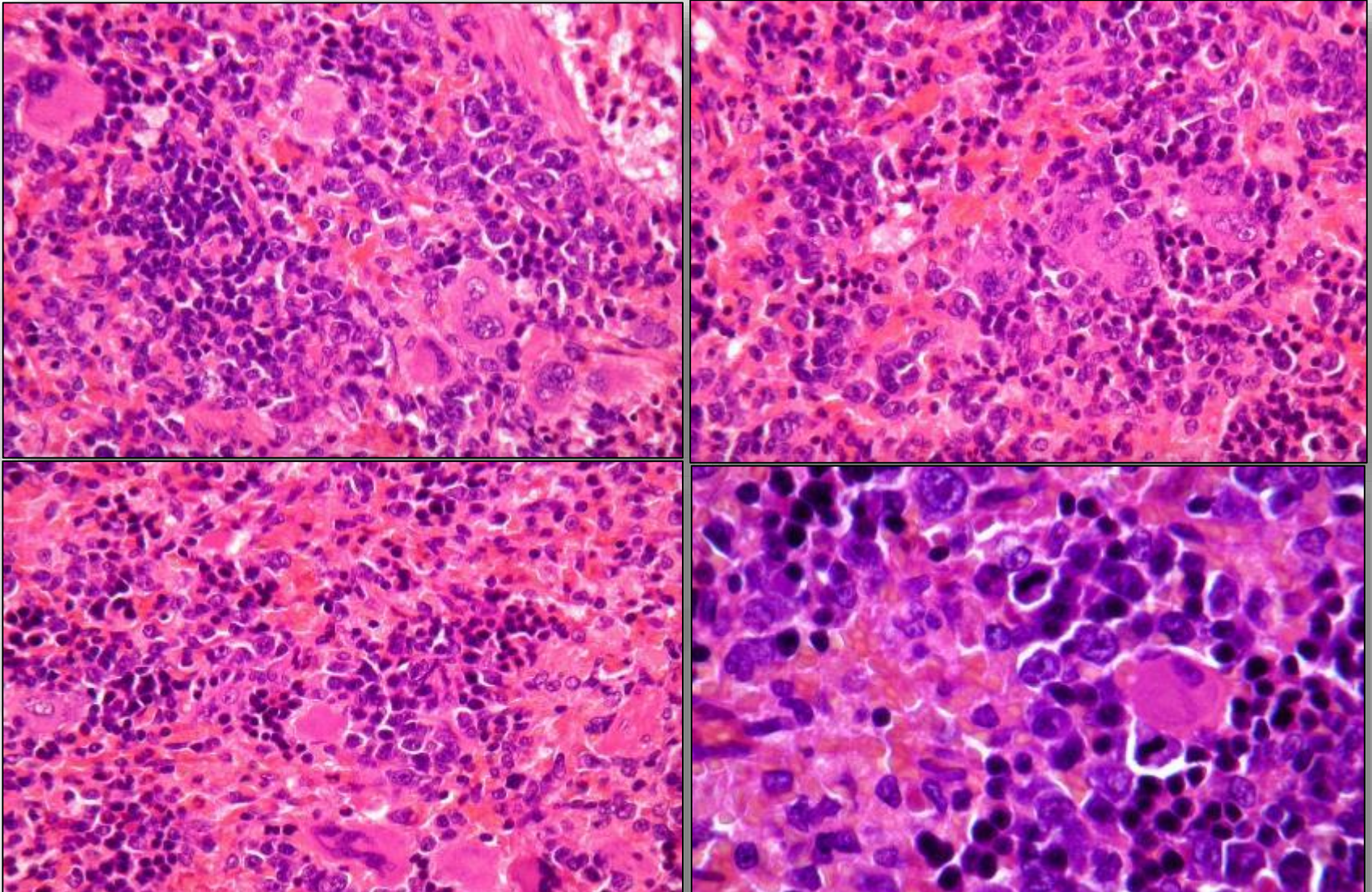
Spleen: Emperipolesis (LPS; Rat)



Note granulocytes in megakaryocyte cytoplasm

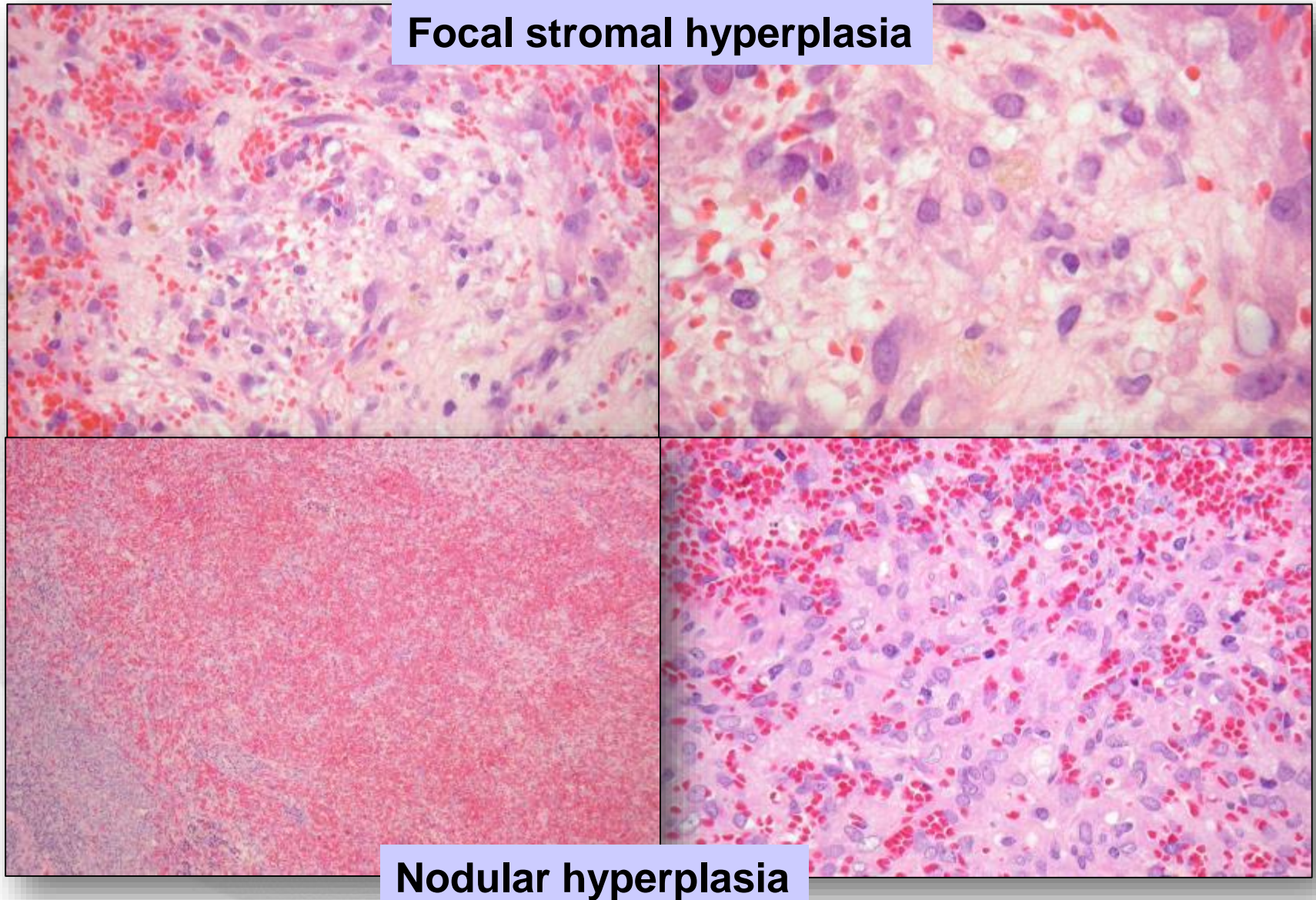


Spleen: Changes in Cell Populations (Rat)



Increased megakaryoctosis and apoptosis (macrocyclic depsipeptide)

Spleen: Red Pulp Lesions(Rat)





Thymus

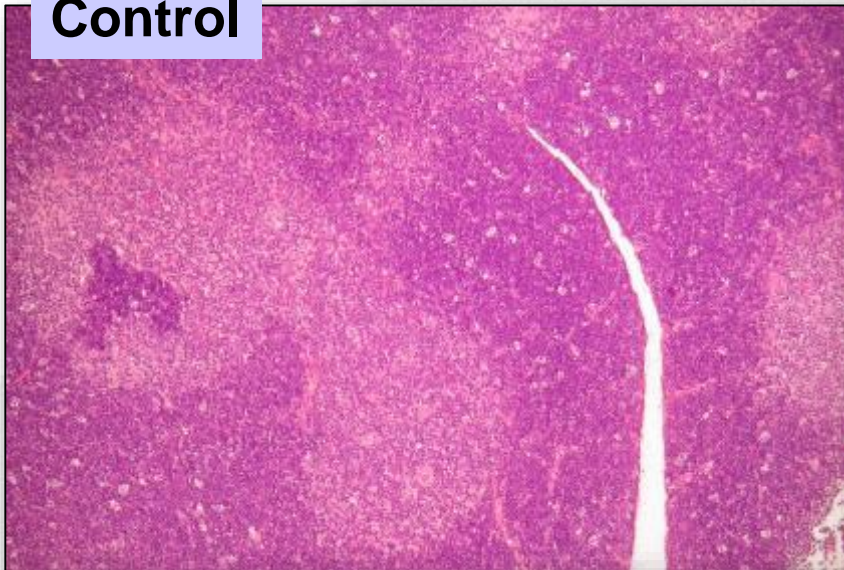
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Functional Structure

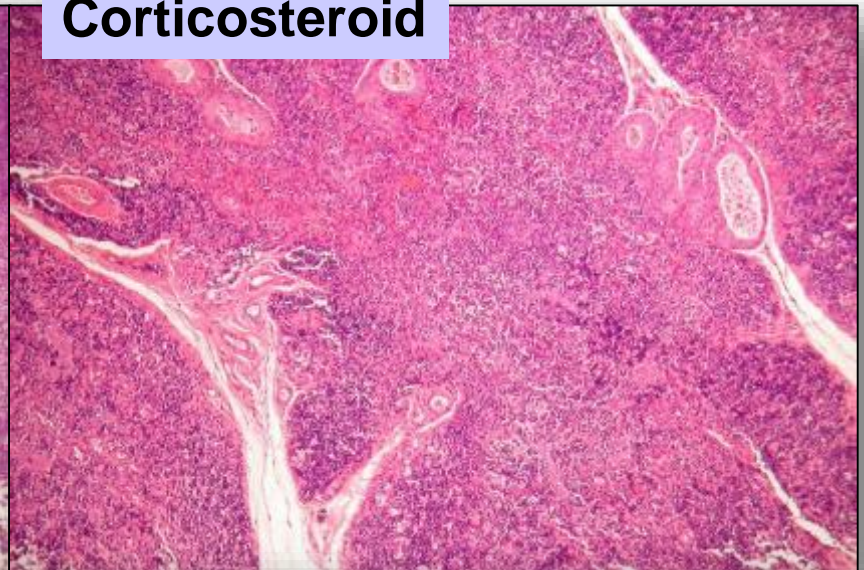
- **Only lymphoid tissue that is an epithelial organ (gland!!)**
- **Epithelial cells forms a layer of 1 or 2 cells deep (subcapsular) and form 4 immunohistochemically distinct populations (subcapsular, inner cortical, medullary, Hassalls corpuscles (not often in rodents)) that produce thymulin, thymosin, thymopoietin, thymic humoral factor**
- **Cortex: small immature lymphocytes and bone-marrow derived population plus tingible body macrophages**
- **Medulla: more mature T-cells and prominent epithelial cells, B-cells**

Thymus: Lymphoid depletion (Rat)

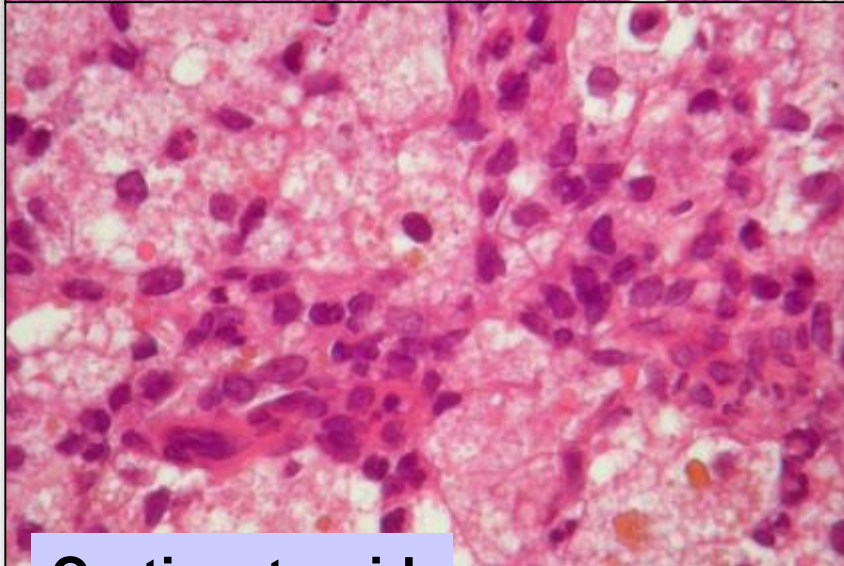
Control



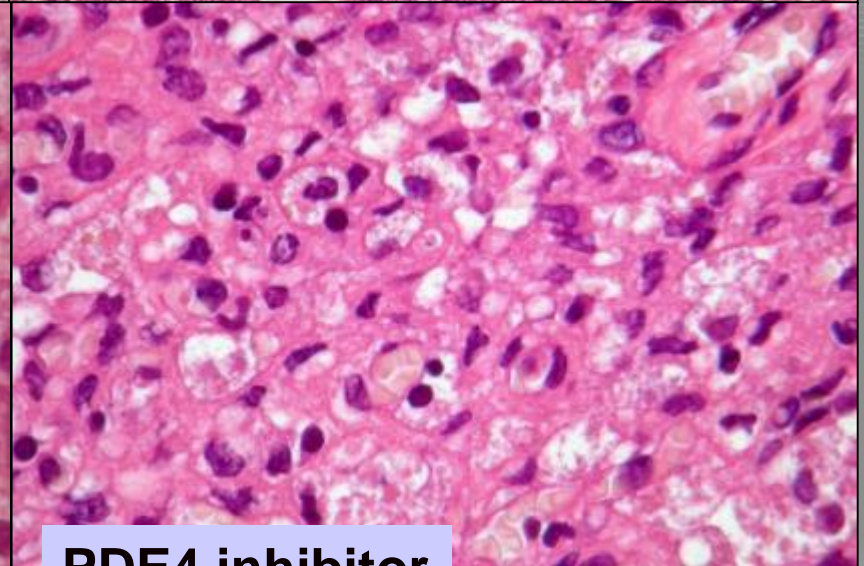
Corticosteroid



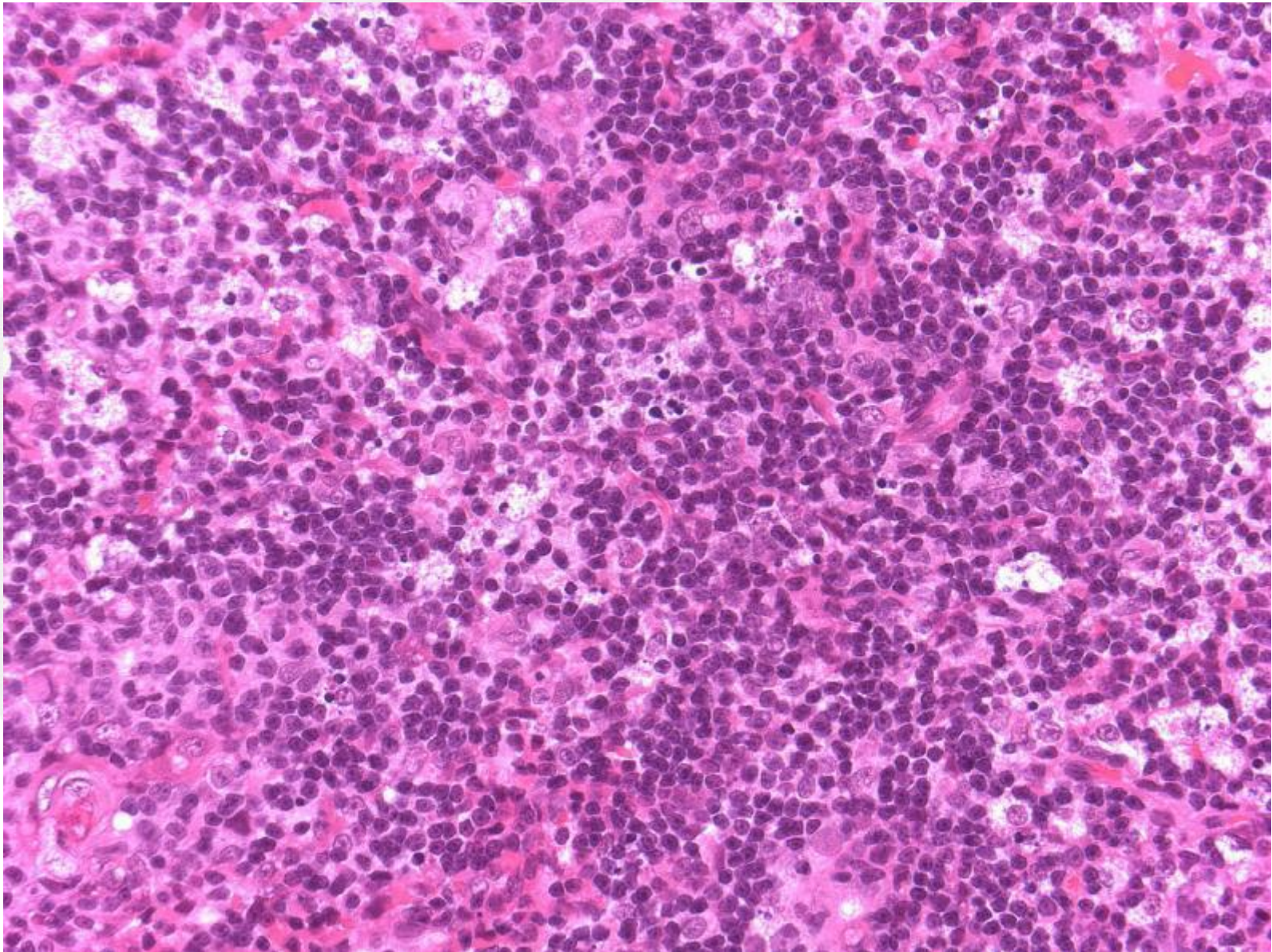
Corticosteroid



PDE4 inhibitor



Thymus: Phospholipidosis (Rat)

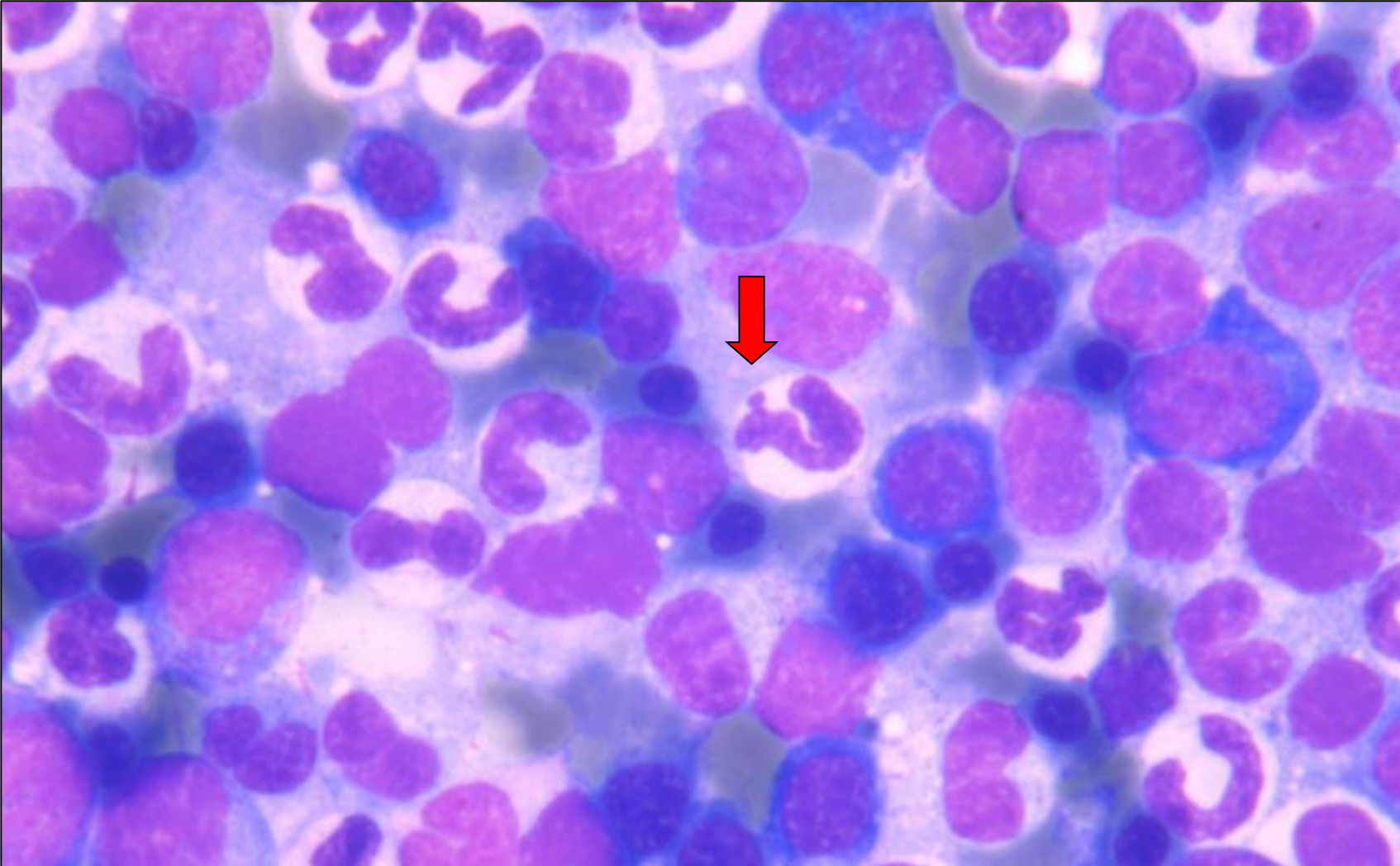




Bone Marrow

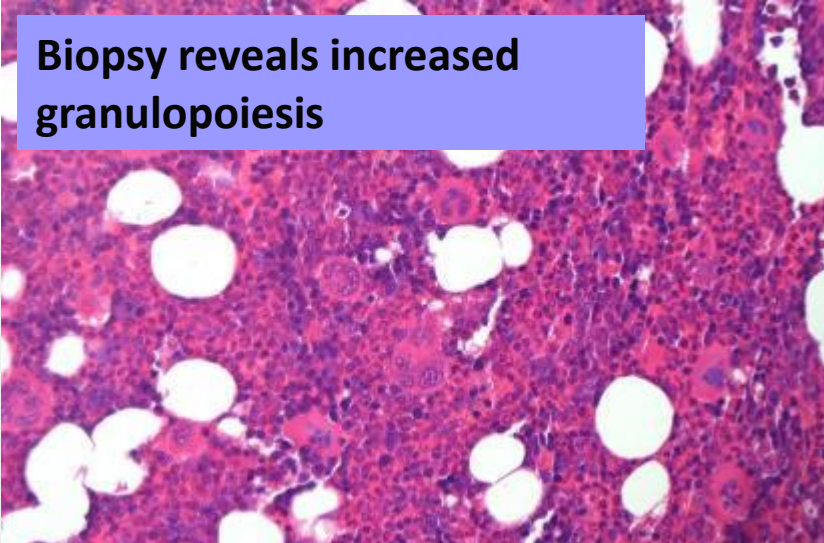
AnaPath

Overview

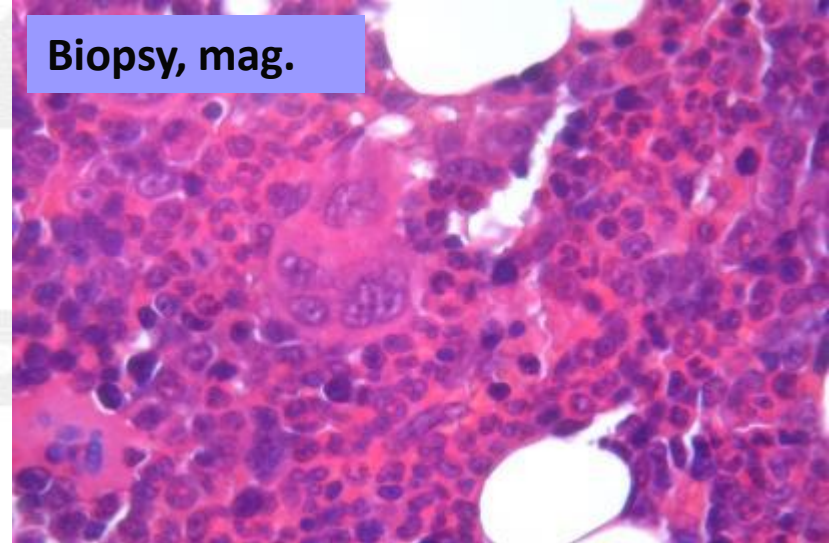


Different Techniques – Different Results

Biopsy reveals increased granulopoiesis



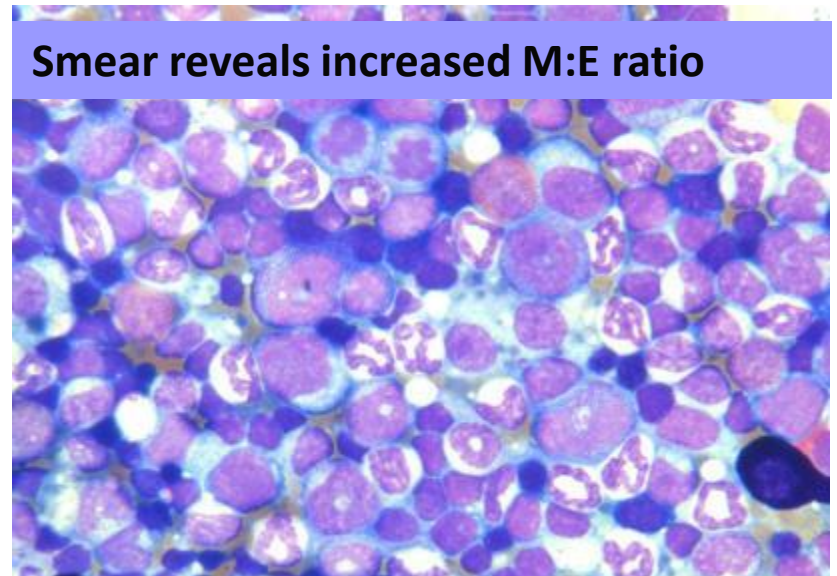
Biopsy, mag.



Section with bone reveals hypocellularity



Smear reveals increased M:E ratio



Differentiation Necessary?

- **Findings/Lesions in peripheral blood**
- **Suspicious compound**
- **Findings in bone marrow (sections)**
- **Findings leading to assessment of immunotoxicity**
- **Bone marrow differentiation is based on evaluation of sections and hematology data.**

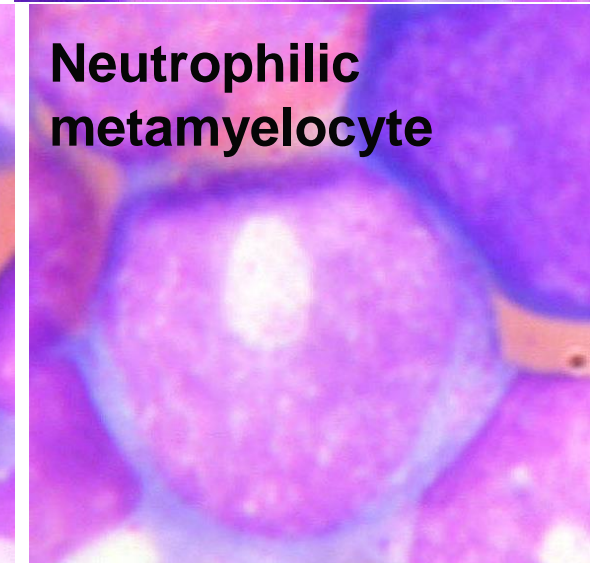
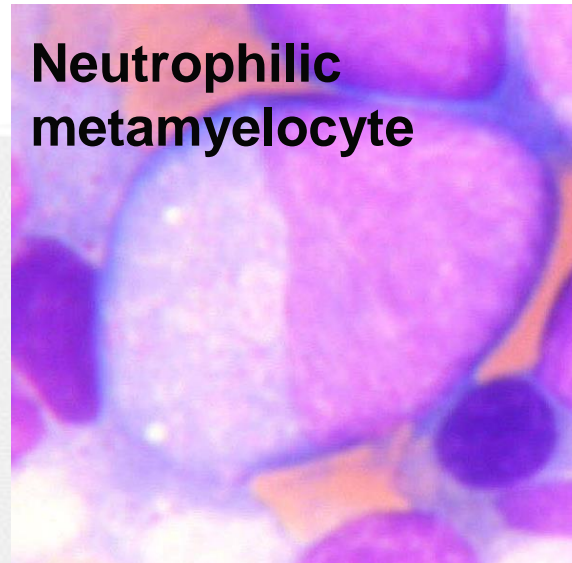
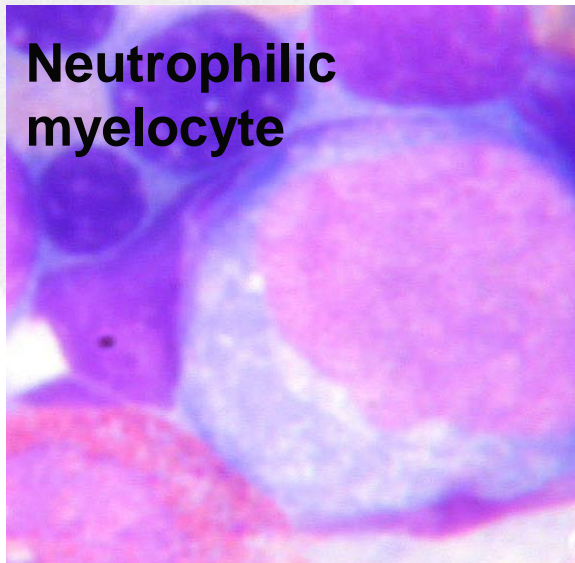
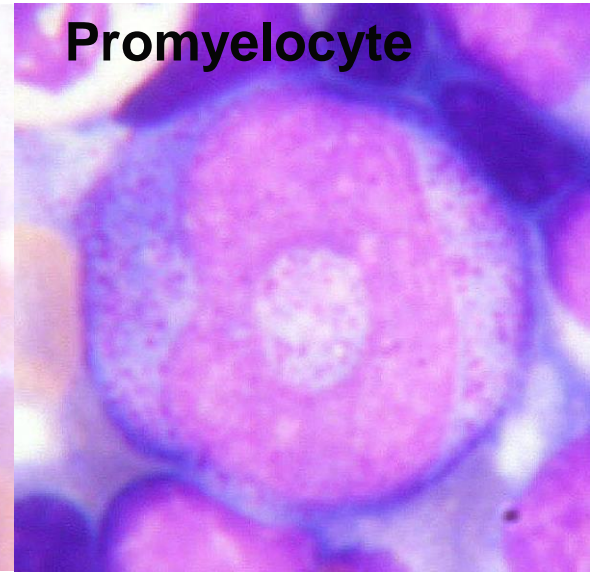
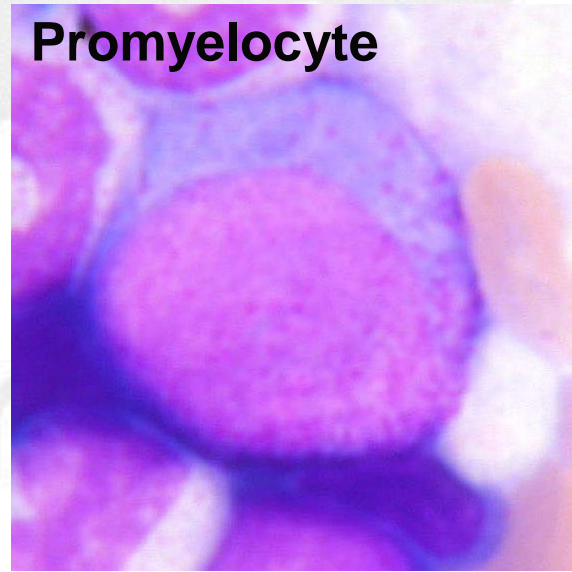
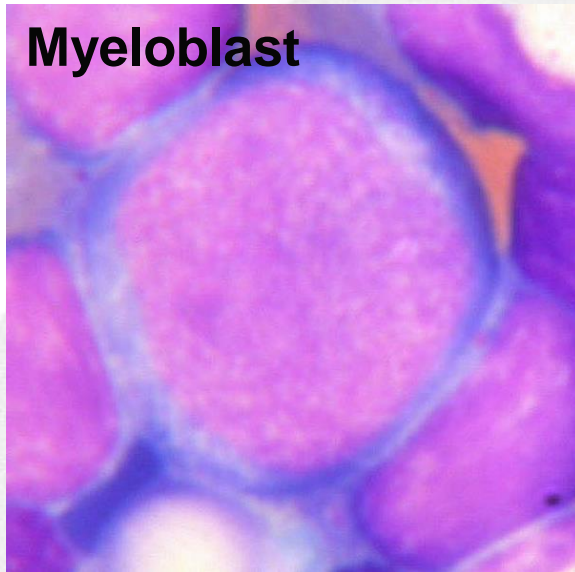
Peripheral blood changes and bone marrow

- **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**

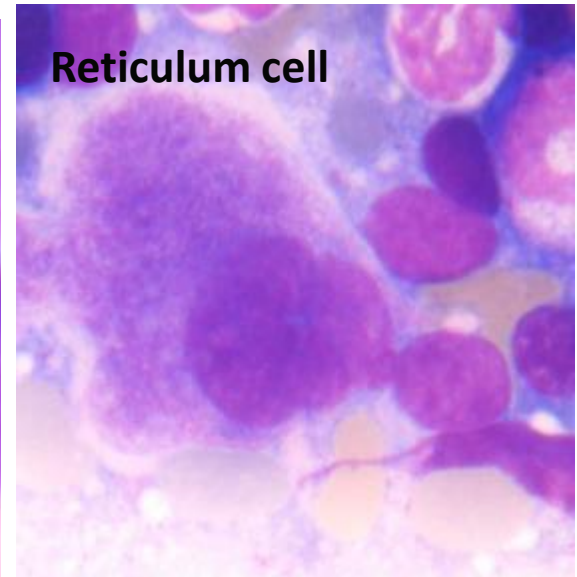
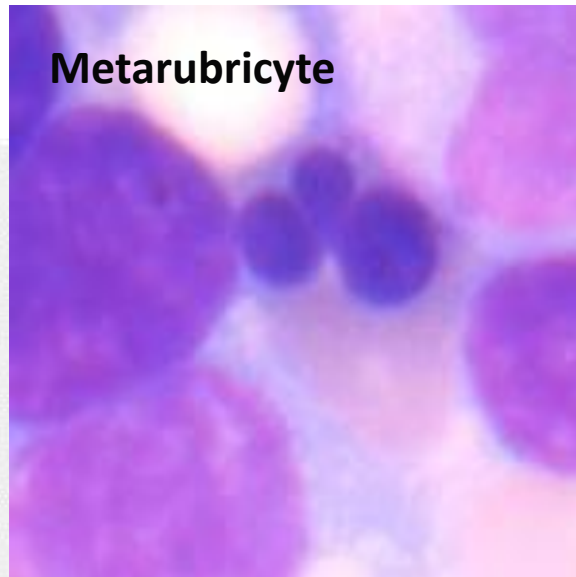
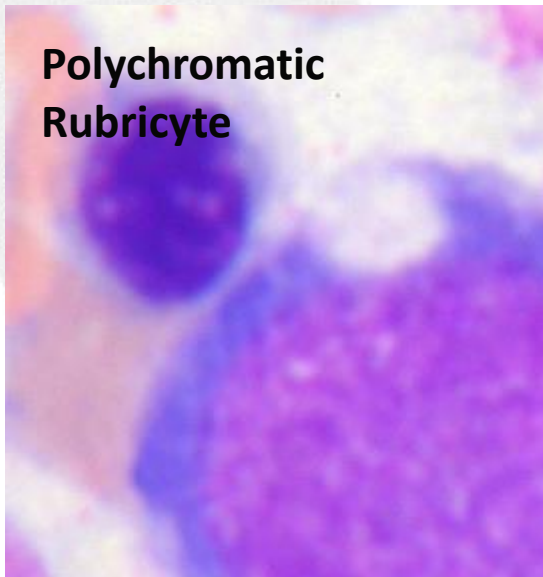
Do or Do Not Perform Bone Marrow Differentiation When:

- **When there are lesions where the mechanism are expected (e.g. corticosteroids)**
- **Only in lower dose groups to establish effects on M:E ratios (e.g. anthracyclins)**
- **Only in groups that are free from any other histological lesion and hematology parameter**
- **In recovery groups, when effects are considered to be irreversible or at least long lasting effects (e.g. platinum compounds)**
- **If the type of anemia is not understood (e.g. LPS)**
- **Special subpopulations or cells e.g. megakarocytes (e.g. inducers of thrombocytopenia)**
- **To establish efficacy (e.g. GCSF, EPO etc.)**

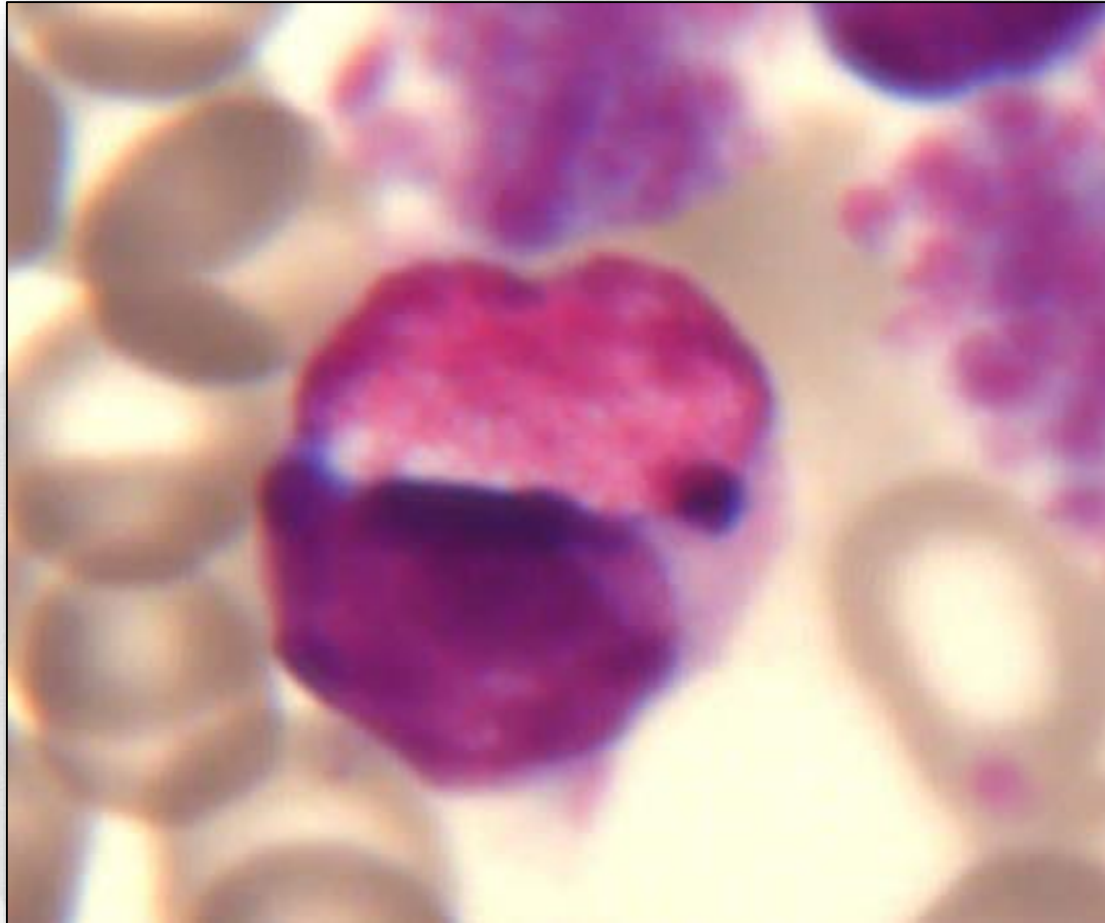
Overview – Granulocytic Lineages, Examples



Overview – Erythroid Lineages, Examples

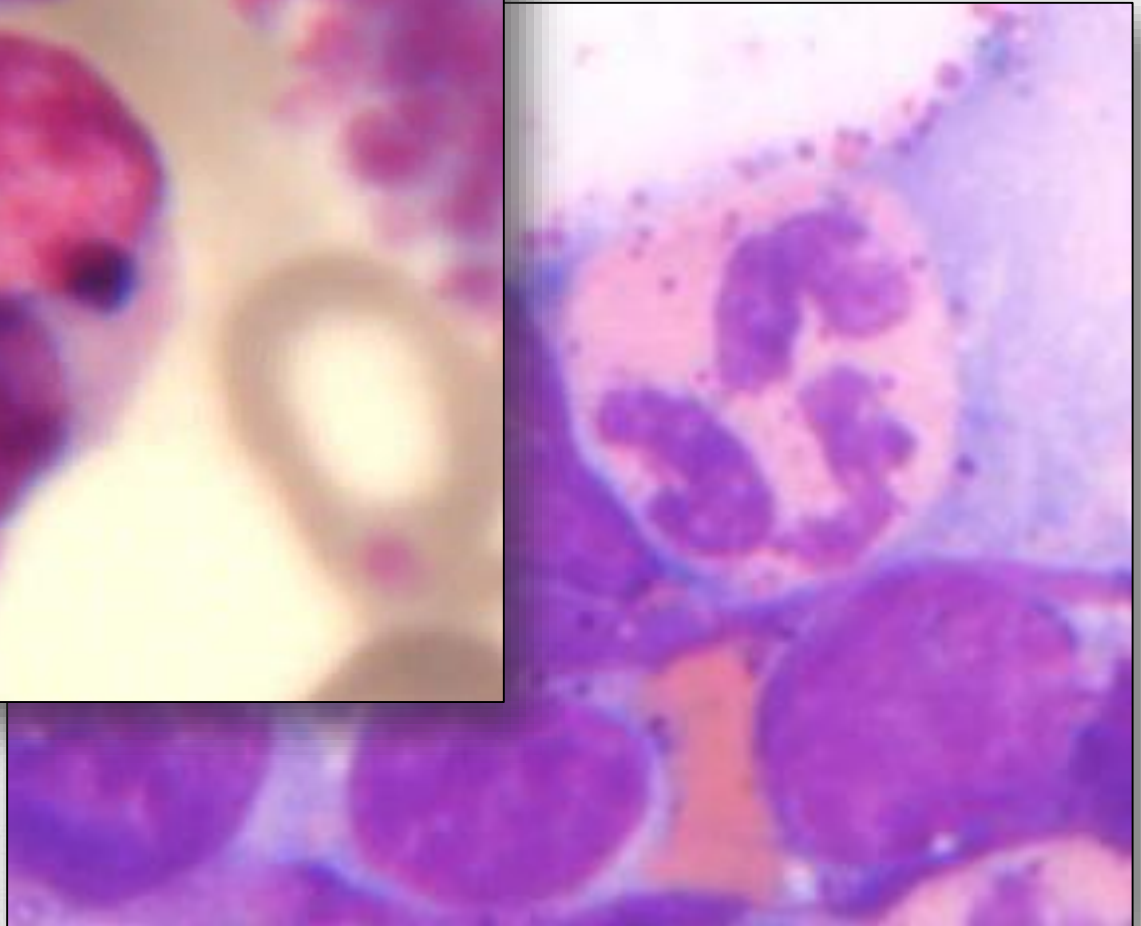


Special Cells in Certain Species

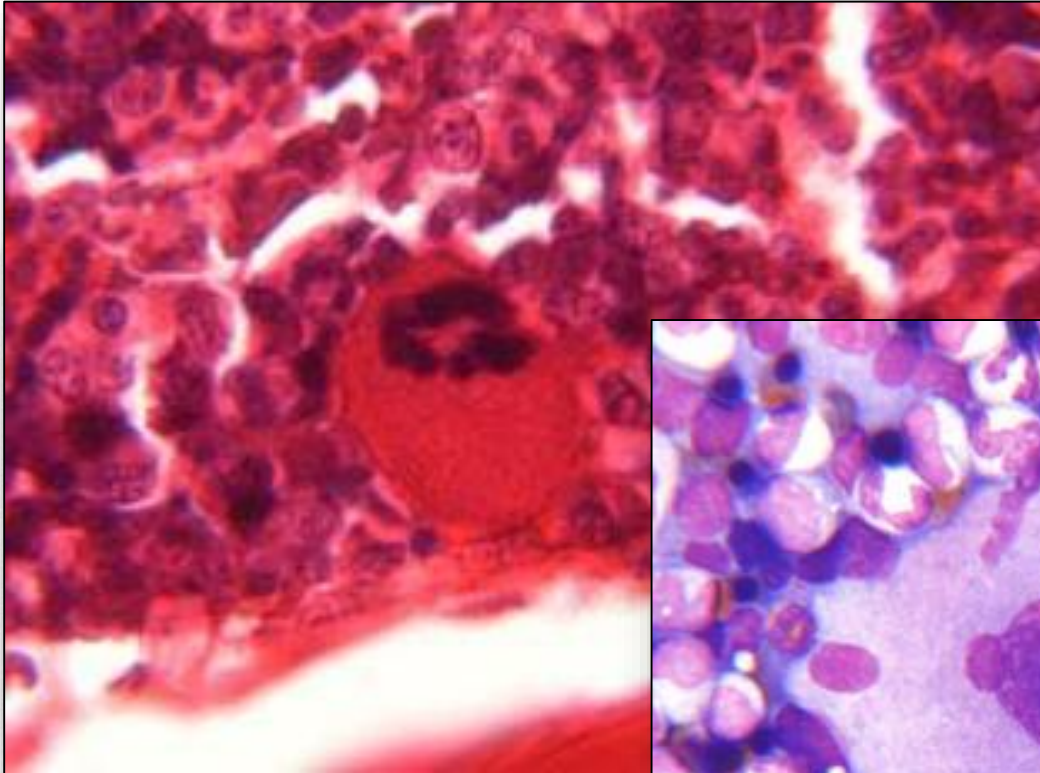


**Foa-Kurloff cells,
Guinea pig etc.**

**Pelgerts Anomaly,
Rabbit**

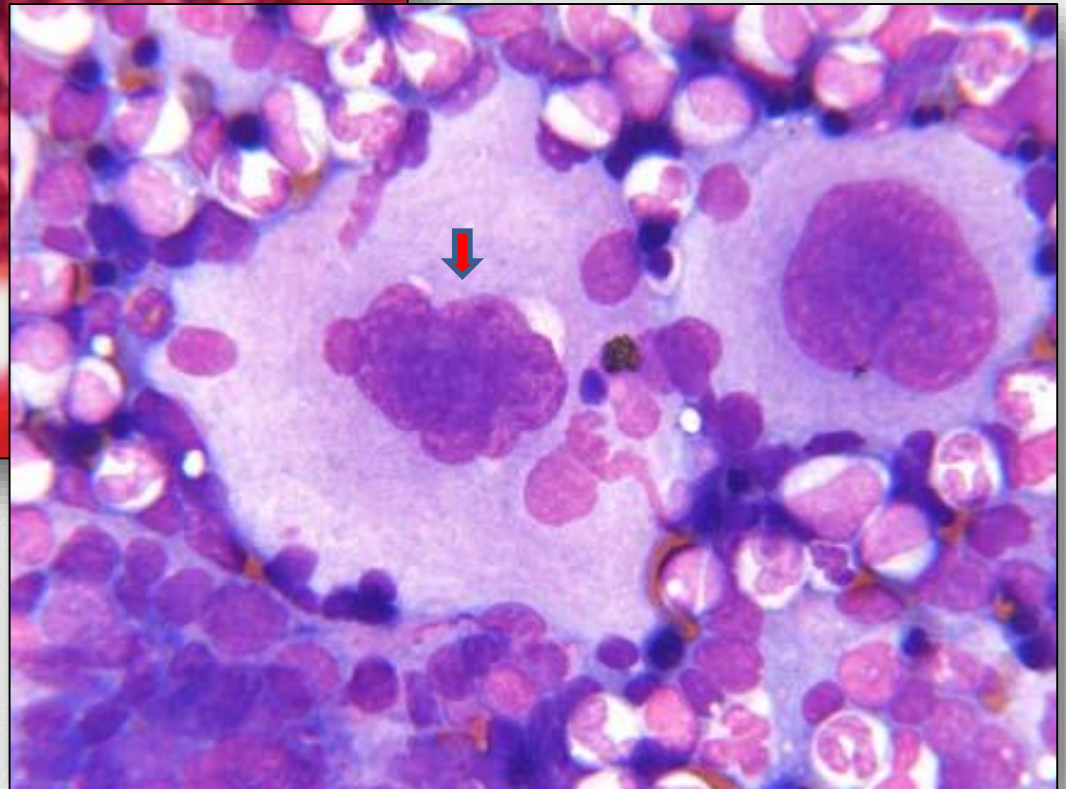


Bone Marrow: Thrombocytopenia (Propofol)

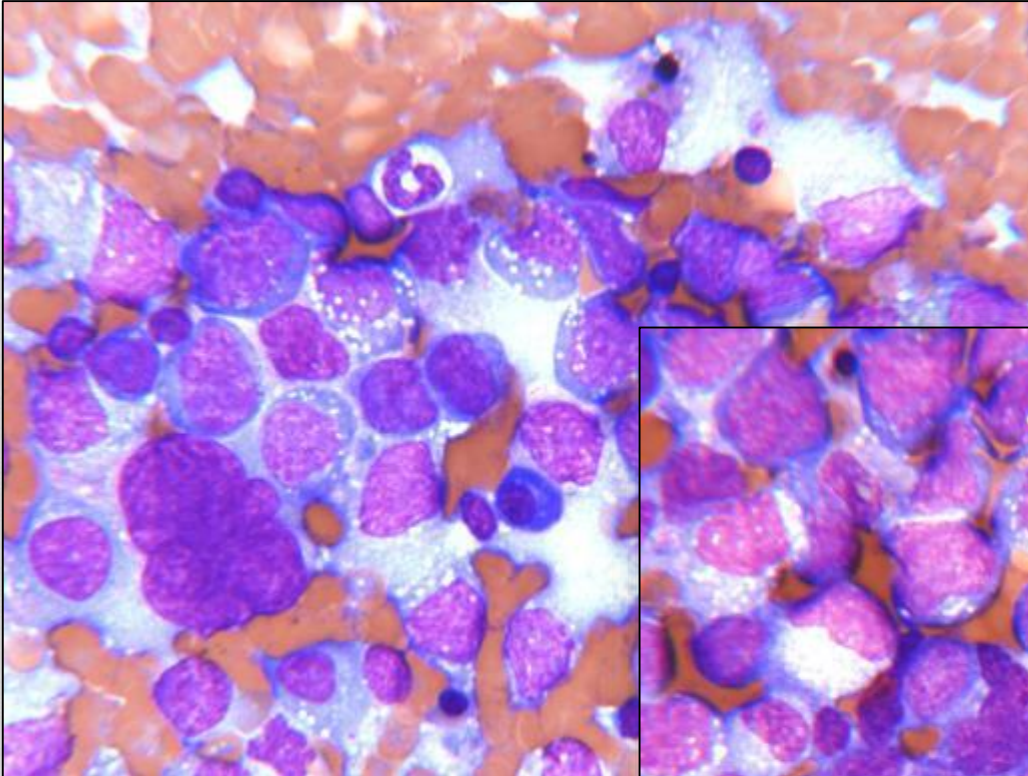


**Dysthrombopoiesis,
Section (H&E): note
abnormal shape of
megakaryocyte**

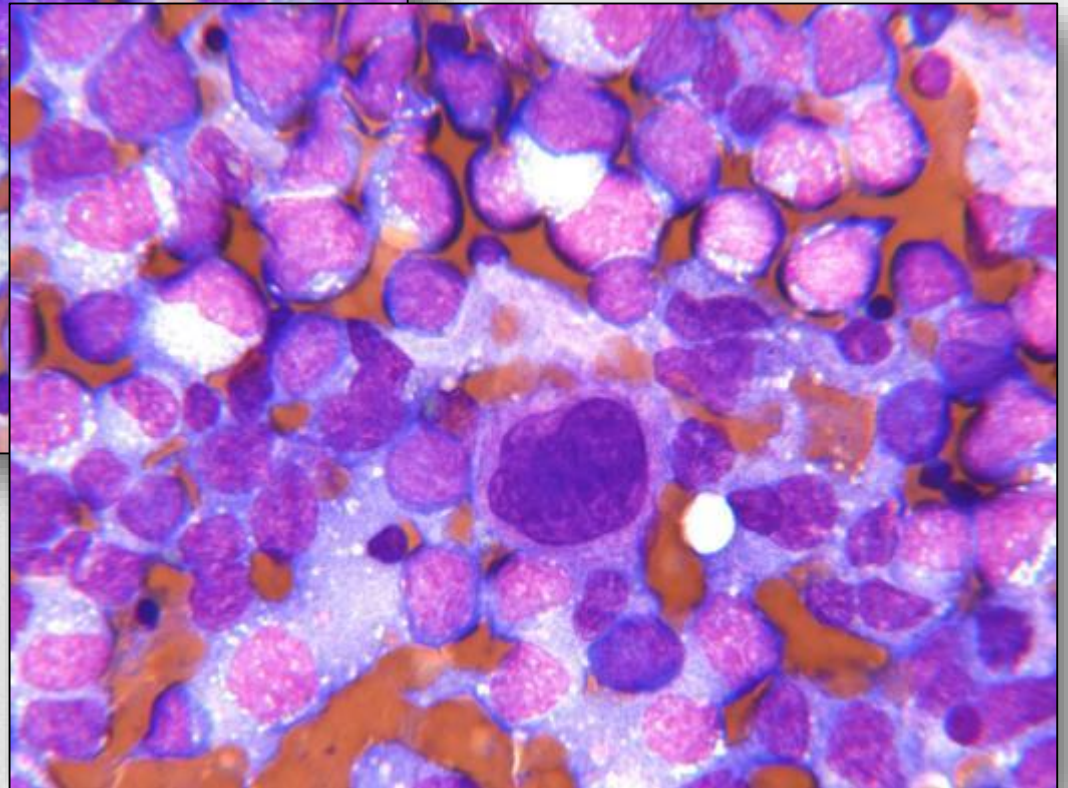
**Dysthrombopoiesis
Smear: note cluster
formation and small
megakaryocytes**



Bone Marrow: Impaired Maturation (Anti-Cancer)

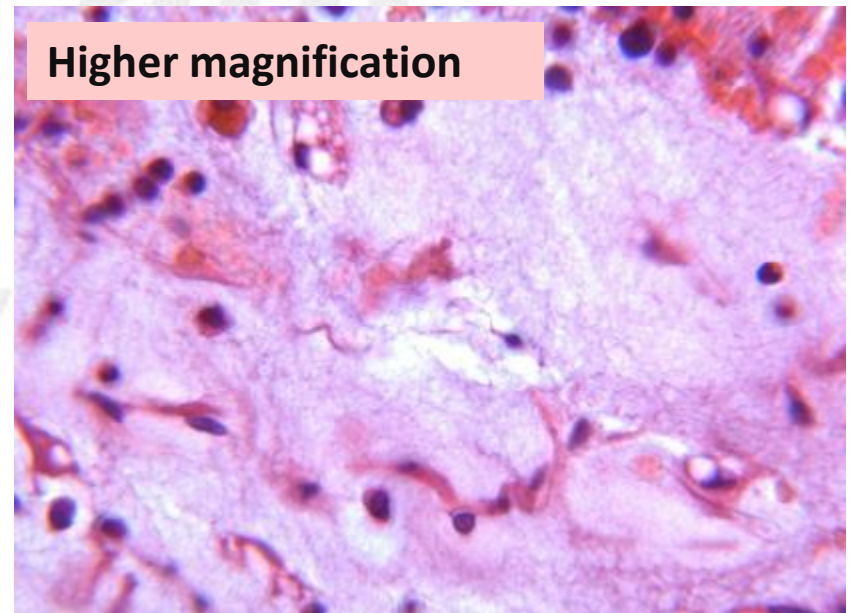


**Granulocytic lineages
blocked at status:
Myelocyte,
Metamyelocyte**

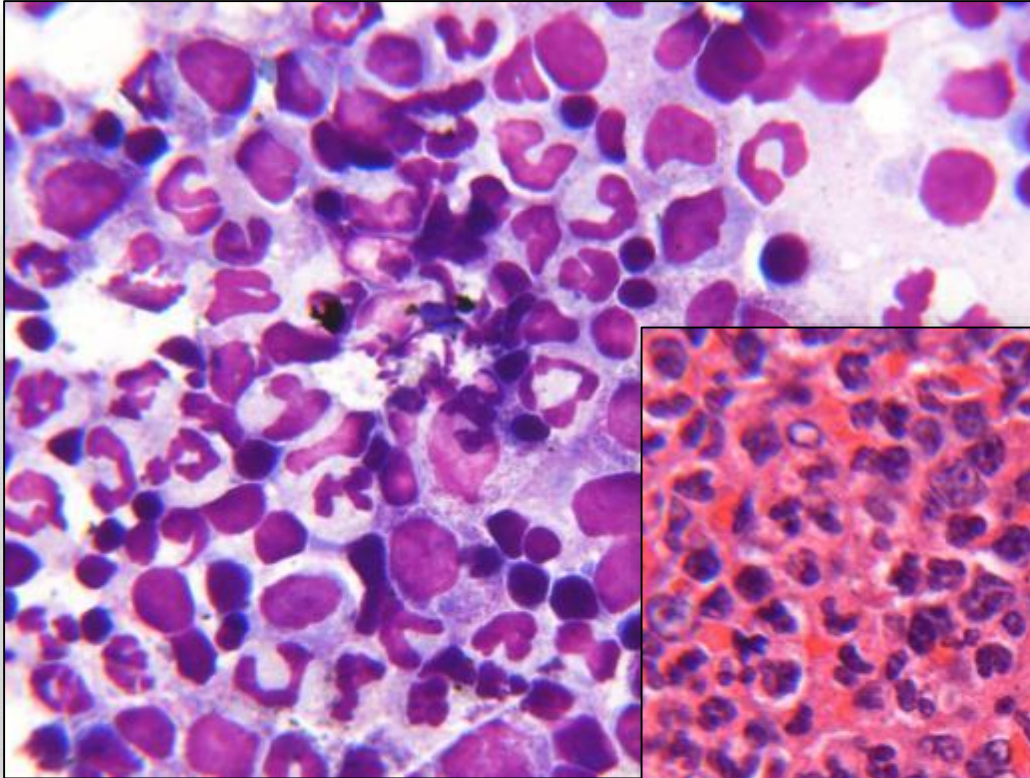


Bone Marrow : Extreme atrophy/degeneration: PPAR agonists

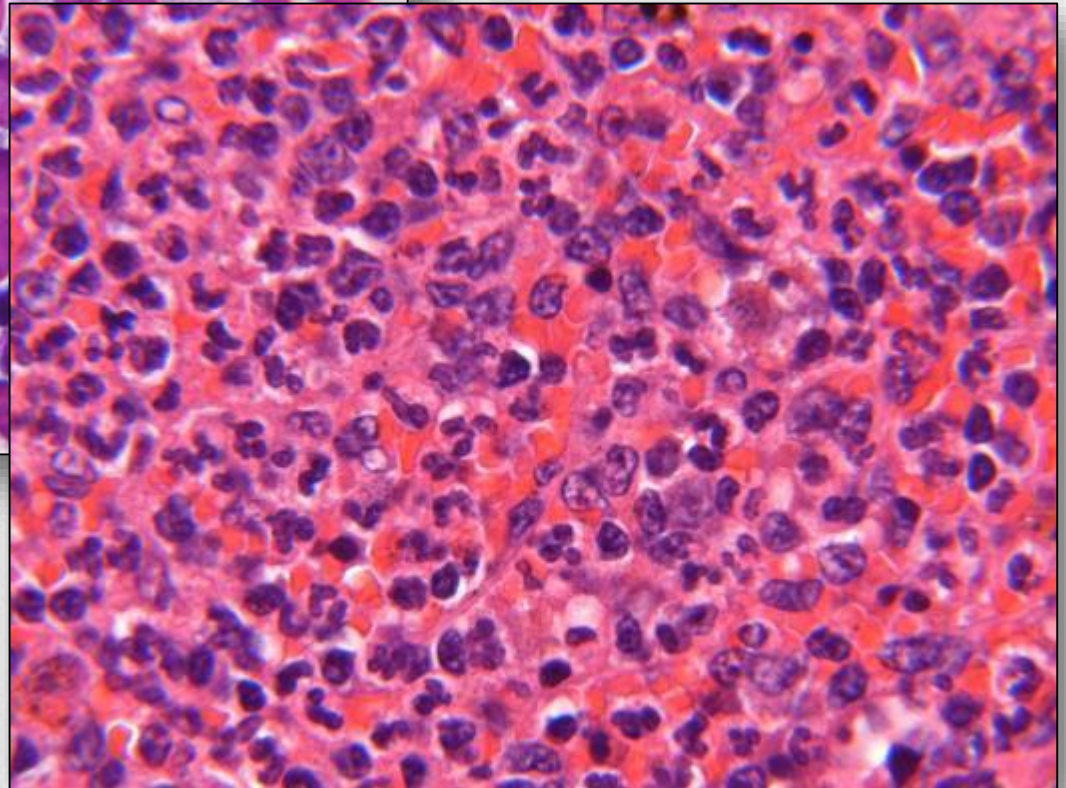
- **Dogs**
- **High dose animals with gelatinous degeneration of bone marrow**
- **Finally additional groups treated with less than 1 mg/kg bw revealed no differences in bone marrow differentiation compared to controls**



Bone Marrow : Changes in Cell populations (Colony Stimulating Factors)



Cynomolgus:
Not extreme increase in
Granulopoiesis.





Enhanced Histopathology of Lymphoid Organs

Best Practice: STP

STP Position Paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System

Thymus	Spleen	Lymph node	Bone marrow
Cortex	White pulp <ul style="list-style-type: none"> • PALS • Lymphoid follicles • Germinal centers 	Cortex <ul style="list-style-type: none"> • Subcapsular sinus • Follicles • Germinal centers • High endothelial venules 	Erythroid component
Medulla		Paracortex	Granulocytic component
Cortex-medulla ratio ²		Medulla <ul style="list-style-type: none"> • Medullary cords • Medullary sinuses 	Fat Lymphoid component
	Marginal zone Red pulp		Stroma Megakaryocyte Other cells

Best Practice: STP

Lymphocytes: increased/decreased

Granulocytes: increased/decreased

Mast cells: increased/decreased

Megakaryocytes: increased/decreased

Tingible-body macrophages

Pigmented macrophages

Vacuolated macrophages

Plasma cells: increased/decreased

Fat necrosis

**Inflammation; specify type as appropriate i.e.,
granulomatous**

Sinus erythrocytosis; designate sinus

Sinus histiocytosis; designate sinus

Hemorrhage

Necrotic cells; designate cell type if possible

Infarct

Erythroid component: increased/decreased

Granulocytic component: increased/decreased

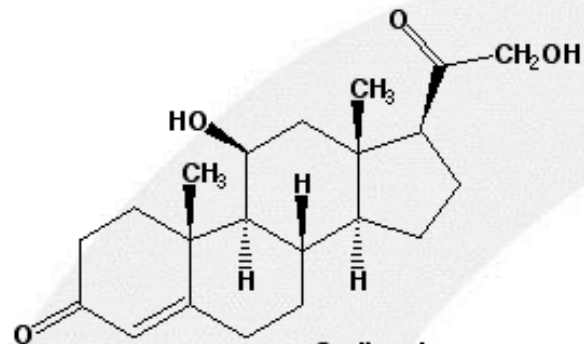


**Example:
Inhalative Glucocorticoids**

Synthesis, Behaviour

- **Steroid hormones synthesized from cholesterol within adrenal cortex**
- **11 β -hydroxylase for corticosterone**
- **Almost pure glucocorticoids as dexamethasone**
- **Compounds with combined effects of mineralocorticoid and glucocorticoid action as prednisone**

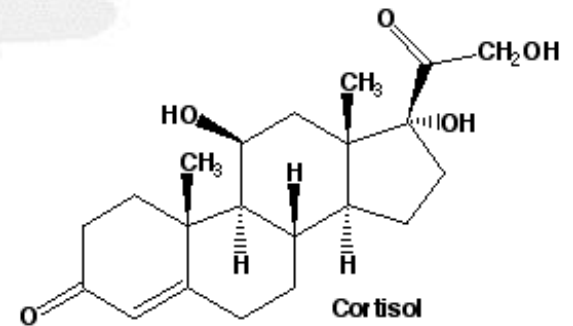
Structures



Corticosteron

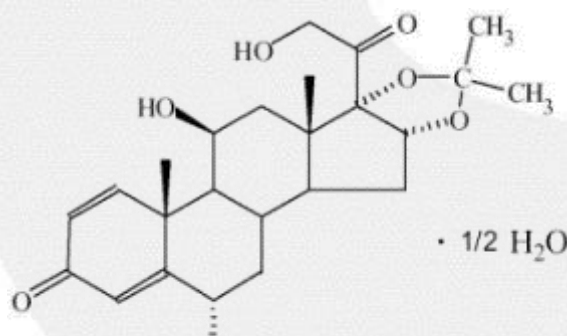
-4-pregnene-3,20-dione

Natural Products



Cortisol

11 β ,17 α ,21-trihydroxypregna-4-ene-3,20-dione (hydrocortisone)

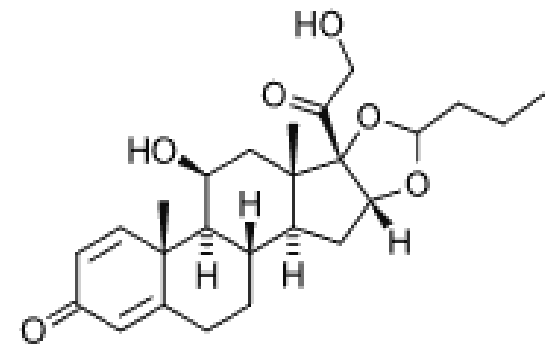


$\cdot \frac{1}{2} \text{H}_2\text{O}$

Flunisolide Hemihydrate

=6 α -Fluoro-11 β , 16 α , 17, 21- tetrahydroxylpregna-1,4-diene-3, 20-dione cyclic-16, 17-acetal

Synthetic Products



Budesonide

(provided as the mixture of two epimers 11-beta,16-alpha)-16,17- (Butyldiene-bis(oxy))-11,21-dihydroxy-pregna-1,4-diene-3, 20-dione

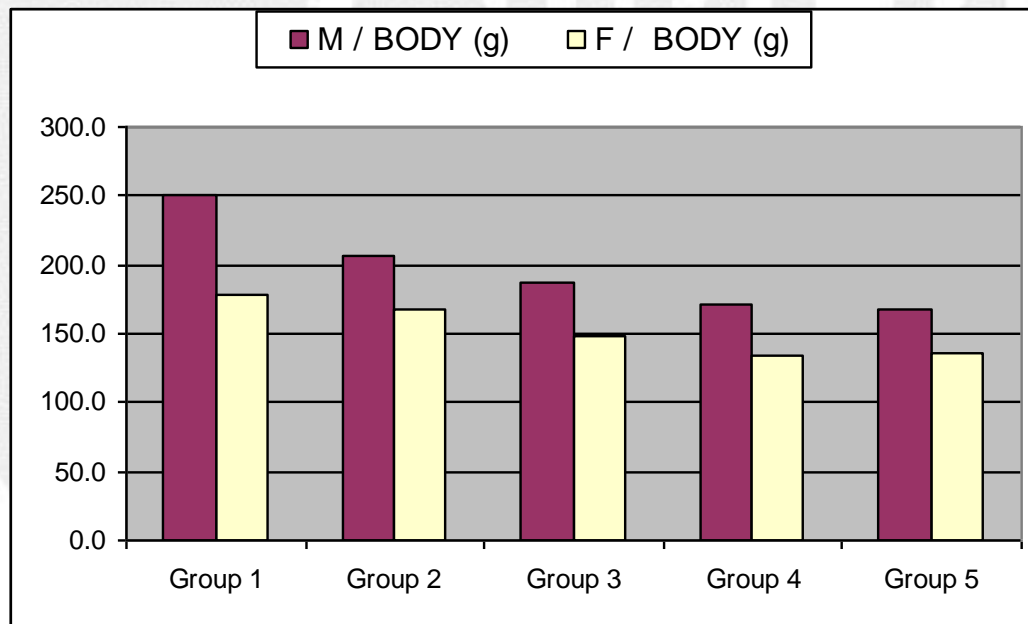
Study Designs: 7 Days/28 Days

Group 1	Group 2	Group 3	Group 4	Group 5
HFA vehicle	CORTICO- STEROID_I/HFA 0.1, mg/kg	CORTICO- STEROID_I/HFA 0.3 mg/kg	CORTICO- STEROID_I/HFA 0.9 mg/kg	CORTICO- STEROID_II SPRAY/CFC 0.9 mg/kg
5 Animals per Sex	5 Animals per Sex	5 Animals per Sex	5 Animals per Sex	5 Animals per Sex

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Air alone	HFA vehicle	CORTIC. _I/HFA 0.03 mg/kg	CORTIC. _I/HFA 0.06 mg/kg	CORTIC. _I/HFA 0.12 mg/kg	CORTIC. _II SPRAY/CFC 0.12 mg/kg
Main: 10 Animals per Sex	Main: 10 Animals per Sex Recovery: 5 Animals per Sex	Main: 10 Animals per Sex	Main: 10 Animals per Sex	Main: 10 Animals per Sex Recovery: 5 Animals per Sex	Main: 10 Animals per Sex Recovery: 5 Animals per Sex

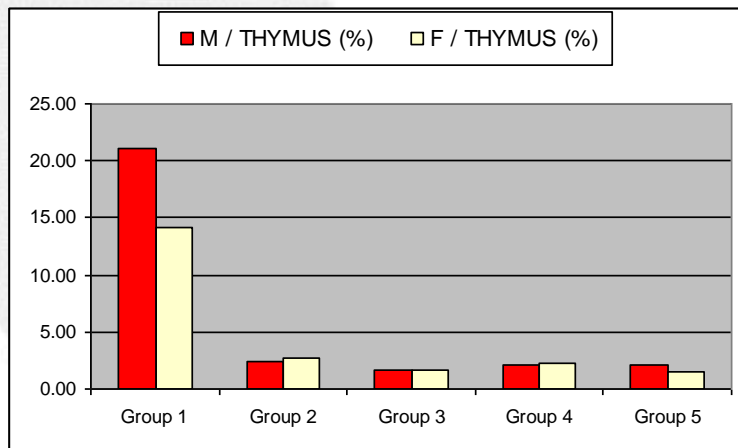
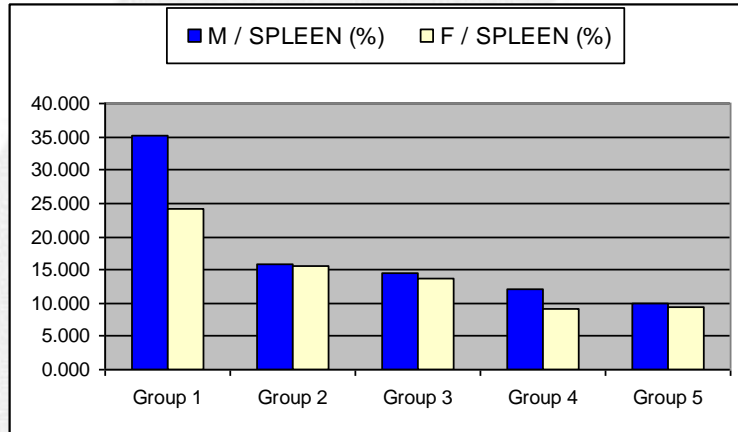
Study Designs: 7 Days

- **Body weight reduced:**
 - 10% in low dose, 25% in high dose groups 4 and 5
- **Food consumption decreased at:**
 - 50% in high dose groups 4 and 5
- **Decedents in groups 4 and 5**

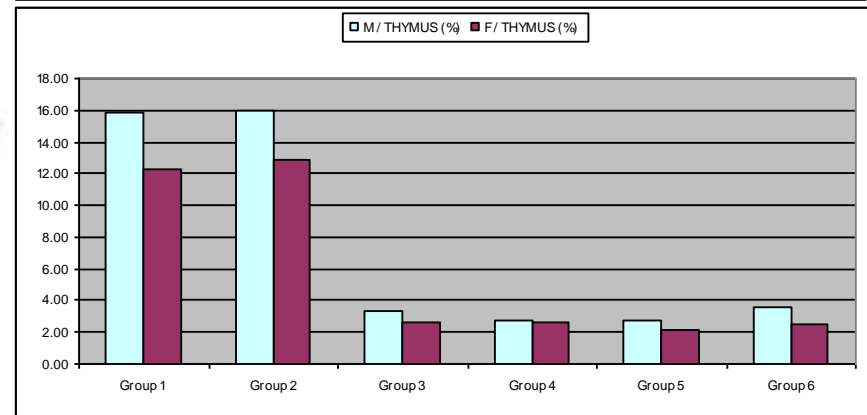
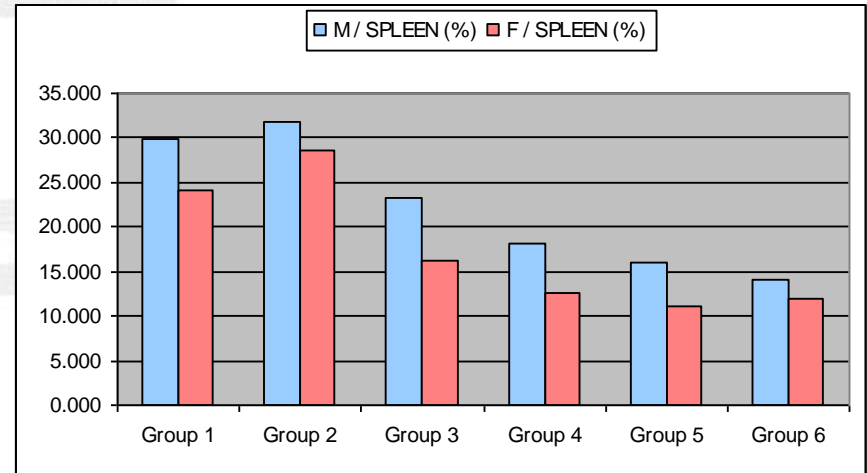


Effects in Lymphatic Organs: 7/28 Days

- Thymus and spleen reduced in size and organ weights.



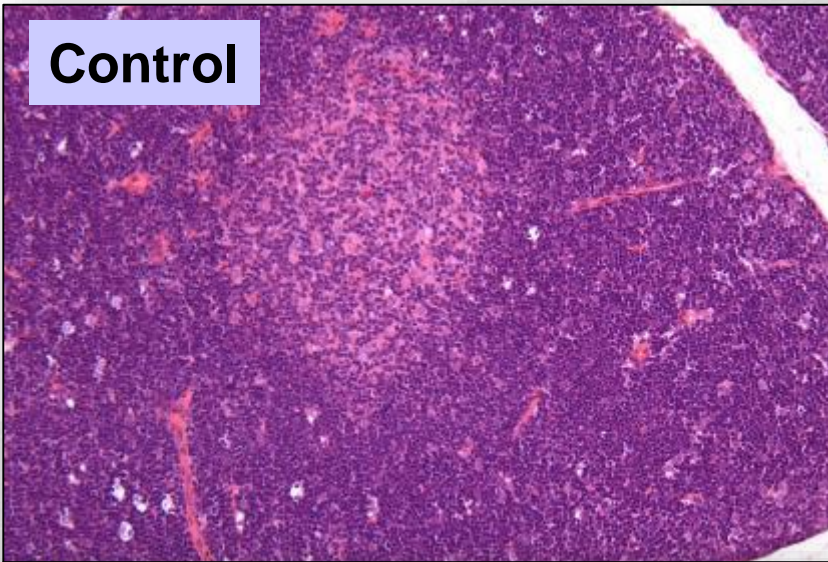
7-Day



28-Day

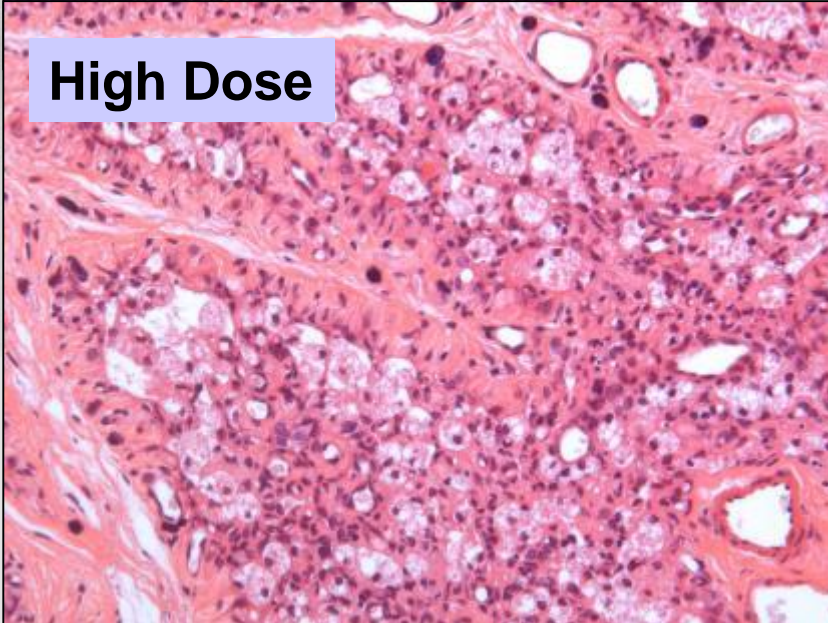
Effects in Thymus: 7/28 Days

Control



- **Cortical and medullary atrophy**
- **Increased histiocytosis**
- **Lymphocytolysis**
- **Lymphocytophagocytosis**
- **Recovery after 14 days!**

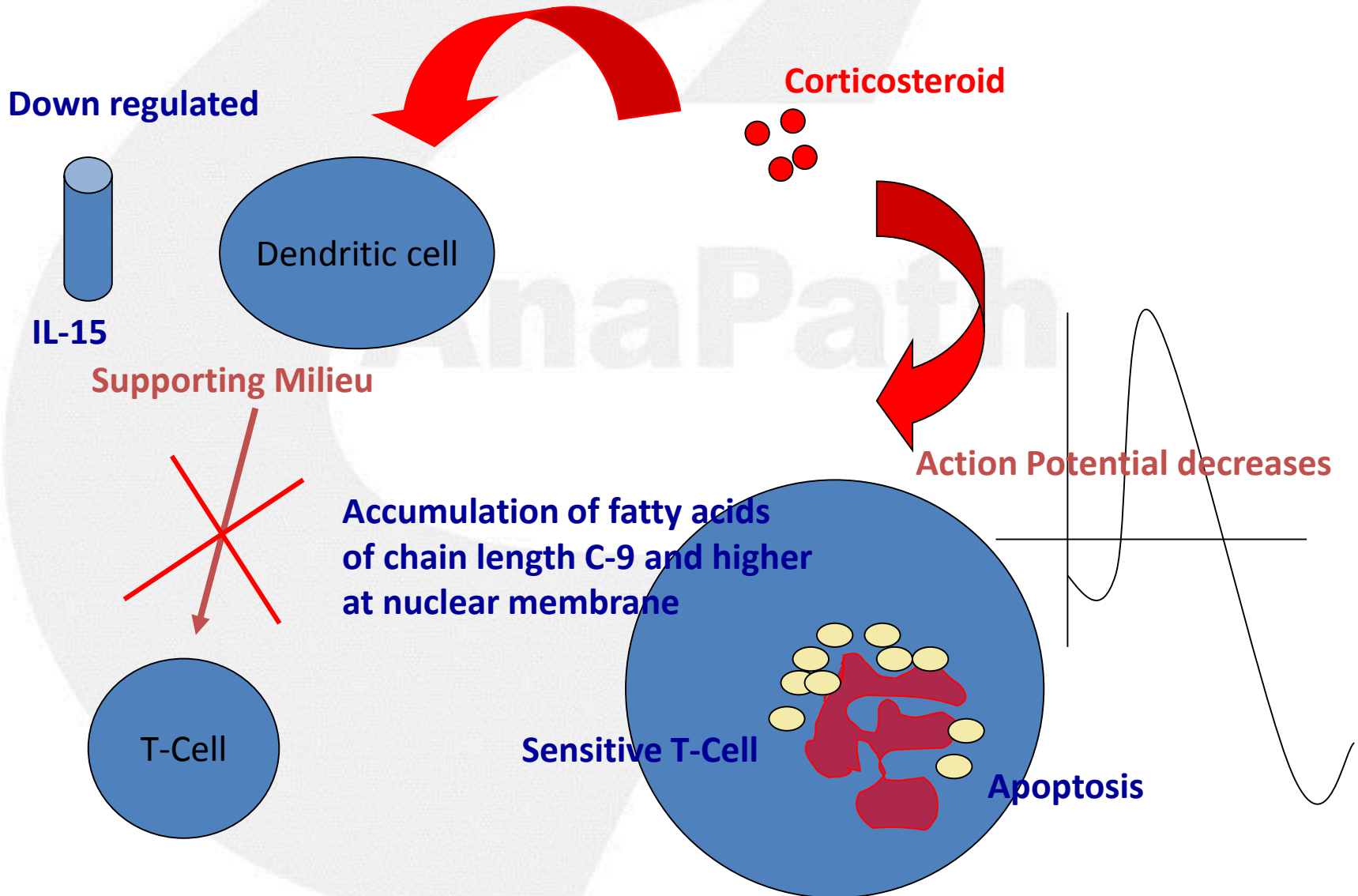
High Dose



High Dose

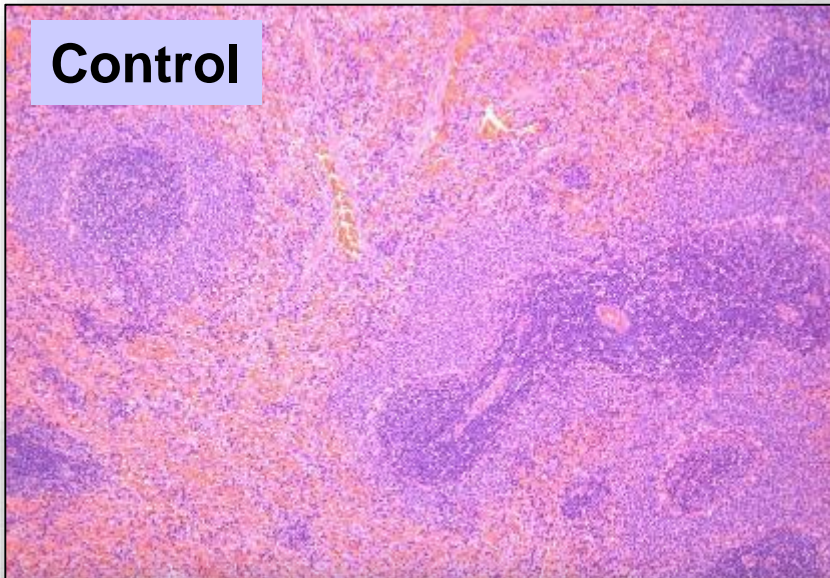


Mechanism



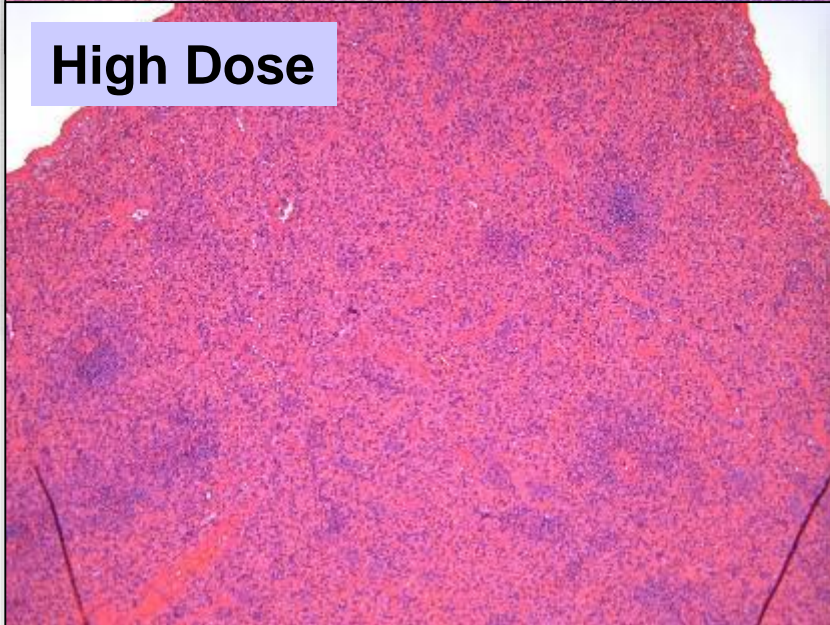
Effects in Spleen: 7/28 Days

Control

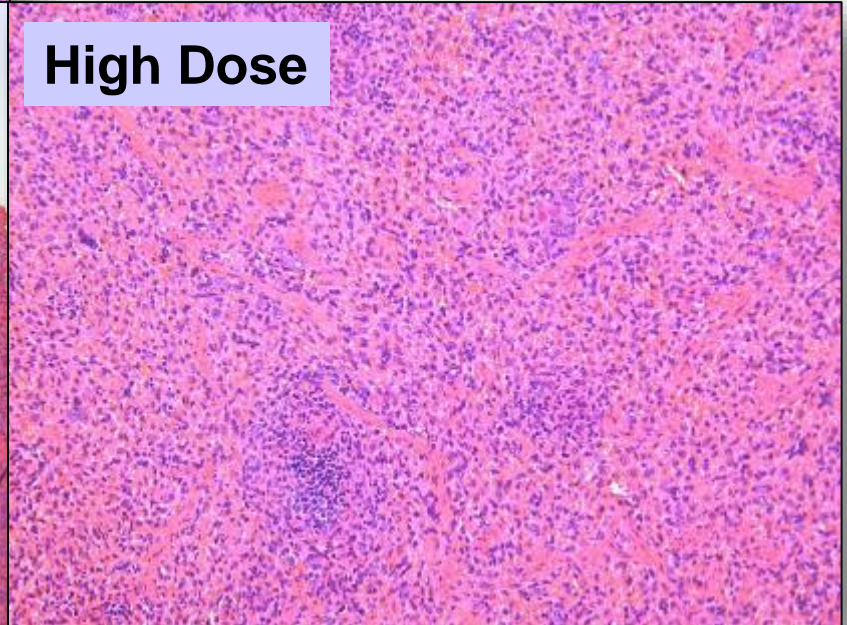


- **Mantle zone and periarteriolar sheets atrophy**
- **Recovery after 14 days!**

High Dose

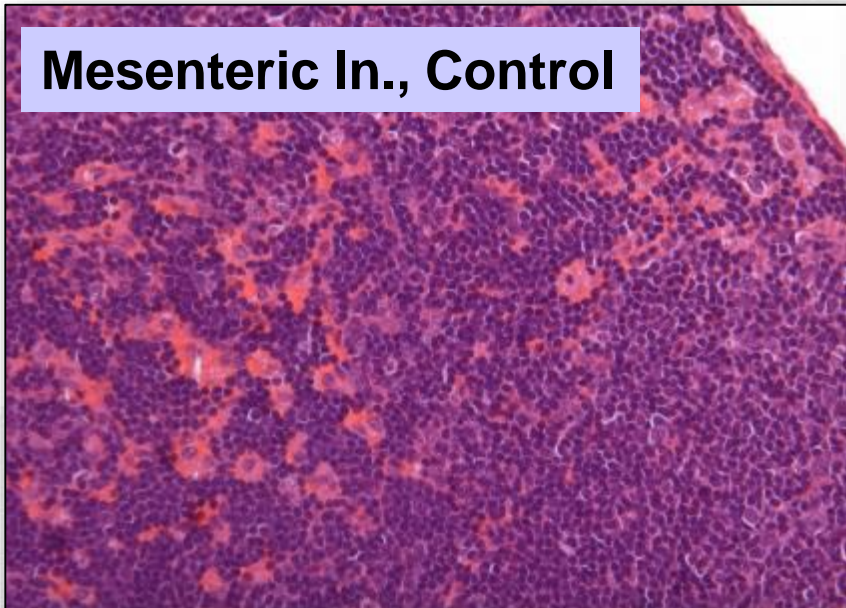


High Dose

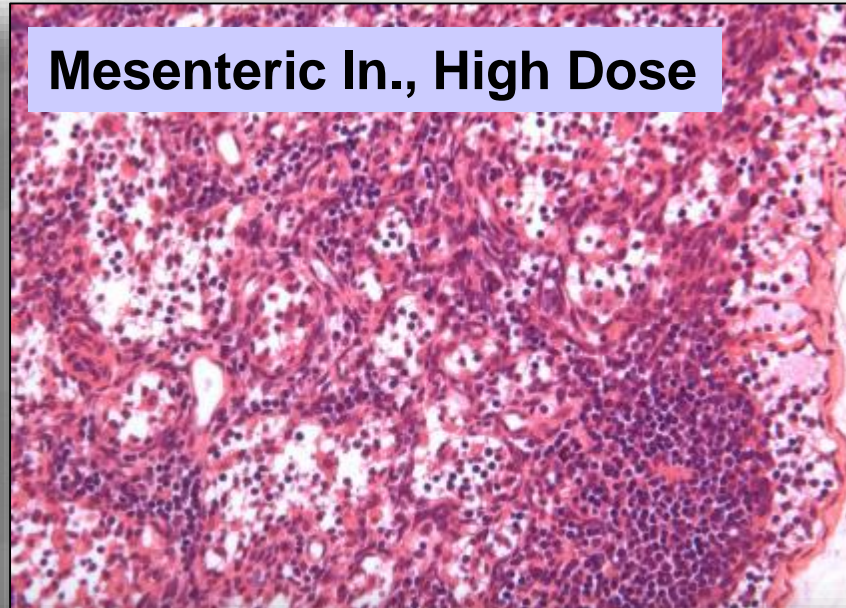


Effects in Lymph Nodes: 7/28 Days

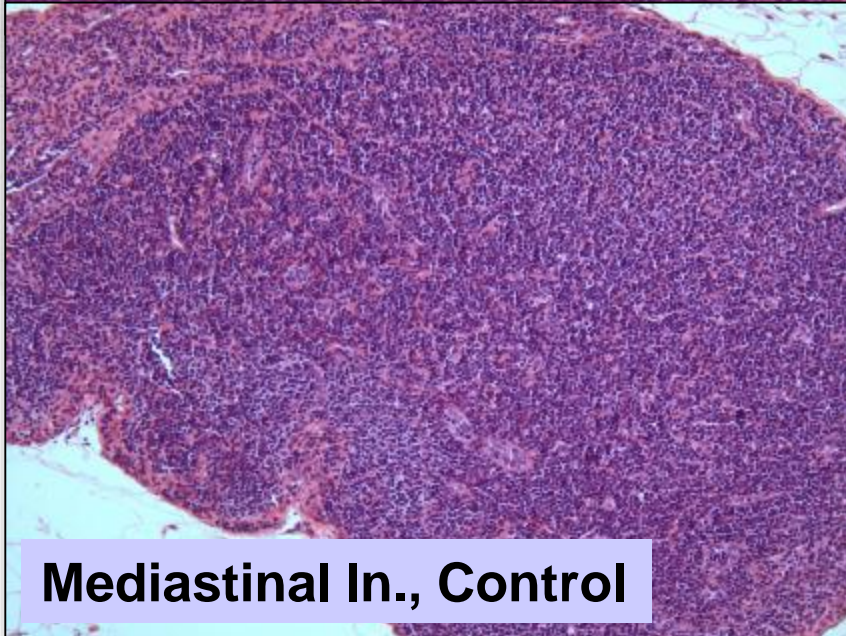
Mesenteric In., Control



Mesenteric In., High Dose



Mediastinal In., Control

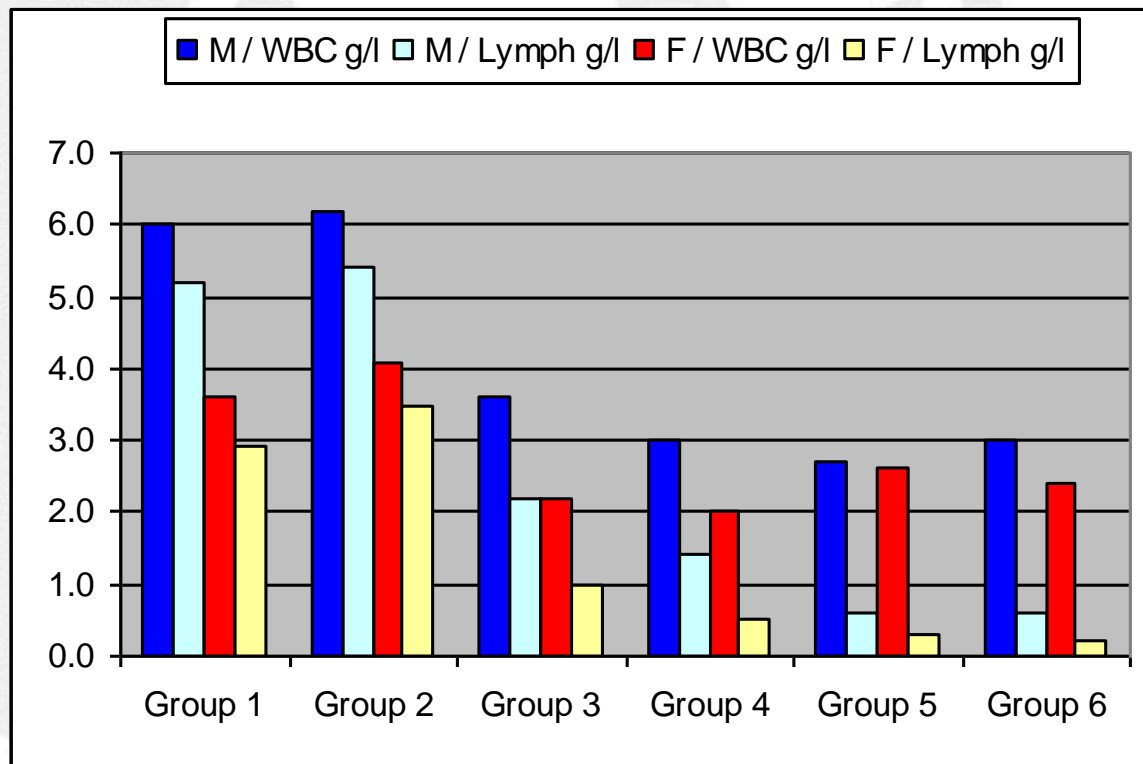


Mediastinal In., High Dose



Hematology: 7/28 Days

- **Decreased WBC in both studies**
- **Mainly lymphocytes were reduced**
- **Segmented granulocytes increased compensatory**



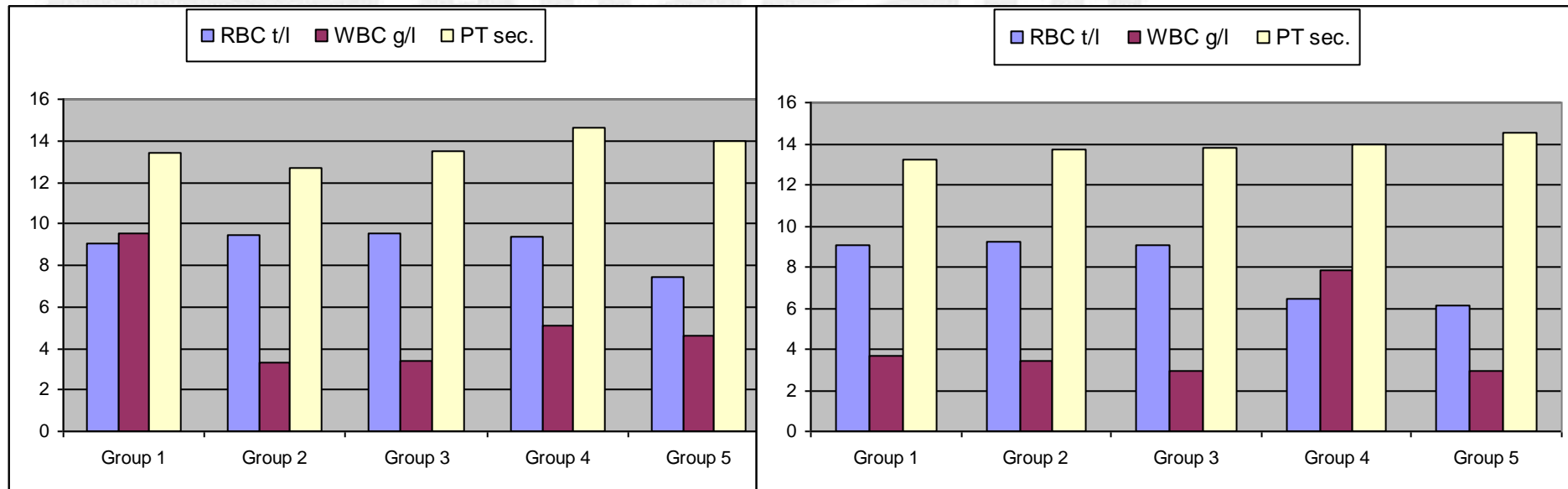
Hematology: 7/28 Days

- Normocytic and normochromic non-regenerative anaemia (reduced RBC, Hb, Hc) in both studies

7-Day Study

- Male

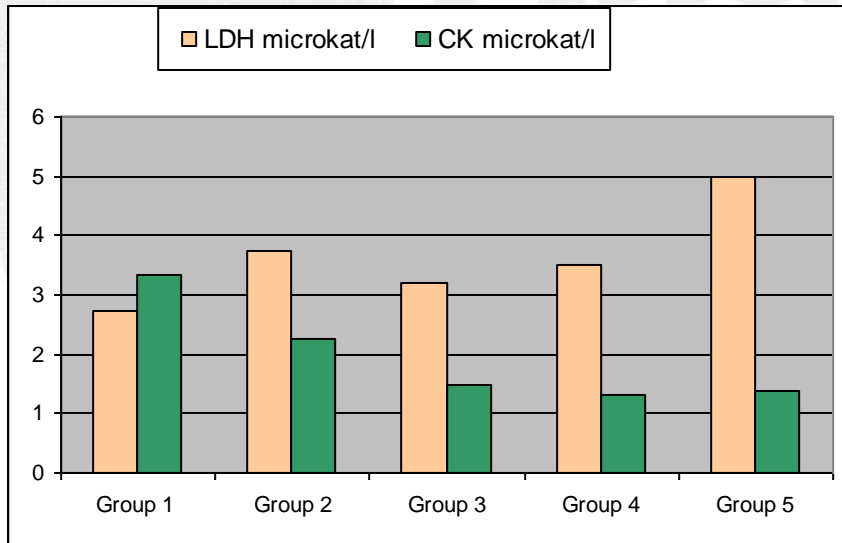
Female



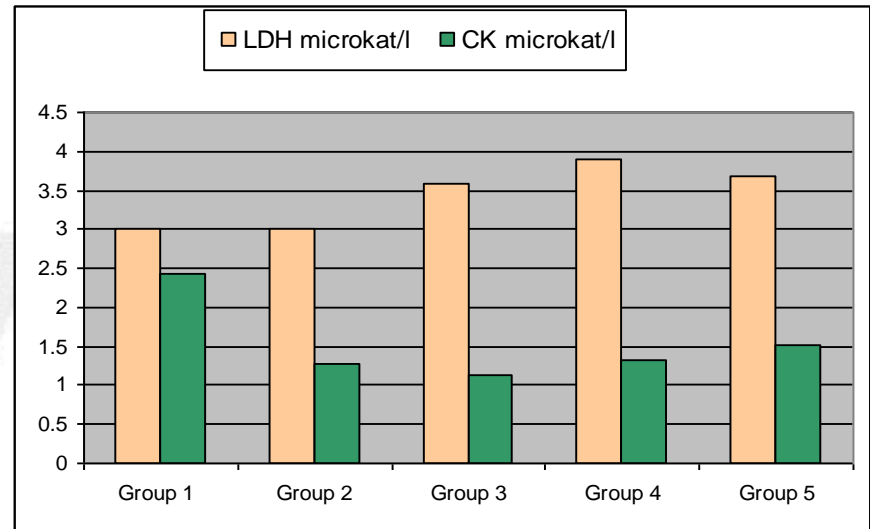
Hematology: 7/28 Days

- Increased LDH (7-day study only) and potassium levels (hemolysis), and total bilirubin in both studies

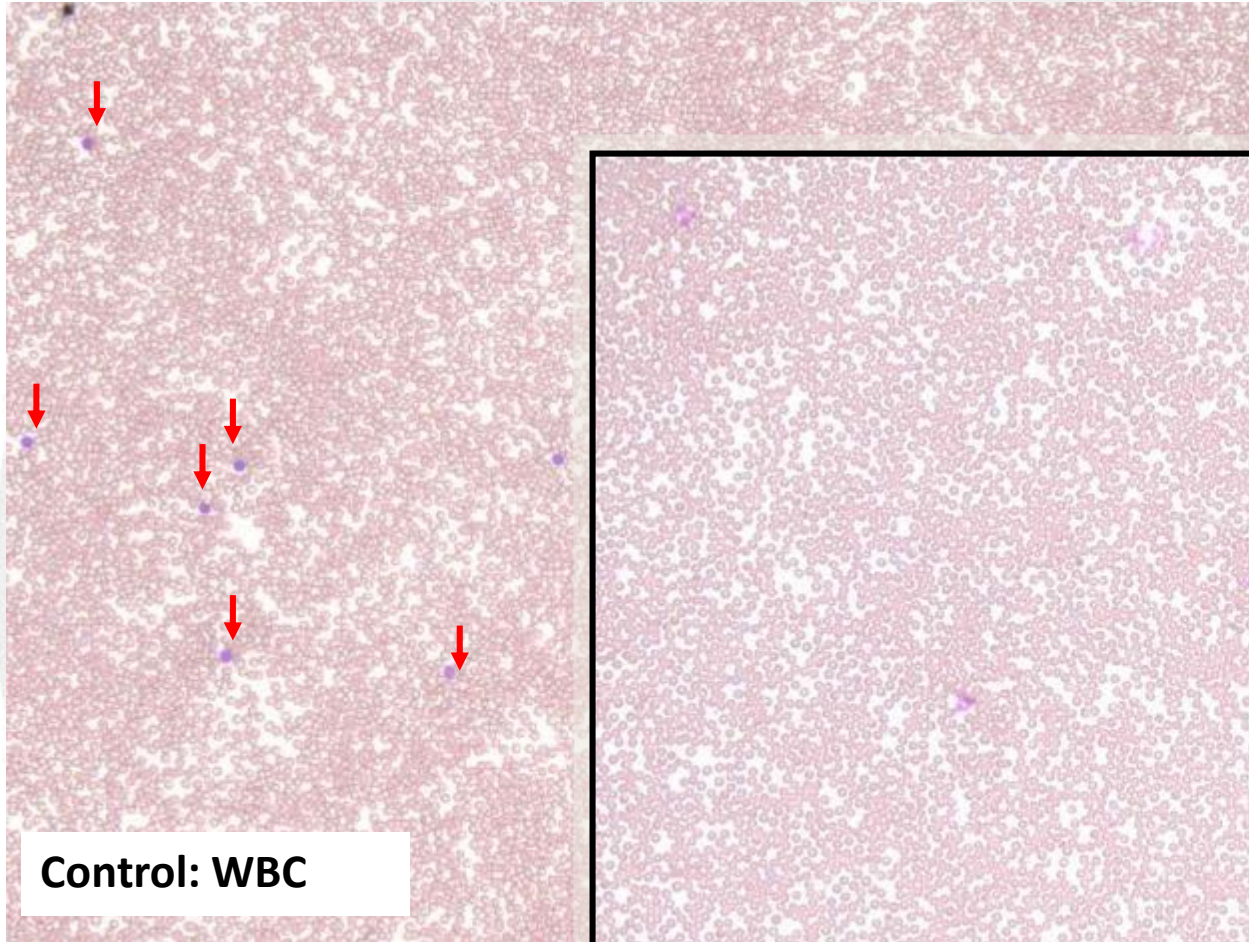
- **Males**



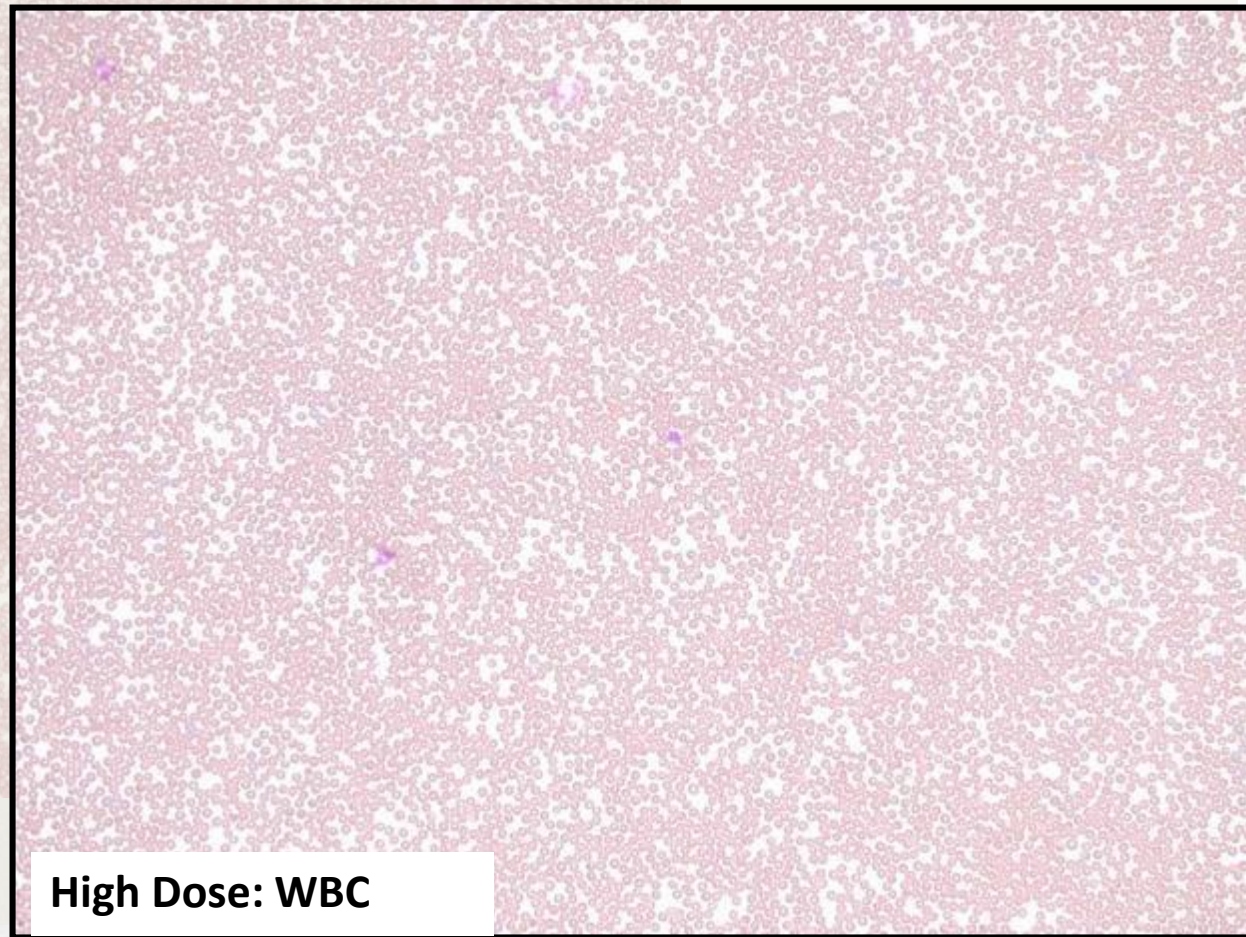
- **Females**



Peripheral Blood: WBC

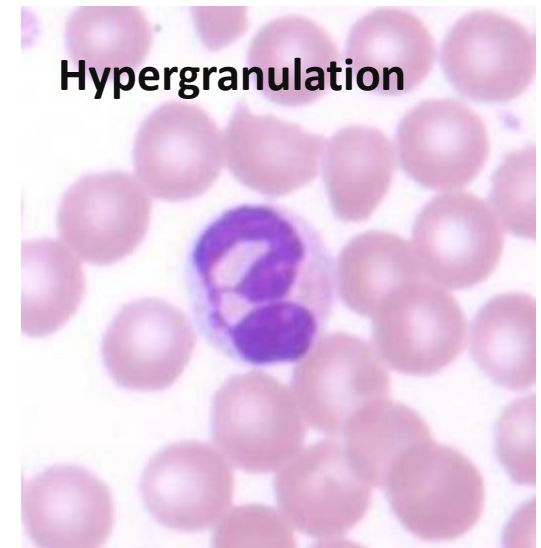
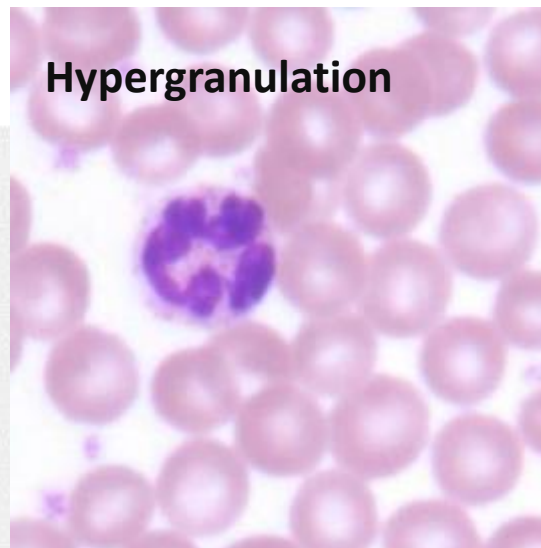
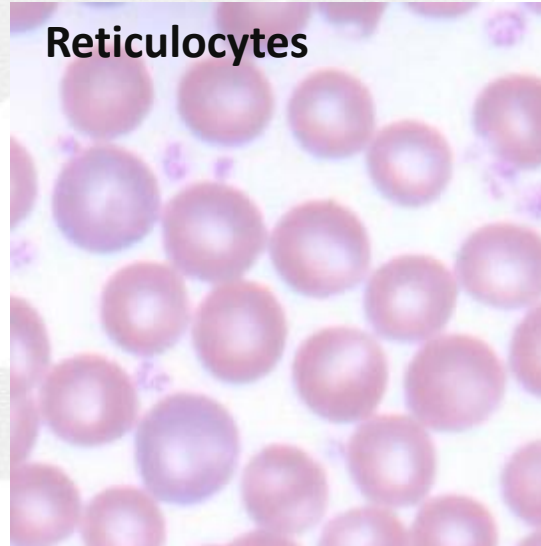
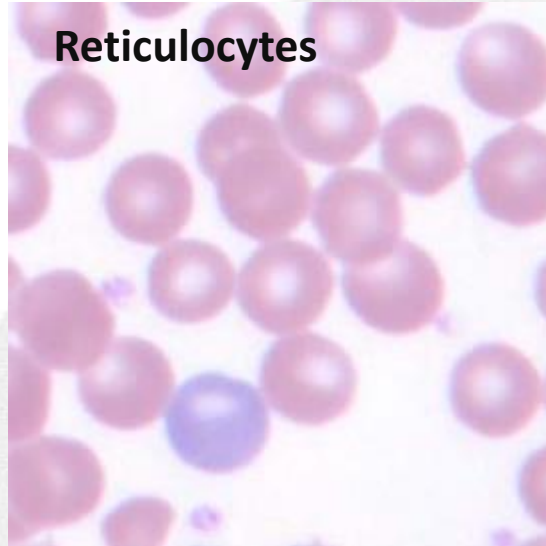


Control: WBC



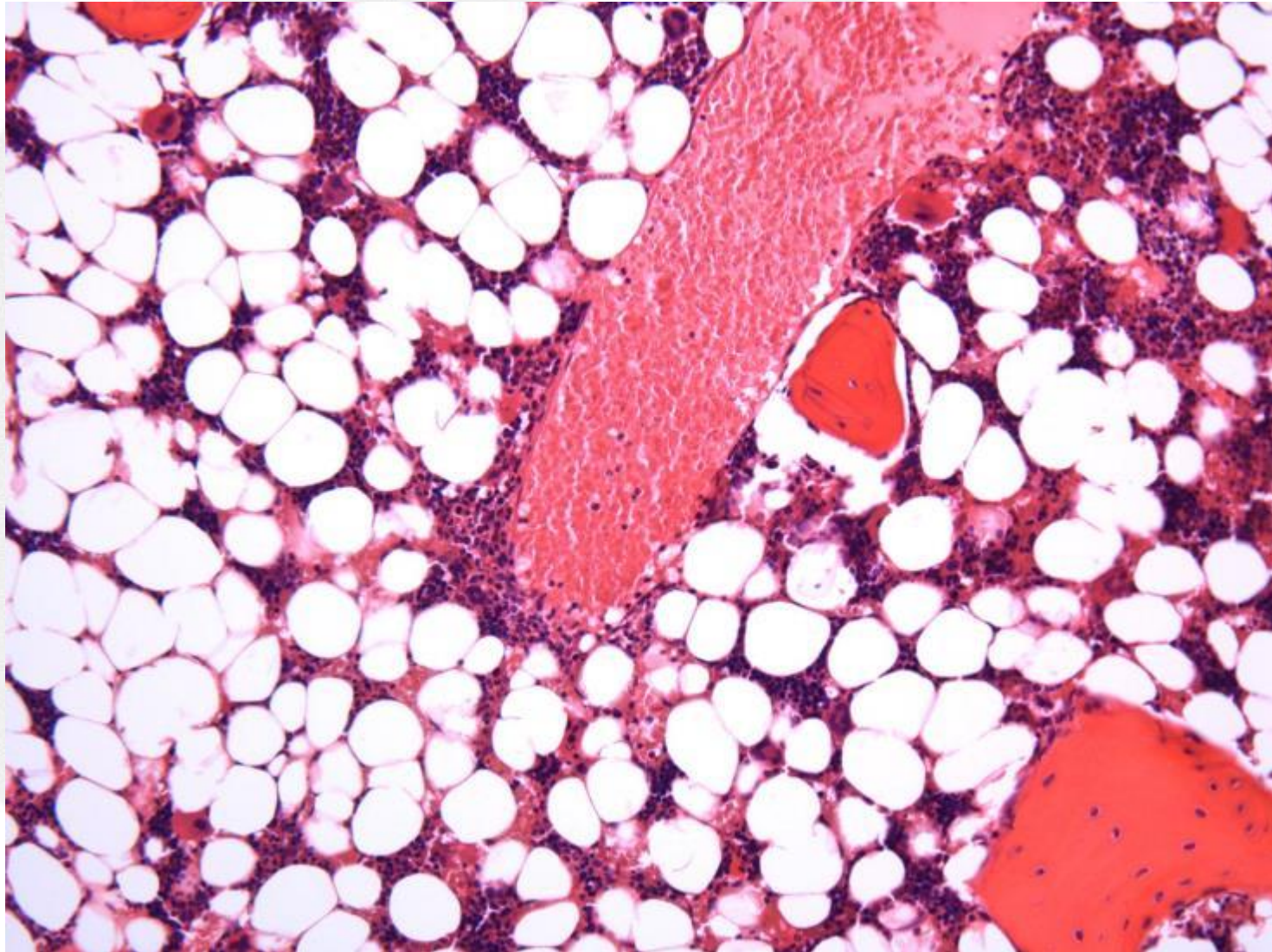
High Dose: WBC

Peripheral Blood: Findings



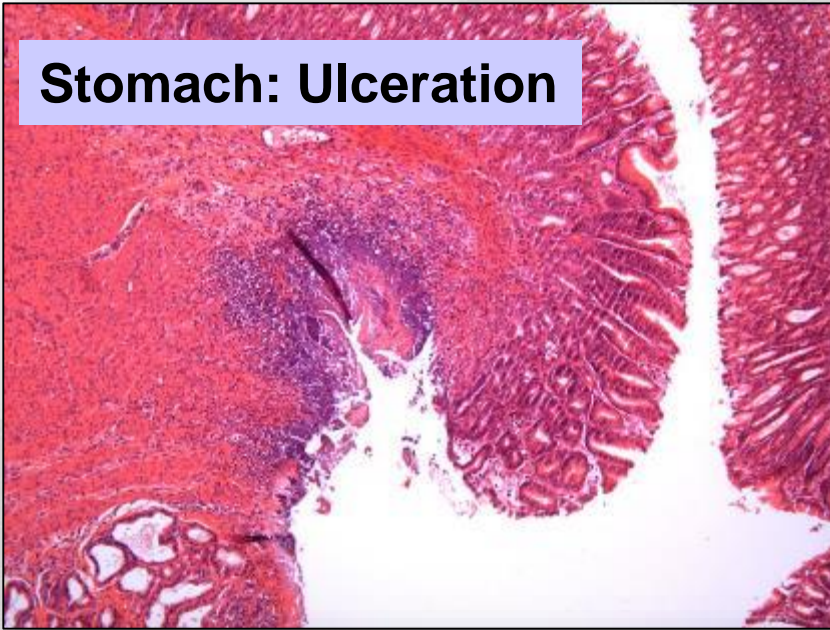
Peripheral Blood: Findings

- **Fatty replacement in 28-Day study (only)**

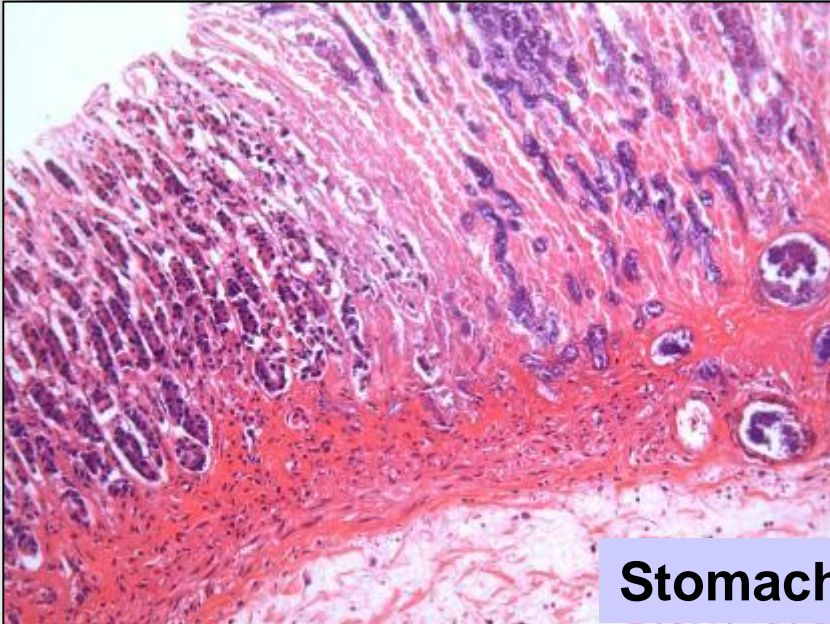
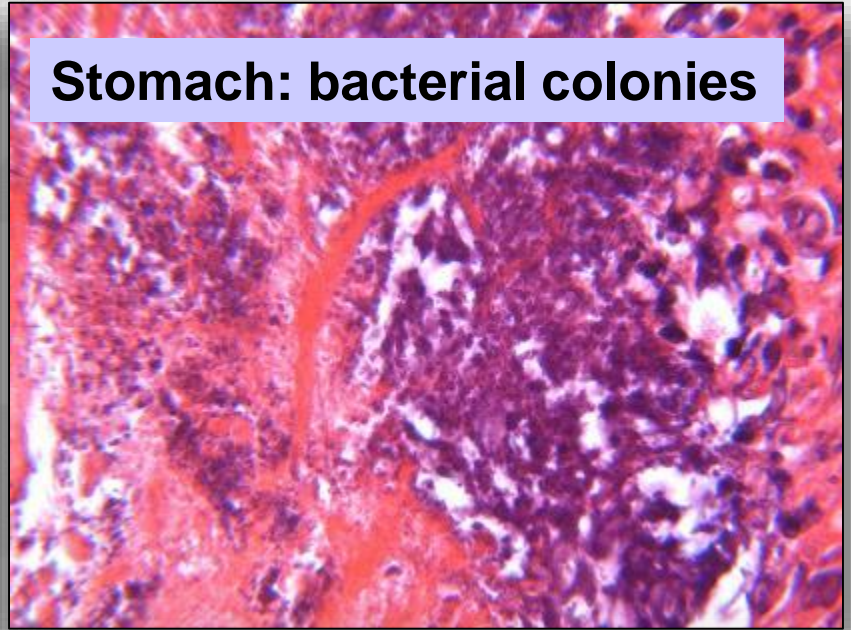


Gastrointestinal Tract: Findings

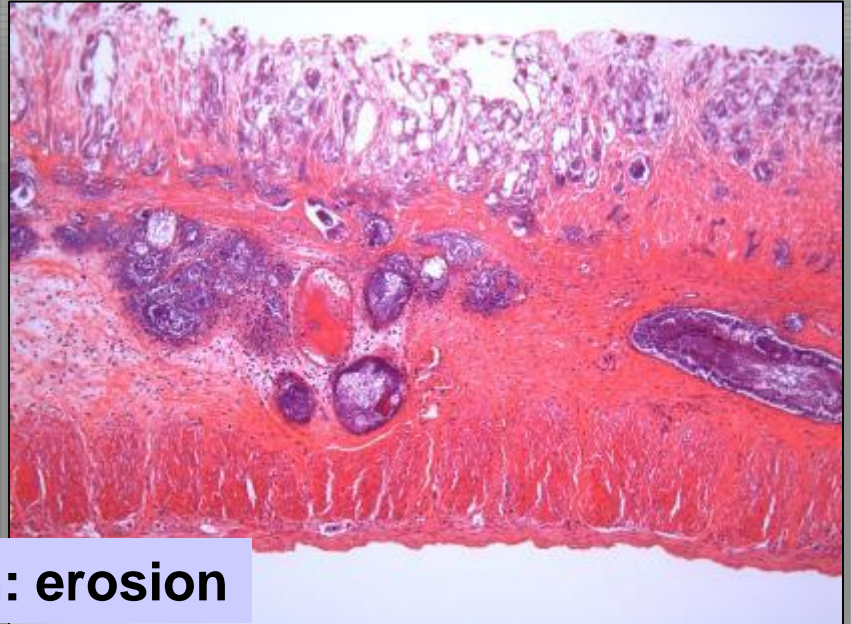
Stomach: Ulceration



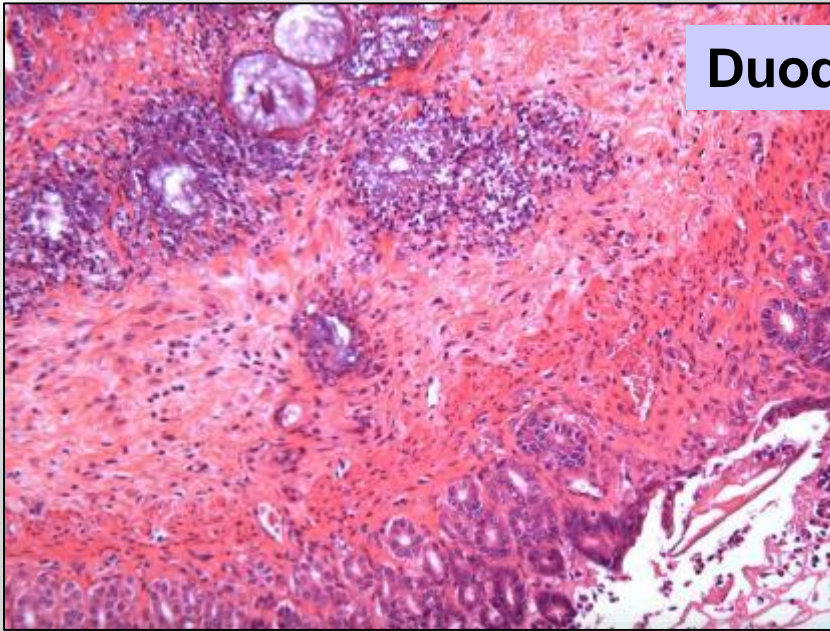
Stomach: bacterial colonies



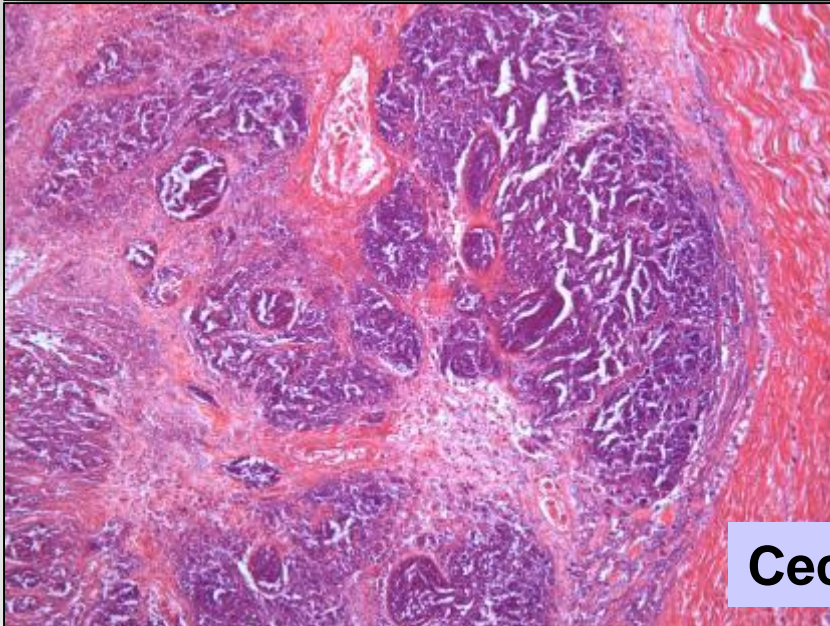
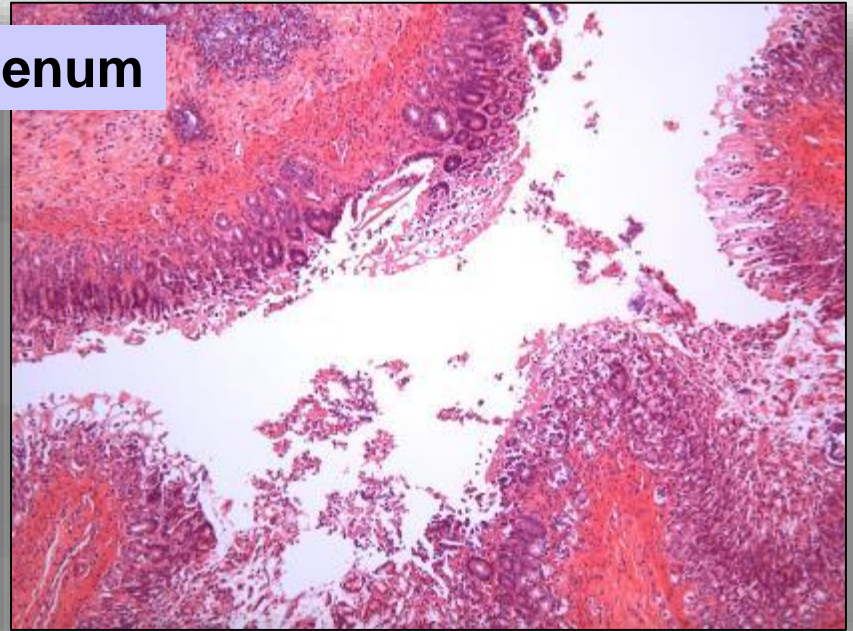
Stomach: erosion



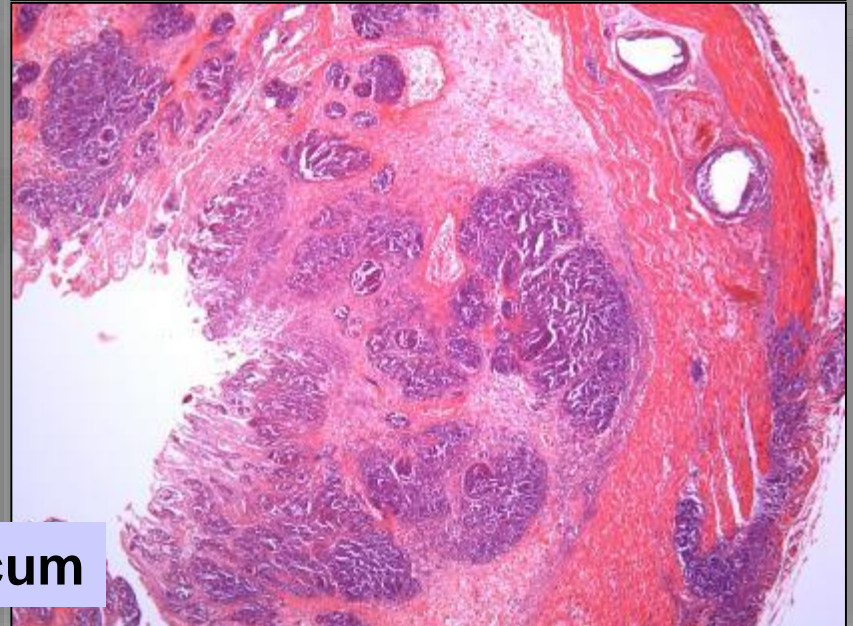
Gastrointestinal Tract: Findings



Duodenum



Cecum

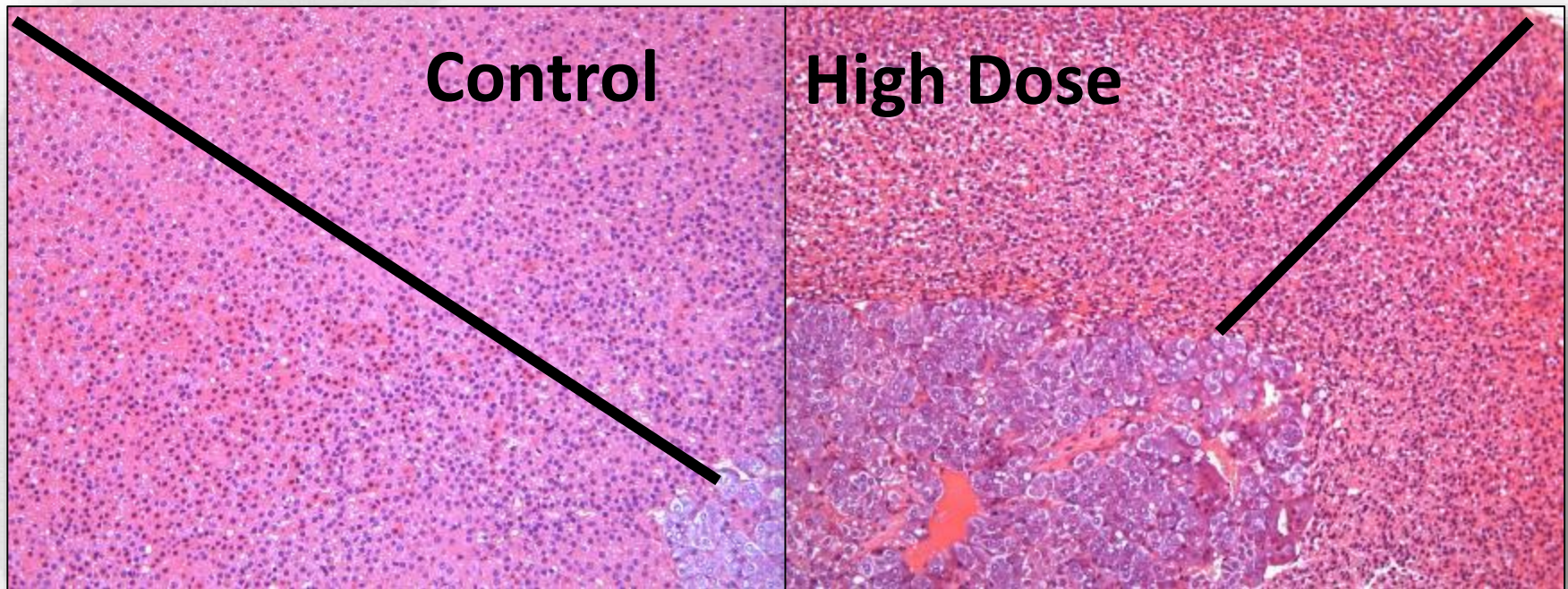


MOA/Mechanistics

- **Elevated levels of histamine-forming histidine decarboxylase**
- **Inhibitory action to PGE2 synthesis**
- **No explanation on large intestinal involvement**
- **The indicators for anemia are most probably related to the GIT-lesions, either directly by blood loss or indirectly maldigestion/malabsorption.**

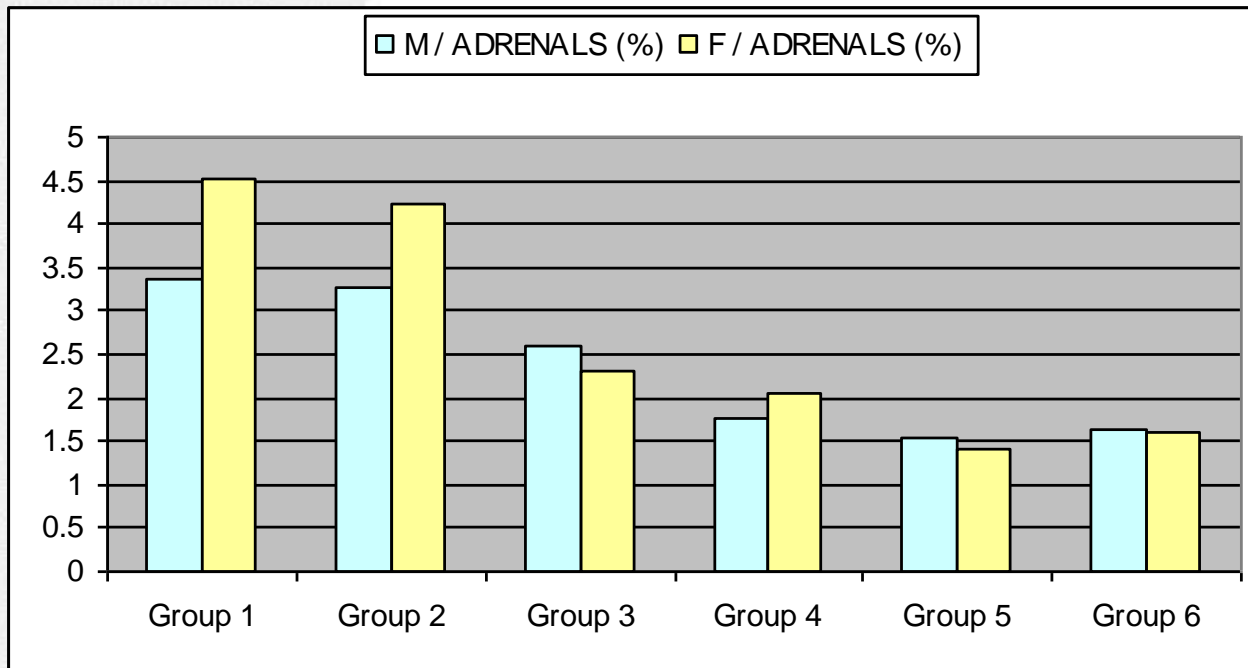
Adrenal Gland Involvement

- Reduced organ weight along with atrophy (zona fasciculata)



MOA/Mechanistics

- Secondary to exhaustive action by glucocorticosteroid
- Glucocorticoid administration enhances adrenal atrophy
- Suppressive effect on the pituitary-adrenal axis



**Organ/Brain Ratio
28 Days**

Other Effects

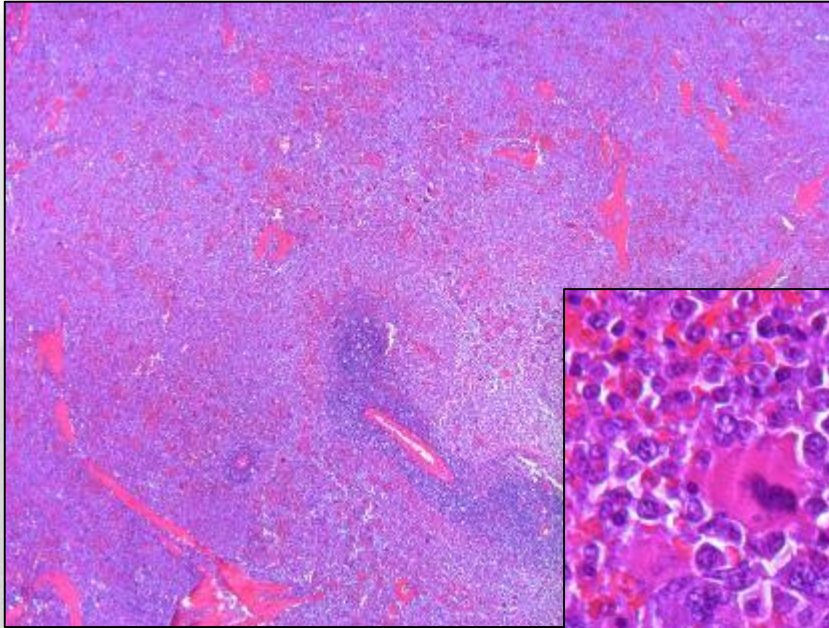
- **Liver hypertrophy and glycogen storage**
- **Atrophy of uterus/cycle blockage**
- **Skin adnexal atrophy**
- **Femoral physis degeneration ect.**

AnaPath

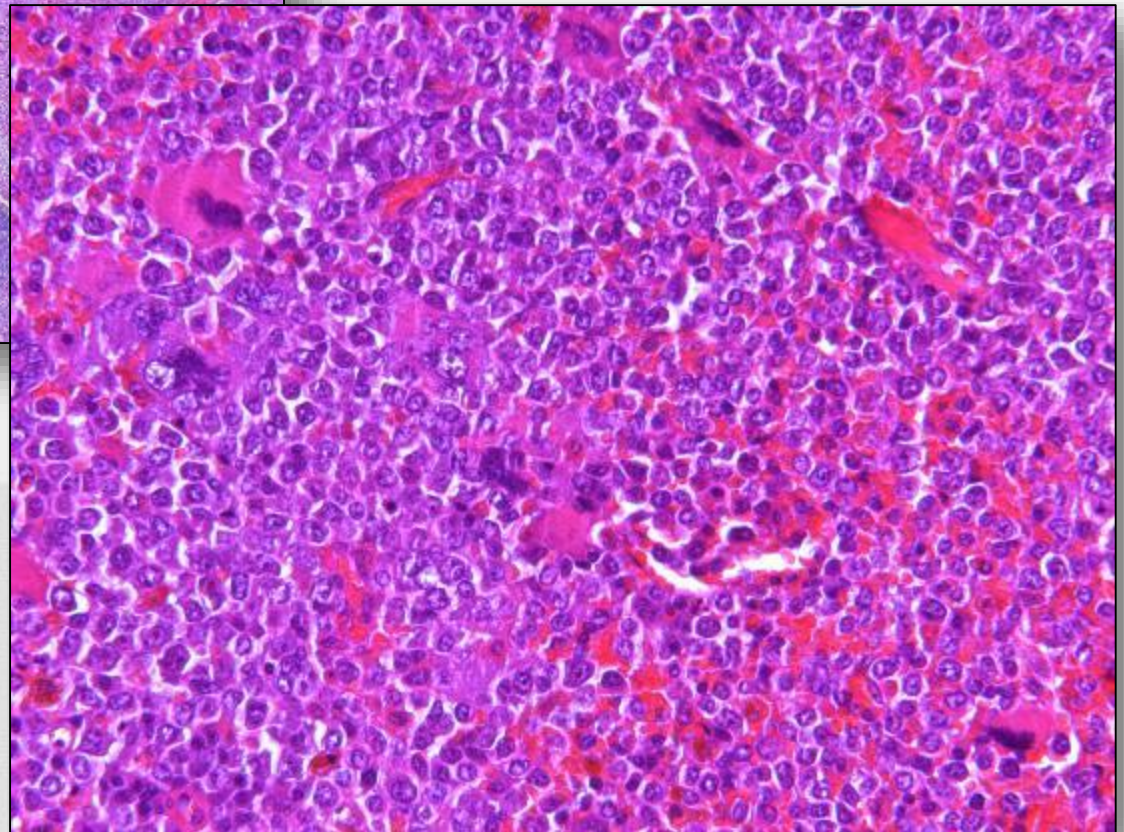


**Example:
Antitumoral Compound**

Unexpected lesions in dog liver and bone marrow

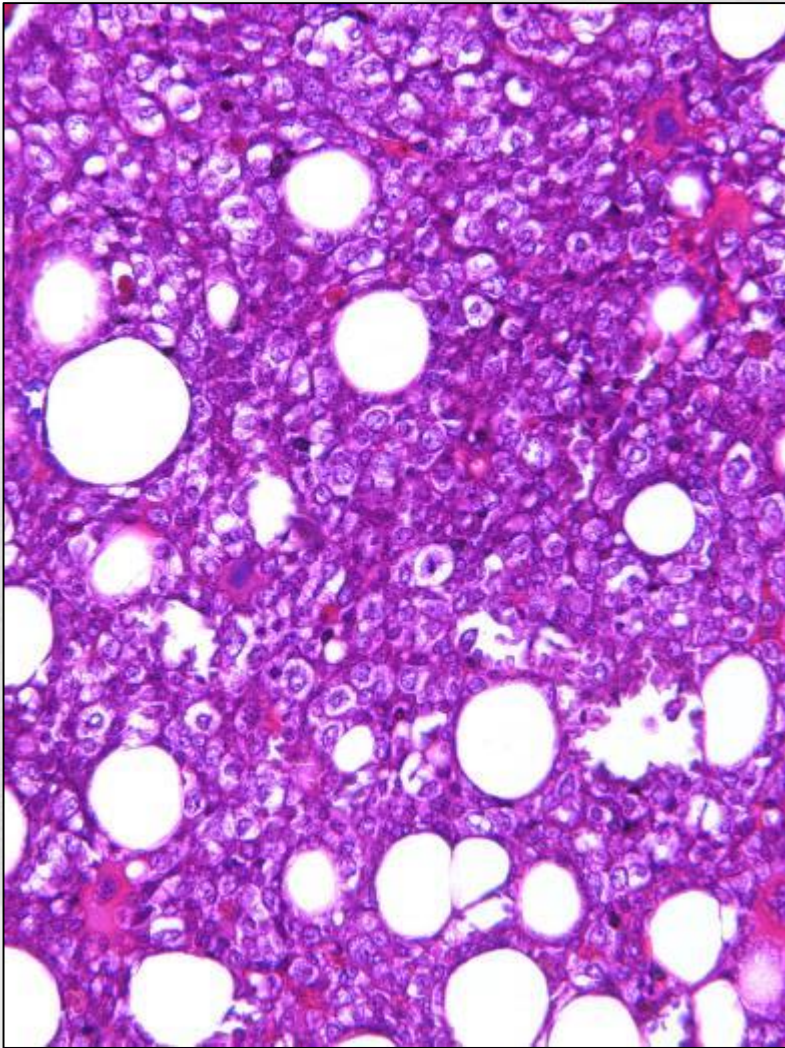


- **Myelodysplasia**

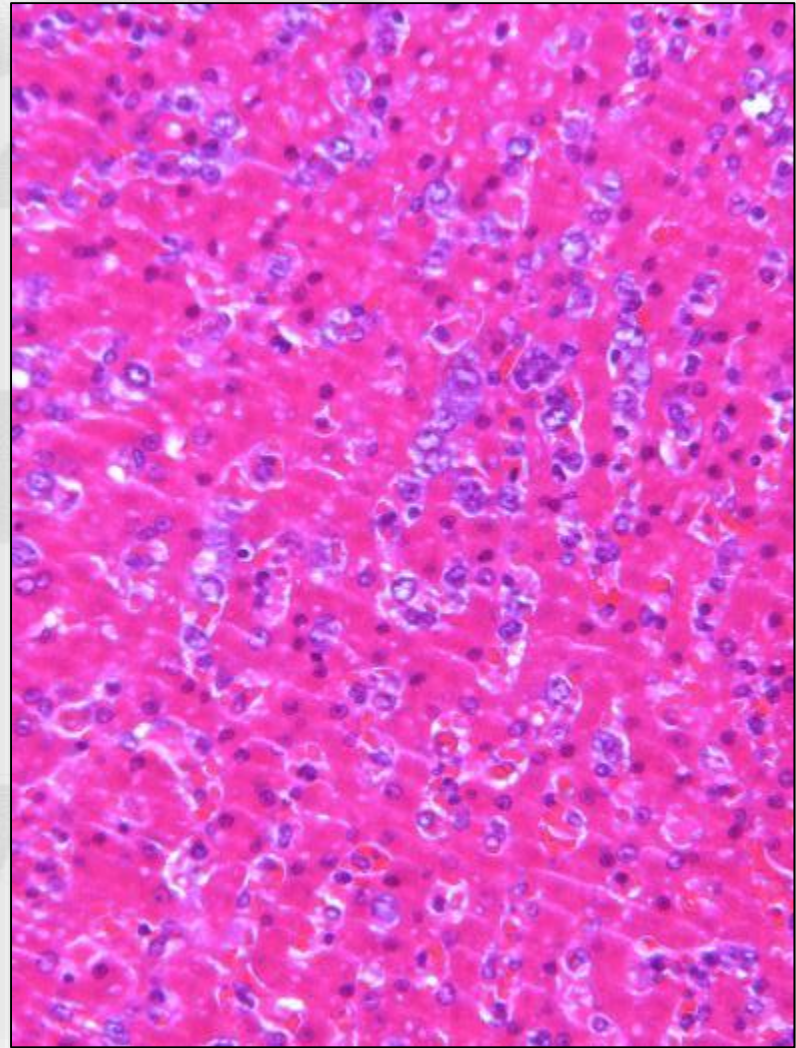


- **Note blasts**
- **Note changes in megakaryocytes**

Unexpected lesions in dog liver and bone marrow

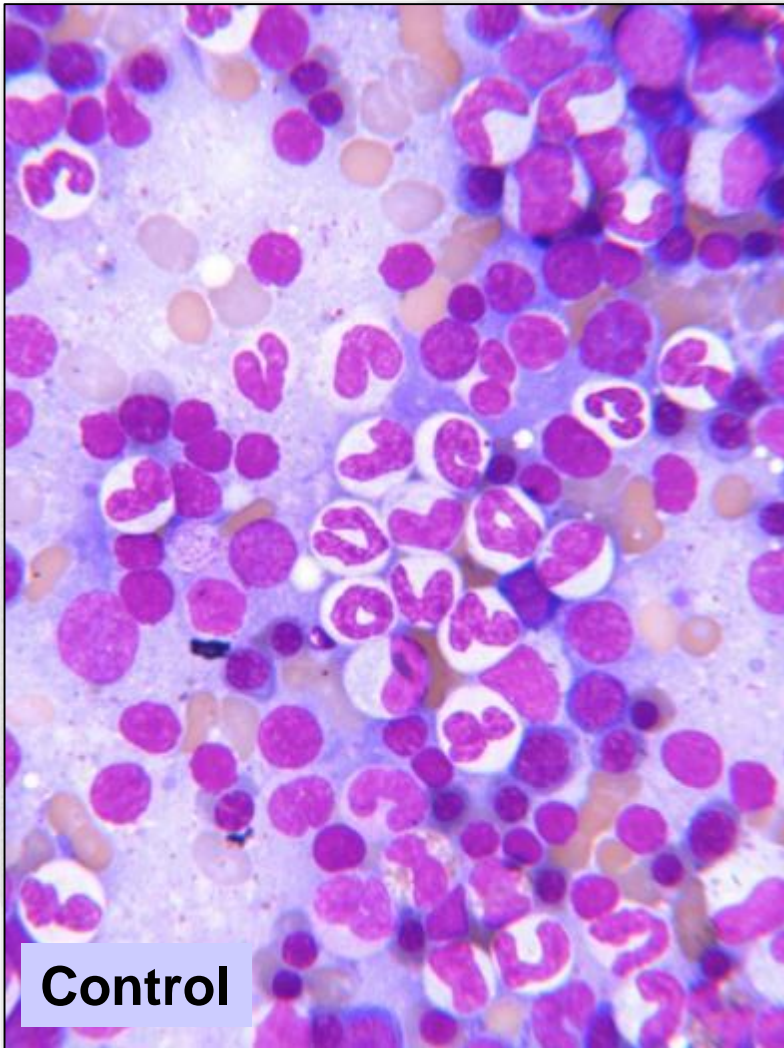


- **Blasts in sternal bone marrow**

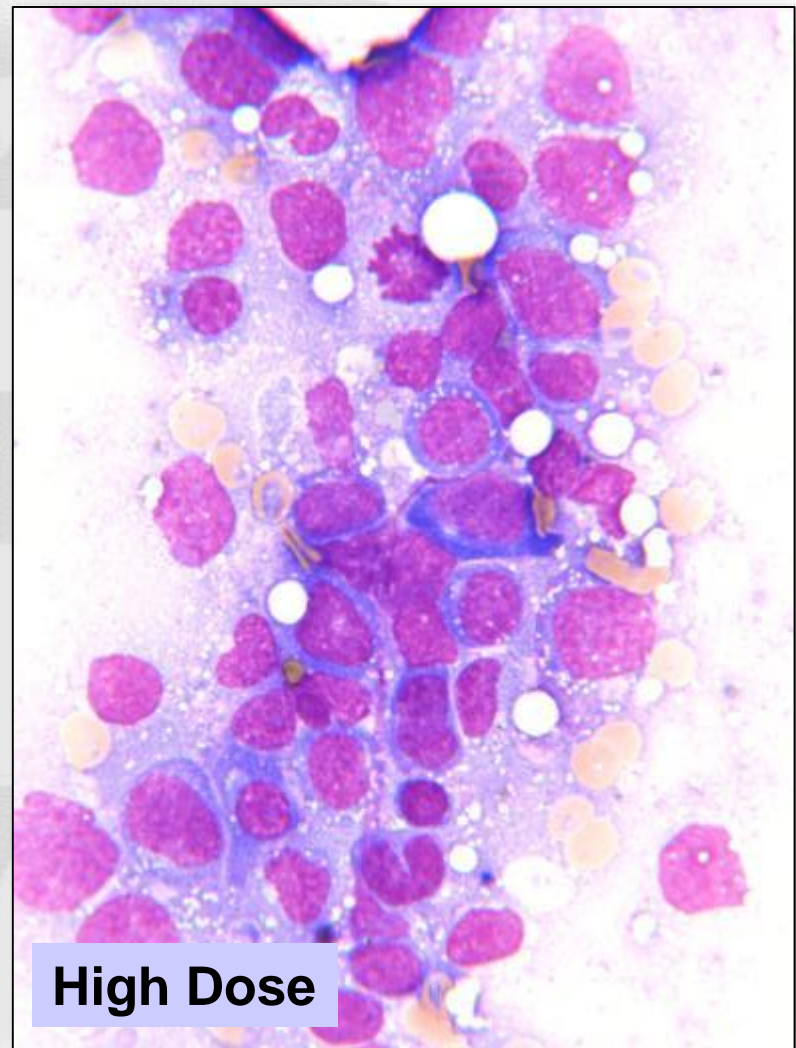


- **Blasts in liver sinusoids**

Unexpected lesions in dog liver and bone marrow



- Normal marrow



- Maturation block



Anaphylaxis

AnaPath

Anaphylaxis: Definitions

- **Anaphylactic shock** associated with systemic vasodilation causing low blood pressure or shock
- **Biphasic anaphylaxis** by the recurrence of symptoms within 1–72 hours without further exposure to the allergen
- **Pseudoanaphylaxis** or anaphylactoid reactions does not involve an allergic reaction but is due to direct mast cell degranulation

Lee, JK; Vadas, P (2011): Clin Exp Allergy. 41: 923–938

Simons FE (2009): J Allergy Clin Immunol. 124: 625–636

Anaphylaxis vs anaphylactoid reaction

- **Anaphylaxis is IgE-dependent**
- **Anaphylactoid reaction is not IgE-dependent, is secondary to the release of cytokines and antibodies bind directly to antigens**
- **Clinical management is the same**
- **Symptoms are the same**
- **Only way to find out are IgE levels (maximally 2.5 days)**

Infusion reaction

- **Any signs or symptoms experienced by patients during the infusion of pharmacologic or biologic agents or any event occurring on the first day of drug administration**
- **May occur after the administration of monoclonal antibodies**
- **Hypersensitivity basis, in which a molecular structure or a component of the drug formulation is recognized as an antigen**
- **IgE-dependent or not**

Kang SP, Saif MW (2007): J Support Oncol. 5: 451–457

Lenz HJ (2006). Oncology (Williston Park). 20(5 suppl 2): 5–13

Shock

- **Quickly progressing disorder resulting from systemic hypo-perfusion due to reduction either in cardiac output or in the effective circulating blood volume**
- **Less supply of oxygen and nutrients and inadequate removal of metabolites.**
- **Increased production of lactic acid**
 - **reversible injury to cells**
 - **irreversible injury with persistence or severe shock**

Differences in shock forms

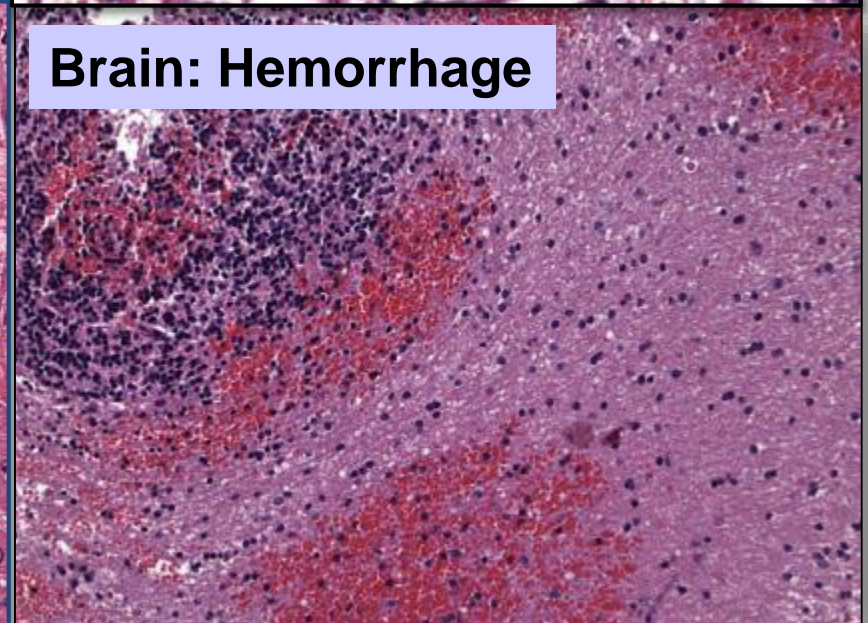
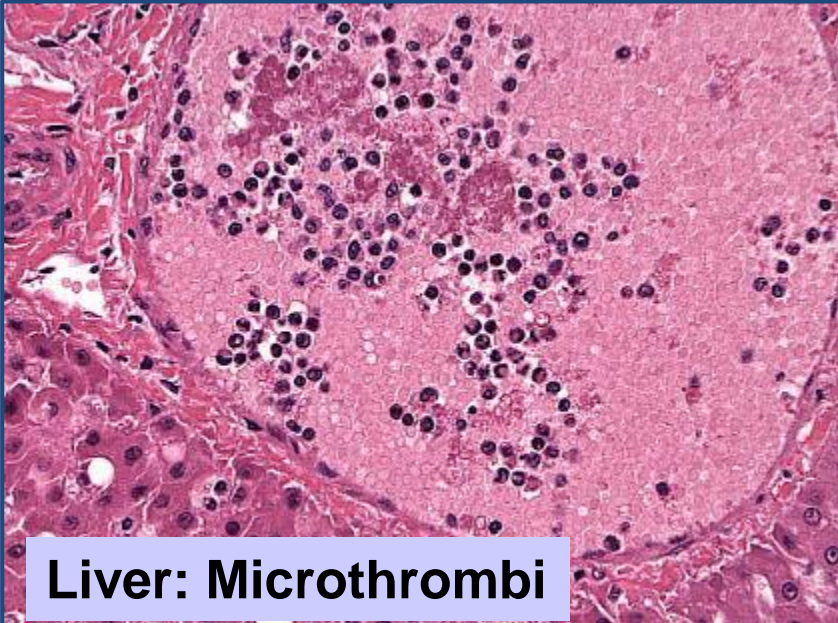
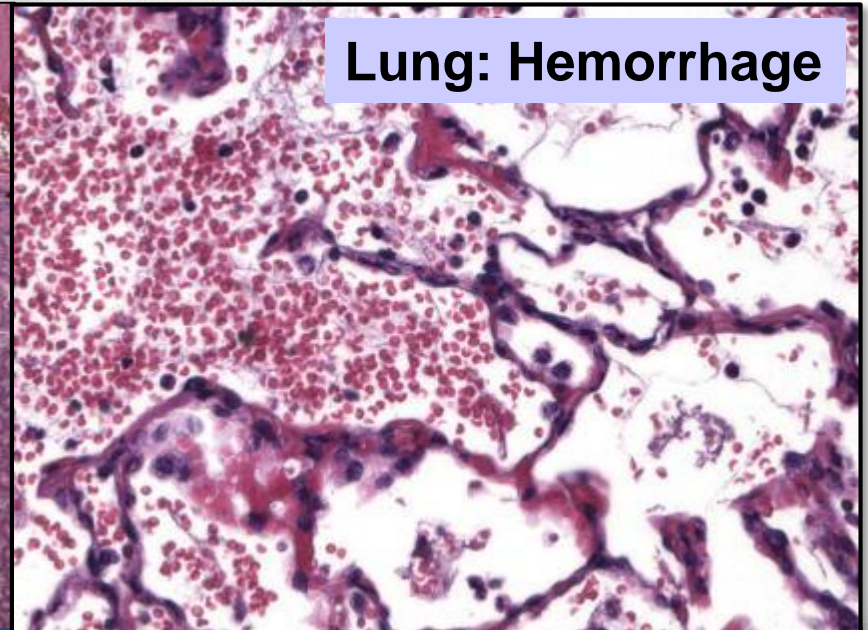
- Cellular hypoxia and end-organ damage may cause MODS

	Pulmonary-Capillary Wedge Pressure	Cardiac Output
Hypovelemic	↓	↓
Obstructive	↑ or ↓	↓
Cardiogenic	↑	↓
Distributive	↓	↑

Shock organs and evidence

- **Clinical signs with typical shock symptoms**
- **Known background**
- **Anaphylactoid Reaction is dose-dependent, Anaphylaxis not generally**
- **Lung, intestine, skin, cerebellum, adrenals, liver, thymus, kidney
(hemorrhage, necrosis, microthrombi)**

Histopathology





To Differ.....

Annapath

Differ Induced Effects from Stress

TABLE 3.—Changes in body weight and standard organ weight parameters potentially associated with mild or severe stress responses in routine toxicity studies.

Tissue	Mild stressors	Severe stressors
Body weight	Unchanged or decreased	Decreased
Thymus	Unchanged or decreased	Decreased
Spleen	Unchanged	Unchanged or decreased
Adrenal glands	Unchanged	Increased
Testes	Unchanged	Unchanged or decreased
Seminal vesicles	Decreased	Decreased
Prostate	Decreased	Decreased
Ovaries	Unchanged	Decreased
Uterus	Unchanged	Decreased

Note: Parameters affected by stress depend on the species, sex, age, and type and duration of stressor; some of these stress responses occur only in rodents. Refer to appropriate text sections for species-specific details.

Differ Induced Effects from Stress

TABLE 8.—Clinical pathology parameters potentially affected by acute or chronic in routine toxicity studies.

Parameter	Acute ^a	Chronic ^b
Neutrophil count	↑	→↑↓
Lymphocyte count	↑	↓
Eosinophil count	→	↓
RBC mass parameters (RBC, HGB, HCT)	→↑	→↓
Reticulocyte count	→↑	→↓
Bone marrow cellularity	→	→↓
Glucose concentration	→↑	↑↓→

Note: Hormones affected by stress depend on the species, sex, age, and type and duration of stressor. Refer to appropriate text sections for species-specific details.

^aAcute: minutes to hours.

^bChronic: days to weeks.

Everds N E et al. *Toxicol Pathol* 2013;41:560-614

TOXICOLOGIC
PATHOLOGY