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Immunotoxicity and the use of enhanced nomenclature

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Multiple sites and organs

but one integrated functional system

Immunotoxicology: References

Enhanced nomenclature

Elmore, S. (2006) Enhanced histopathology of the spleen. Toxicol.Pathol. 34: 648 – 655

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Elmore, S. (2006) Enhanced histopathology of the bone marrow. Toxicol.Pathol. 34: 666 – 686

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Harleman, JH (2000) Approaches to the identification and recording of findings in lymphoreticular organs indicative for immunotoxicity in regulatory type studies. Toxicology 33: 404-407.

Kuper CF, Harleman JH, Richter-Reichhelm HB, Vos JG (2000): Histopathologic approaches to detect changes indicative of immunotoxicity. Tox. Path. 28: 454- 466.

Schulte A, Althoff J, Ewe S, Richter-Reichhelm HB; BGVV Group Investigators (2002) Two immunotoxicity ring studies according to OECD TG 407-comparison of data on cyclosporin A and hexachlorobenzene. Regul Toxicol Pharmacol.36:12-21.

Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Published on October 16, 2008

The ICICIS Group Investigators (1998) Report of validation study of assessment of direct immunotoxicity in the rat. Toxicology 125: 183–201

Immunotoxicology:

Identification of immunomodulating potential

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Repeated dose toxicity studies

OECD 407

advanced histopathology of lymphoid organs

CPMP/SWP/1042/99 "Repeated dose toxicity"

immune function test in 28 day study (or 3 months study)

ICH S8 Immunotoxicity studies for human pharmaceuticals

EPA OPPTS 870.7800 "Immunotoxicity"

immune function test in 28 day study

Positive results trigger for extended studies

Immunotoxicology

CPMP/SWP/1042/99

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Initial screening phase

- advanced histopathology
- bone marrow cellularity
- lymphocyte subsets AND NK cell test OR primary antibody response to T-dependent antigen
- T-dependent antibody response: SRBC, KLH, CRM197

- **Trigger for extended studies**
  - "… changes in the above mentioned parameters may be a trigger … "
  - "… interpretation of the initial immunotoxicity screen should be based on an integrative view of changes in lymphoid tissues and lymphocyte subsets"
  - "… involution of the thymus in the presence of overt systemic toxicity may be too easily explained as a secondary (stress-related) response …"

Extended Studies (functional assays)

CPMP/SWP/1042/99

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- **Delayed type hypersensitivity (DTH)**
  - ☞ established (contact allergy model)
- **mitogen-stimulated lymphocytes**
  - ☞ can be connected to a regulatory study (PWM, ConA, …)
- **macrophage function**
  - ☞ can be connected to a regulatory study (ROI, particle uptake, …)
- **primary T-dependent antibody response**
  - ☞ SRBC, KLH, CRM197
- **host resistance models**
  - ☞ tumor models, parasite models, virus models

Different types of lymphoid organs - tissues

Identification of immunomodulating potential

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- Primary lymphoid organs - bone marrow and thymus

- Secondary lymphoid organs - spleen, lymph nodes, tonsils

- Organ-associated lymphoid tissues
  - Gut (GALT), Skin (SALT), Mucosa (MALT), Bronchio (BALT)

- Tertiary lymphoid structures - Inflammation associated

- Serosa associated lymphoid clusters (SALCS) - (milky spots)

Lymphoid tissues routinely examined in safety studies

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- Hematology
- Bone marrow (smear and section)
- Spleen
- Mesenteric lymph node
- Peripheral lymph node
- Gut (incl. GALT)
- Bronchial lymph node (respiratory studies)

THE HEMATON

MARROW		CIRCULATION		TISSUE
ERYTHROPOIESIS	→ RETICs	R.B.C.	100-120 DAYS (canine)	
GRANULOPOIESIS	→ BANDS	NEUTROPHILS EOSINOPHILS BASOPHILS	FEW HOURS →	SEQUESTRATION INFLAMMATION SITES BODY "FRONTIERS"
Stores ?				
PROMONOCYTE	→	MONOCYTES (HOURS)	→	MACROPHAGES
THROMBOPOIESIS	→	PLATELETS	6-10 DAYS	
LYMPHOCYTES PLASMA CELLS	? ↔	LYMPHOCYTES "T" AND "B"	↔	LYMPHOCYTES
R.E. CELLS (MACROPHAGES) STORAGE OF IRON				
MARROW / BIOPSY ASPIRATION		**BLOOD SAMPLE**		**EXUDATES**

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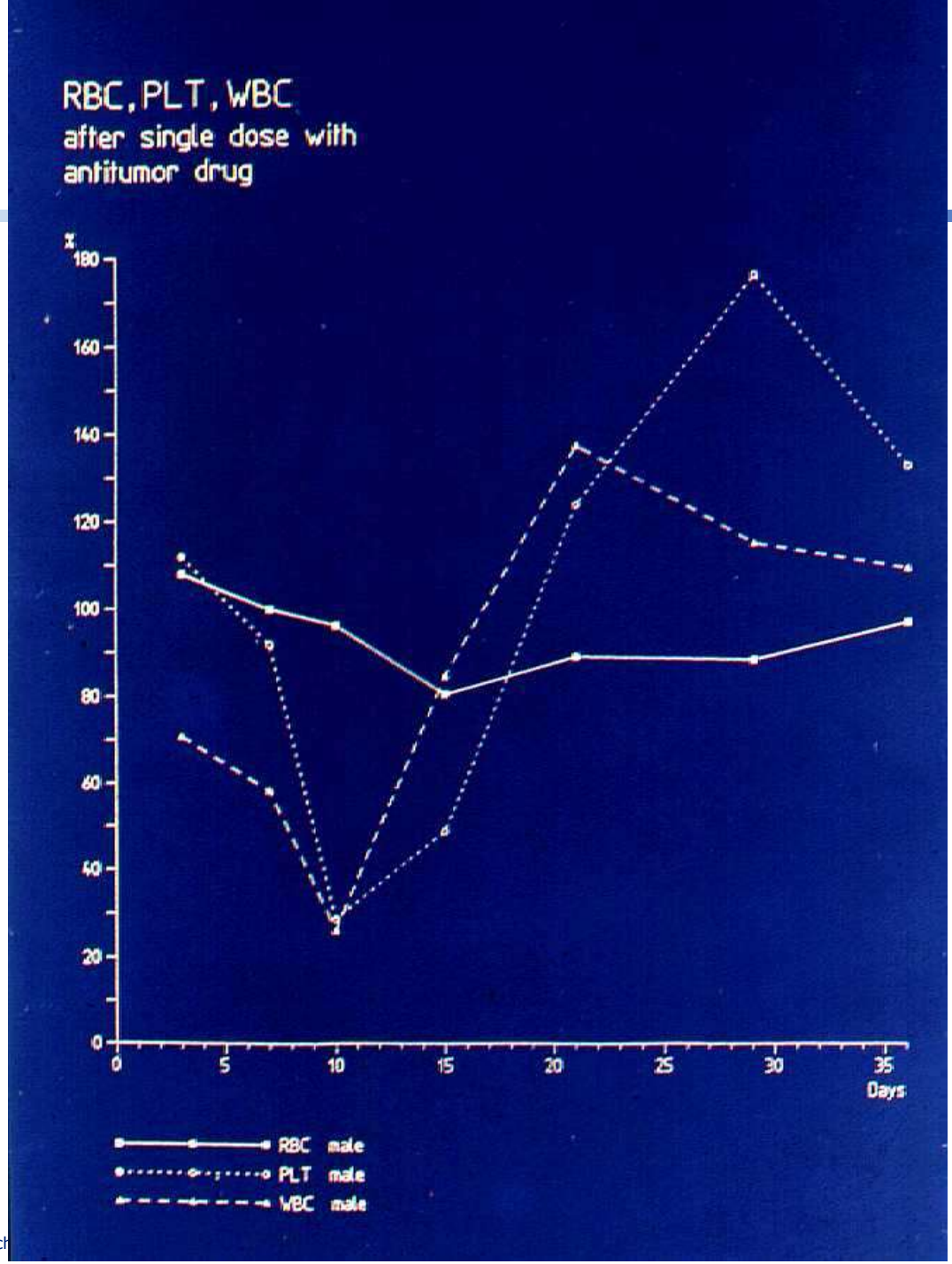

Morphology of the bone marrow - both a section and a smear are needed!

Both are needed for evaluation together with hematology and other data

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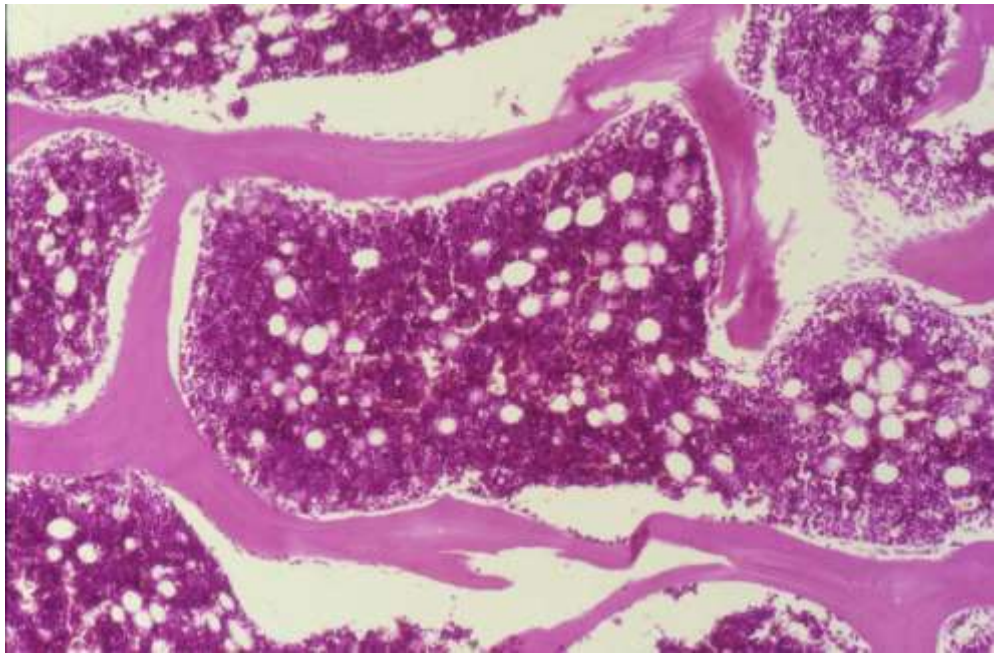

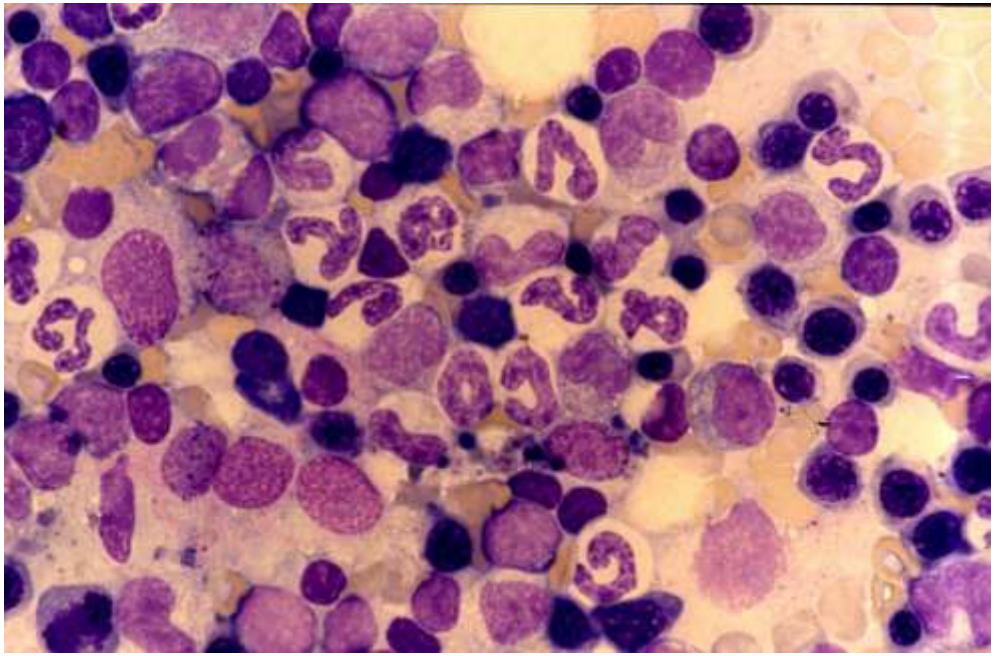

Effects on the bone marrow after acute treatment with an antineoplastic compound

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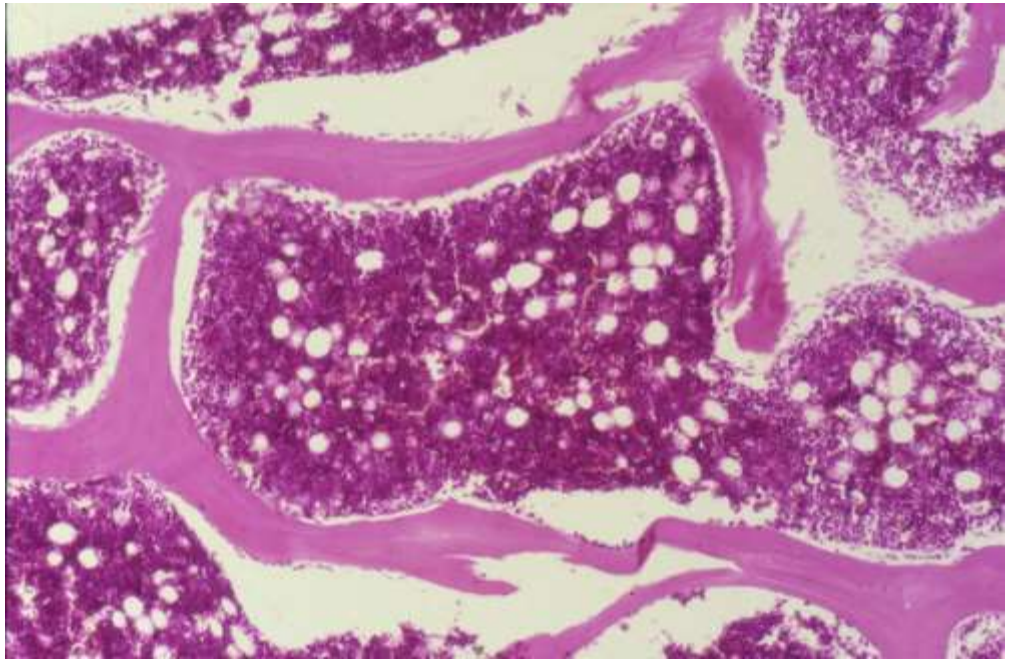

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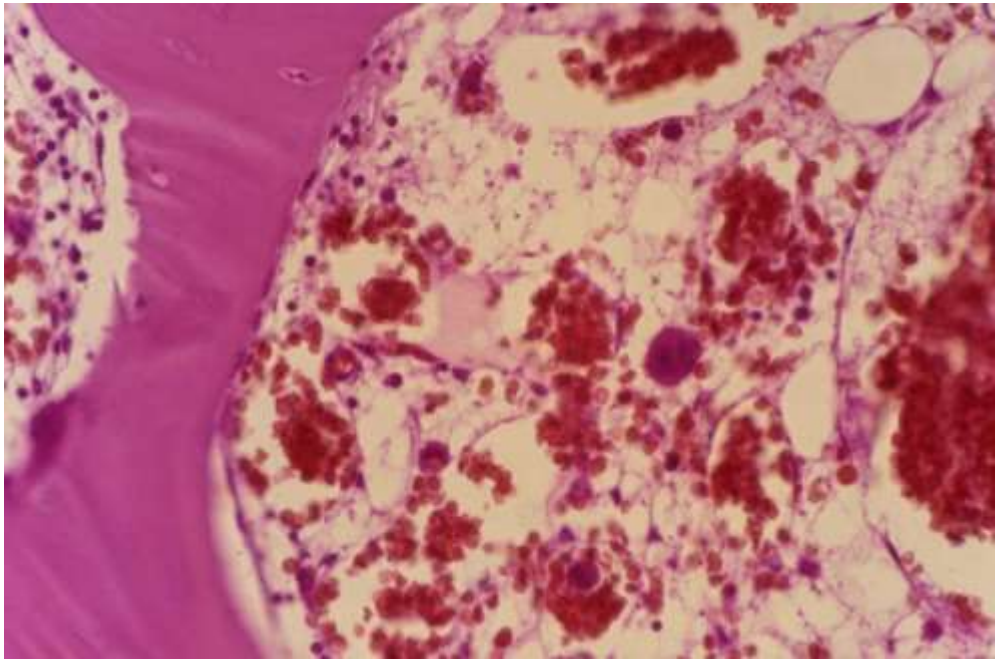

Treated

Blood smear of a control dog and a dog with an induced autoimmune hemolytic anemia (mid-dose, week 8, anti-epileptic)

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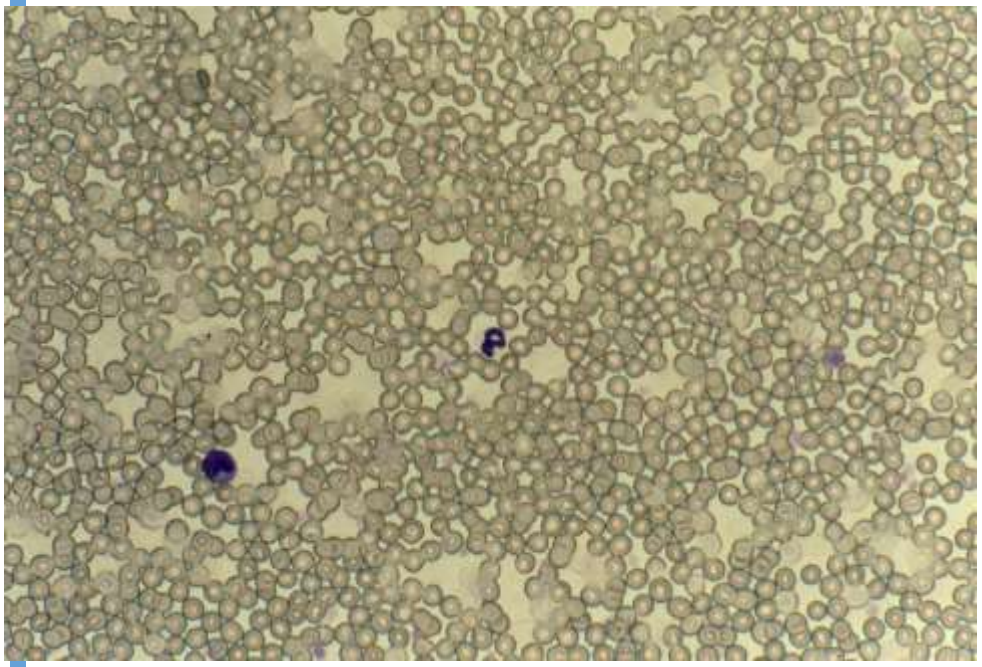

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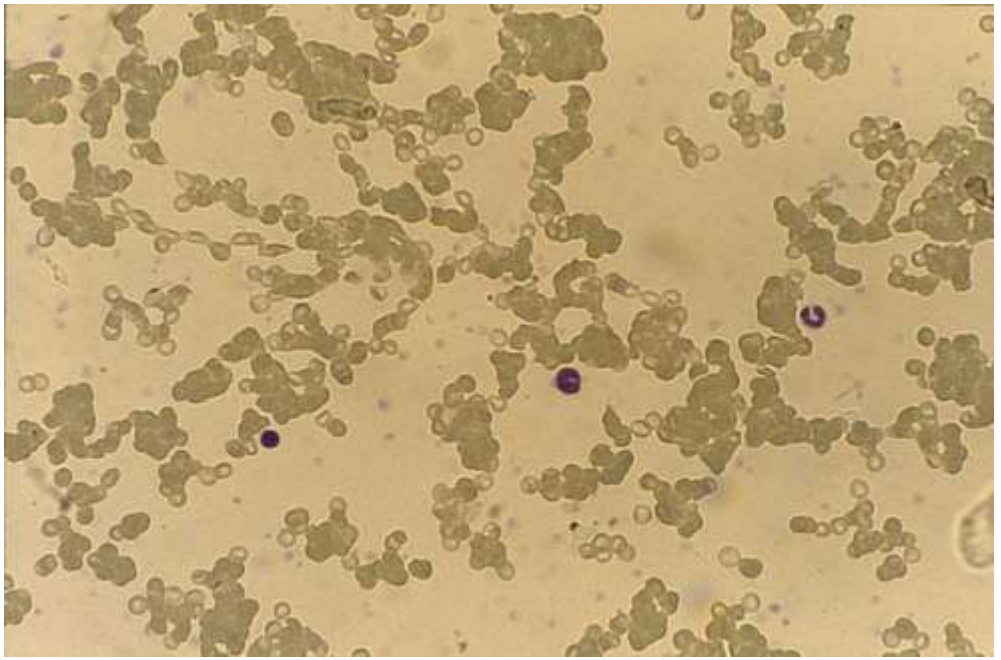

Treated

Low power of blood smear of a dog with an induced autoimmune hemolytic anemea (mid-dose, week 8, anti-epileptic)

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Blood smear of two dogs with an induced autoimmune hemolytic anemea (mid- and high-dose, week 7 and 9, anti-epileptic)

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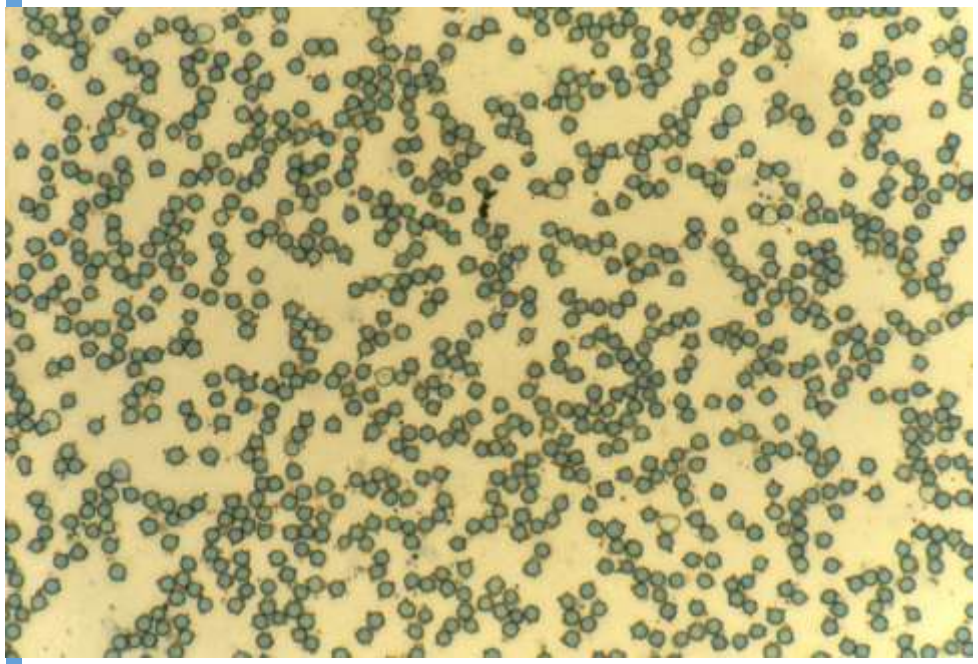

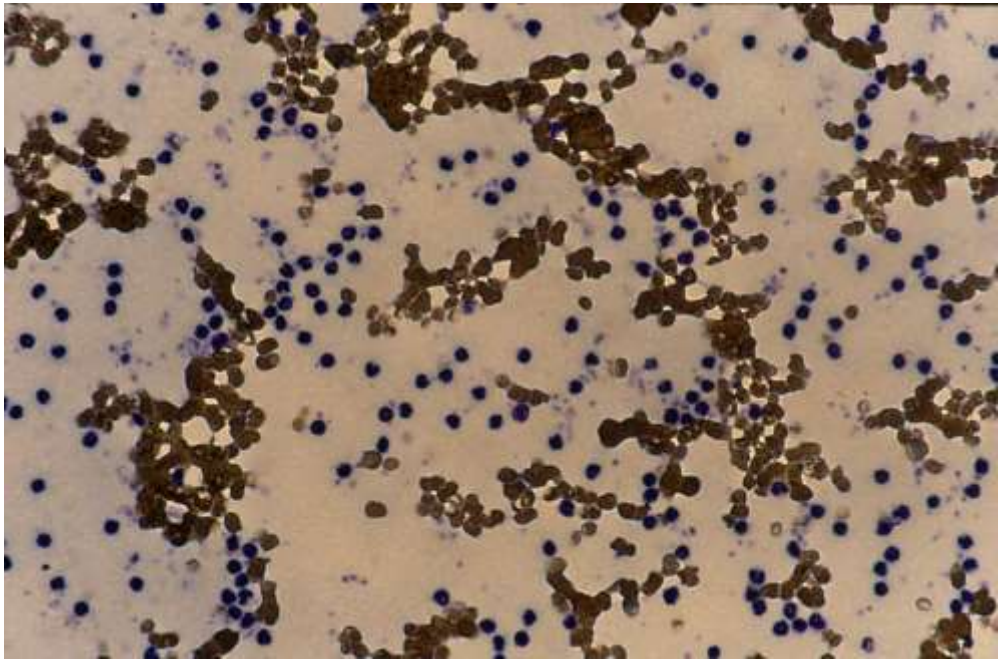

Liver and spleen of a dog with an induced autoimmune hemolytic anemia (high-dose, week 8, anti-epileptic)

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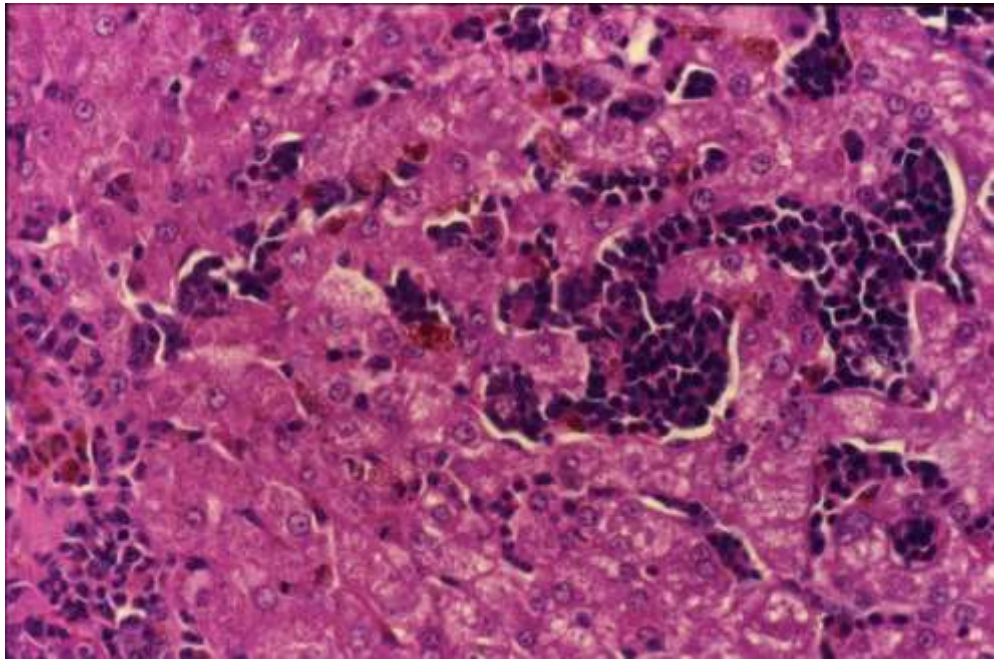
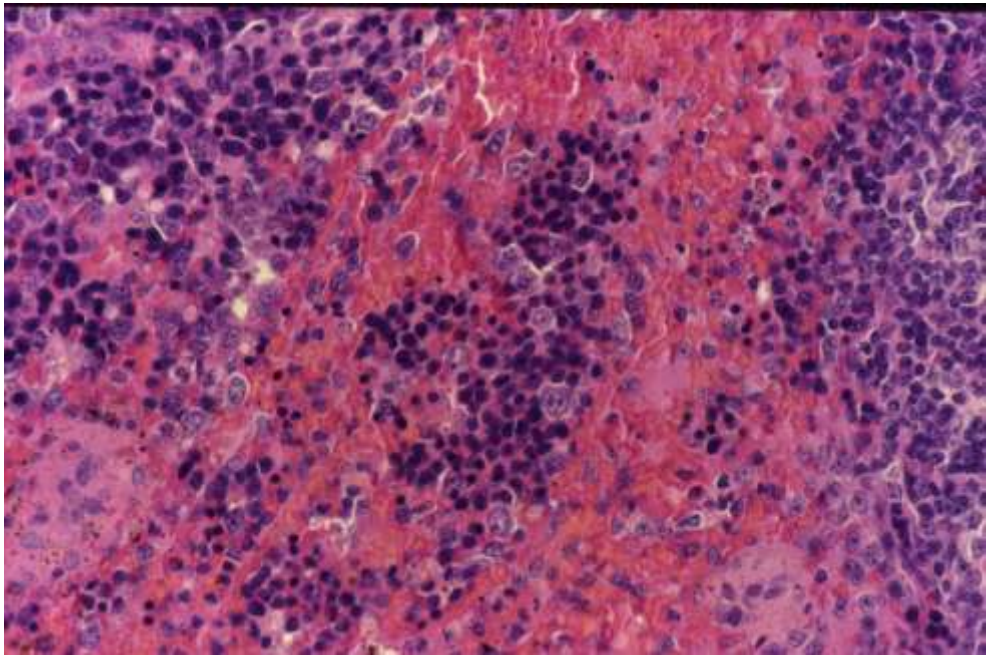
Note the marked extramedullary hematopoiesis and pigment accumulation





Lymphoid organs have anatomical markers and functional areas e.g. lymph node rat

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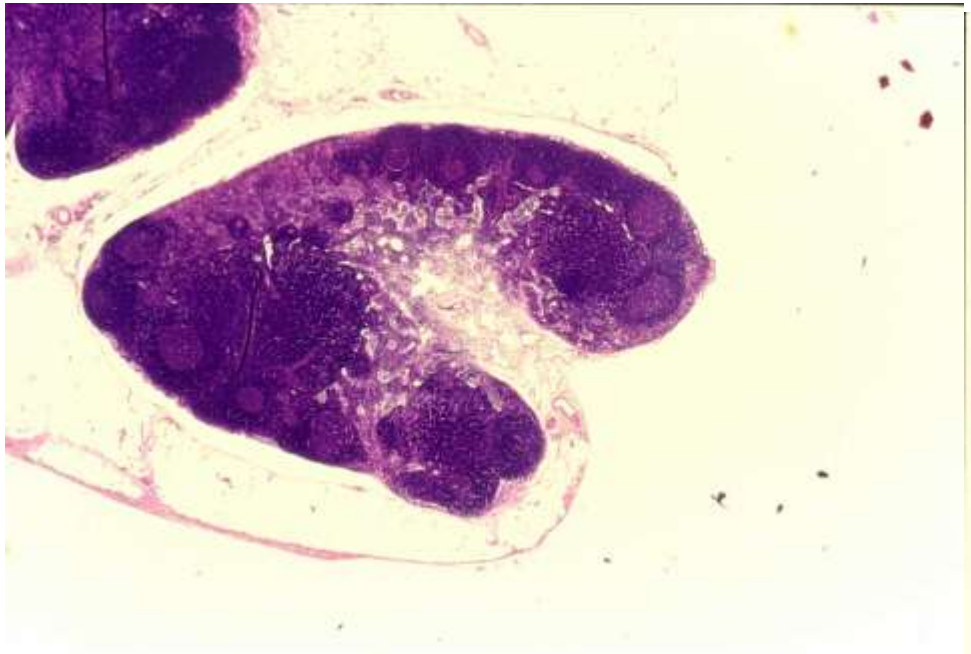

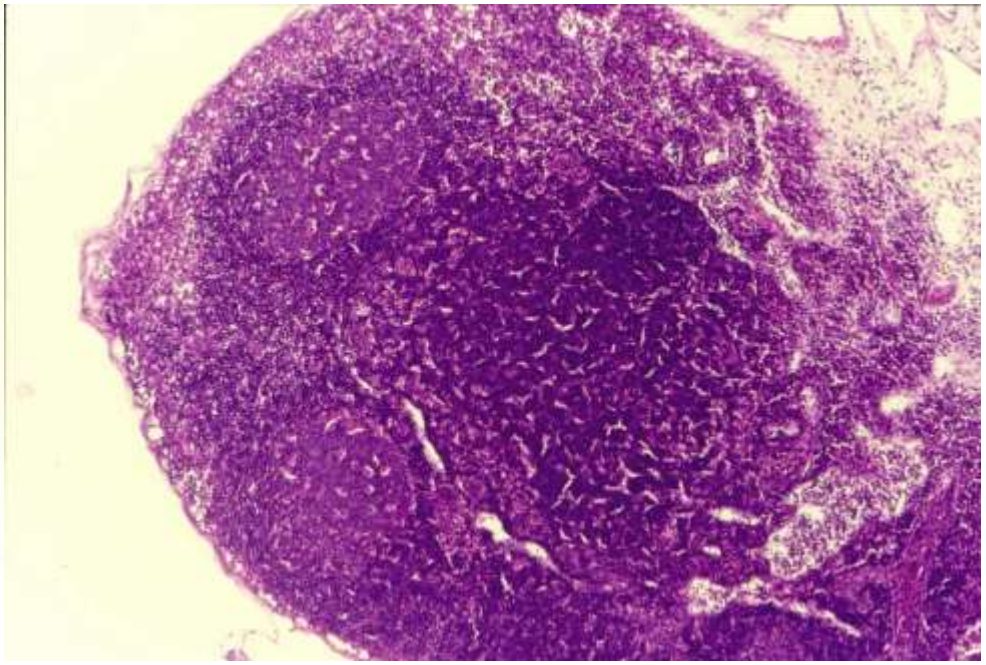

Use of specific monoclonal antibodies for localisation of specific sub-populations in the lymphoid organs

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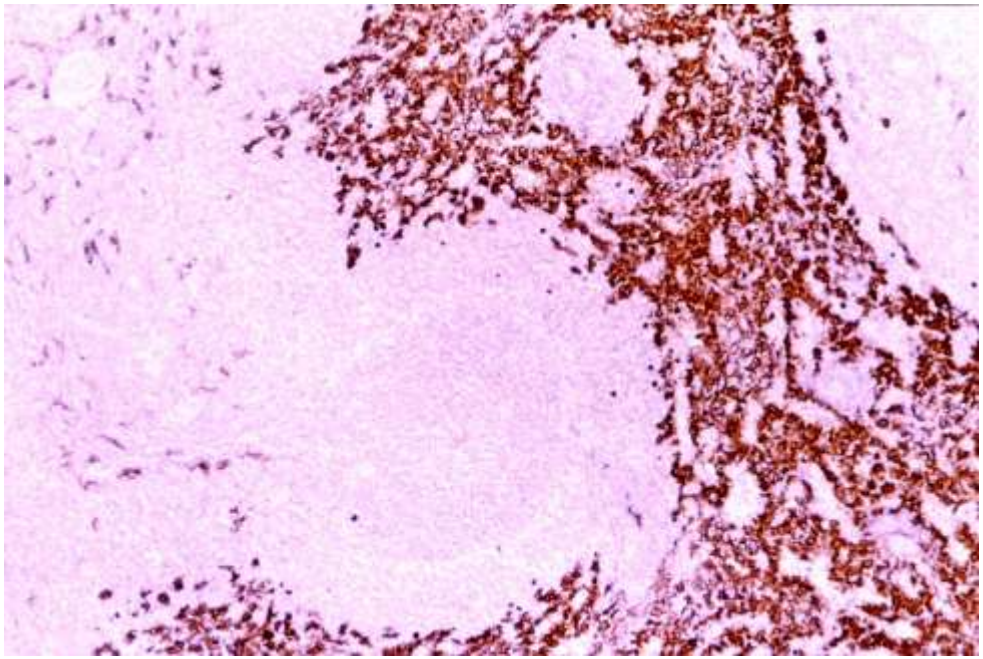
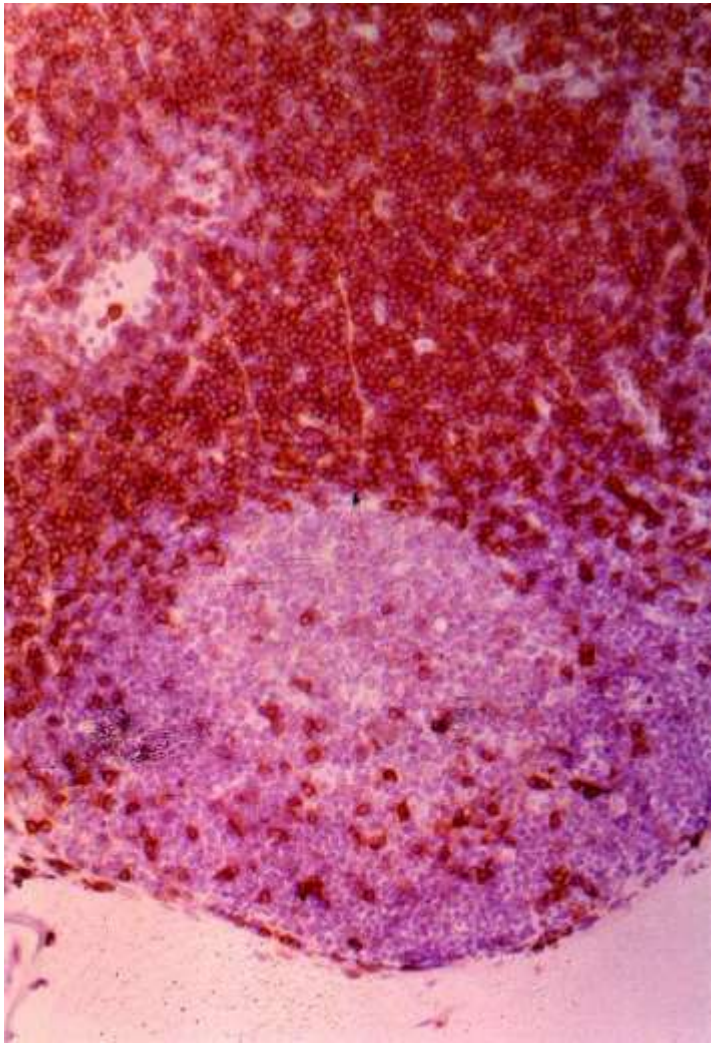


Spleen macrophage marker



Lymph node, T-cell marker

Microenvironments in Lymphoid Tissue

Microenvironment	Cells present	Function
Thymus		
Cortex	Reticular epithelium, immature T-cells	Generation of T-cell competence: T-cell receptor rearrangement, positive selection (MHC restriction), negative selection (auto-reactive cells), phenotypic changes
Medulla	Reticular epithelium, dendritic cells, T lymphocytes	Final generation of T-cell competence (negative selection); thymic hormone synthesis, antigen presentation
Lymph node and spleen		
Paracortex (lymph node)	Interdigitating cells, T_H and T_S cells	Lymphocyte entry through high endothelial venules (lymph node) or central arteriole (spleen), antigen presentation to T_H cells, T-cell proliferation/differentiation/regulation (T_S cells)
Periarteriolar lymphocyte sheath (spleen)		
Primary follicles Follicle mantle of secondary follicles	Dendritic cells (subtype of follicular dendritic cells), dendritic macrophages, B cells, small number of T cells	Storage of virgin/memory B cells, recirculating B cells (surface IgM + IgD+)
Germinal center	Follicular dendritic cells, dendritic macrophages (starry-sky macrophages), B cells (centrocytes, centroblasts), T_H cells	T-cell-dependent B-lymphocyte differentiation, antigen presentation in the form of immune complexes (with/without complement C3)

Microenvironment	Cells present	Function
Lymph node and spleen		
Medulla (lymph node) Red pulp (spleen)	Plasma cells, T effector cells, reticular cells, polymorphonuclear granulocytes	Termination of antigen-specific reaction; antibody synthesis and immune-complex mediated clearance, T_{DTH} and T_C response
Marginal zone (spleen)	Marginal zone macrophages, marginal metallophilic cells, marginal zone B cells	T-cell-independent B-lymphocyte proliferation/differentiation, for example, to bacterial plysaccharides B-cell memory (surface IgM+IgD- cells)
Mucosa-associated lymphoid tissue		
Epithelium covering lymphoid tissue (e.g. Peyer's patches)	M (microfold) cells	Transport (uptake) of exogenous substances
Follicles and inter-follicular areas	See lymph node and spleen	For antibody-synthesis: precursors of IgA-plasma cells
Mucosal epithelium	Epithelial cells, T_C cells, natural killer cells, T γ-δ cells	First line of defence, synthesis of secretory component, transport of IgA (IgM) to lumen
Lamina propria	Plasma cells, macrophages	Synthesis of IgA antibody, phagocytosis and killing

Proposed diagnostic terms for identifying histopathological changes in lymphoid organs and tissues (Kuper et al., 2000)

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Organs and compartments	(Semiquantitative) changes
All organs, all compartments	Increased or decreased size Increased or decreased cellularity (lymphocytes, plasma cells, blast cells) Increased numbers of cells otherwise not present or in low numbers: tingible body macrophages (“starry-sky appearance”) phagocytizing or pigmented macrophages mast cells and granulocytes apoptotic cells (Mircro)granulomata or macrophage aggregates
Thymus	Cortex: medulla ratio Increased epithelial cords and tubules
Thymus; EFA	Increased or decreased number of EFA
Lymph nodes, spleen, MALT	Erythrocyte rosette formation
Lymph nodes, spleen and MALT; follicles	Increased or decreased germinal centre development
Lymph nodes and MALT; paracortex; interfollicular area	Prominent HEV
Lymphatics and lymph nodes	Lymphatic ectasia

Use of enhanced nomenclature

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It is up to the responsible pathologist

- Immune toxicity studies
- Up to 3 month repeat dose studies
- Studies with immune modulators
- Not recommended for chronic toxicity and carcinogenicity studies

Terminology	Study Type	Examples
Conventional Terminology	Chronic	Hyperplasia, lymphoid, thymus
Enhanced Terminology	Short term (up to 3 months) Immunotoxicity	Lymphocytes, increased, cortex, thymus
Alternative Terms	All study types except immunotoxicity	Increased cellularity, cortex, thymus

Thymus (H&E) control animal

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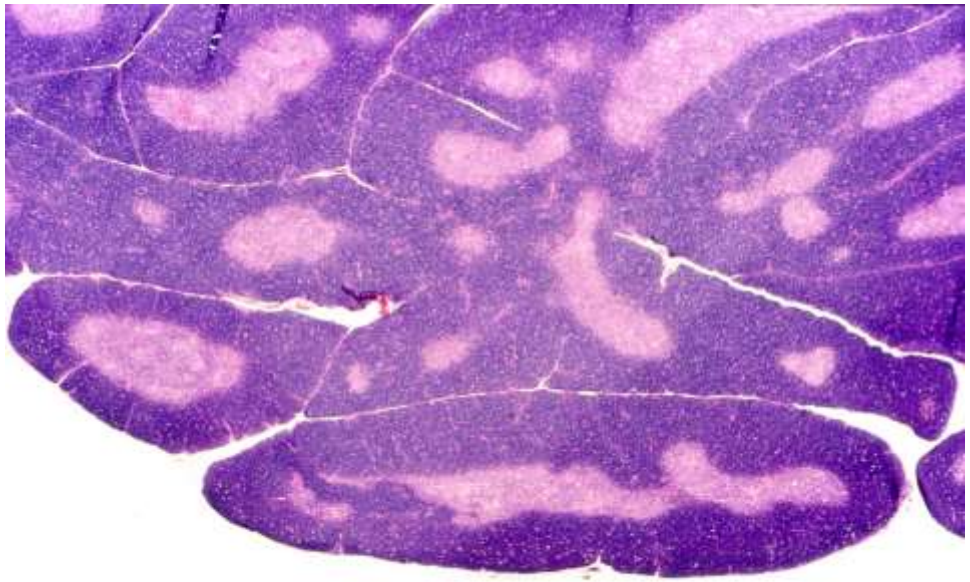

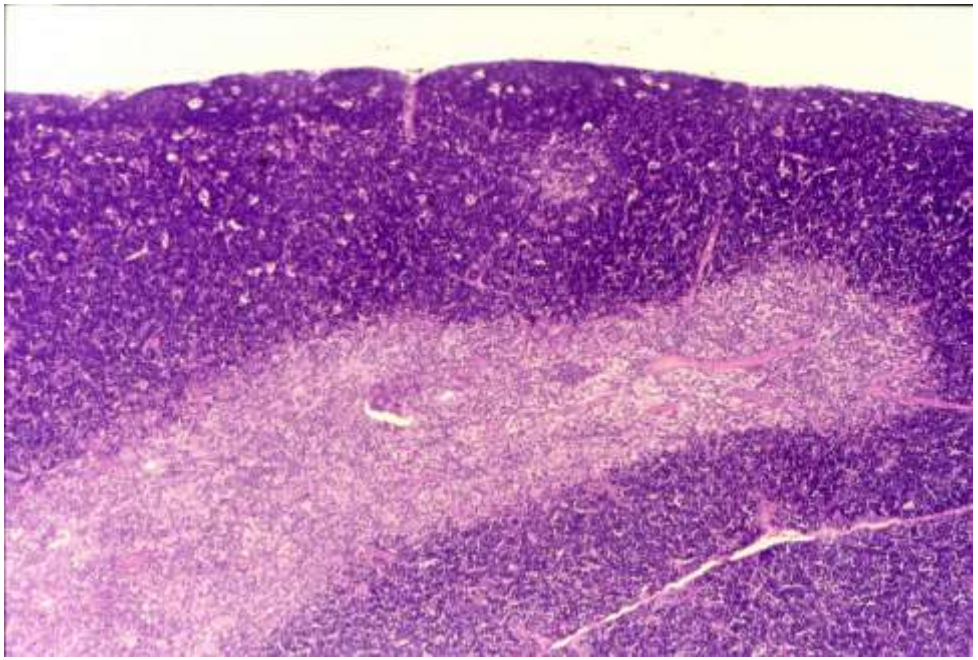

Thymus (H&E) cyclosporin treatment

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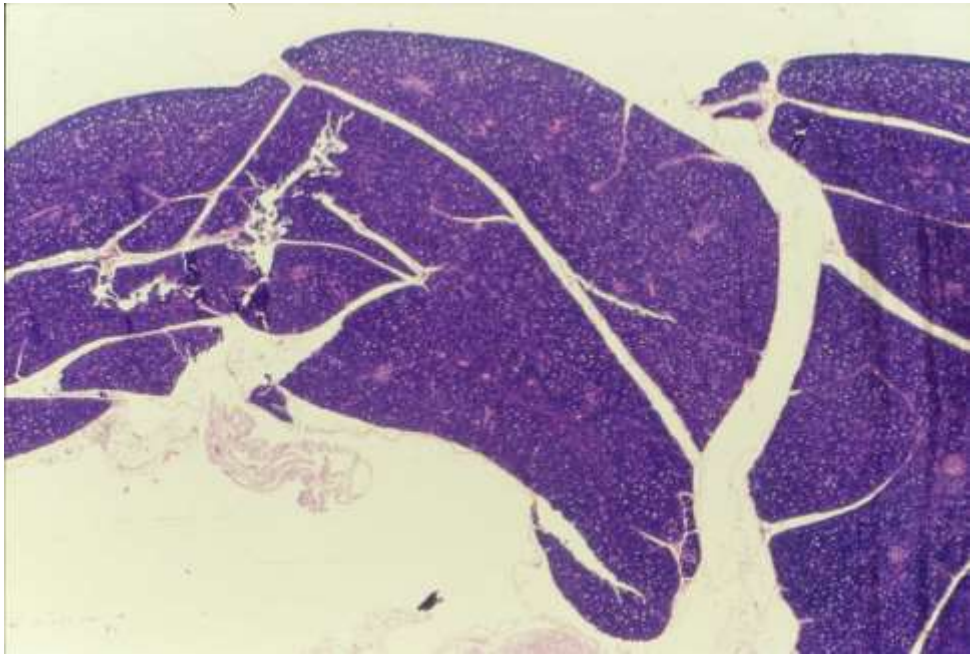

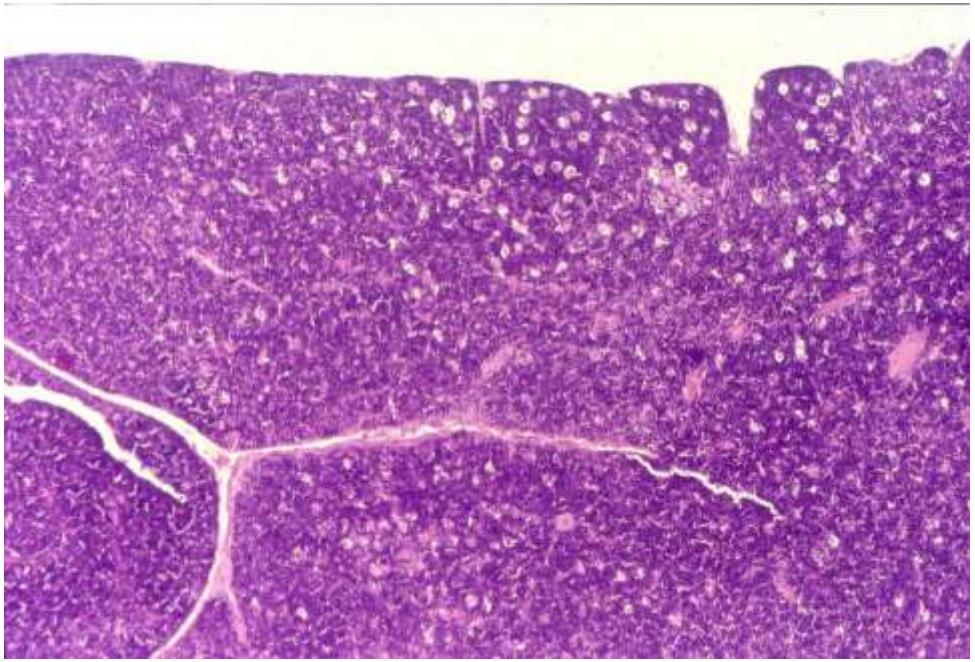

Spleen (H&E) rat, control animal

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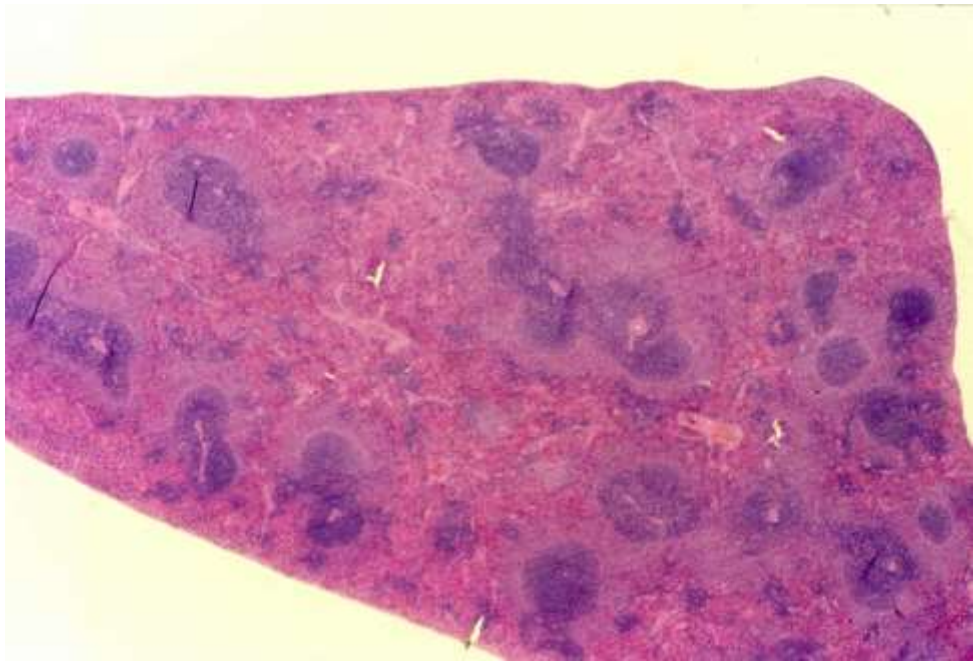

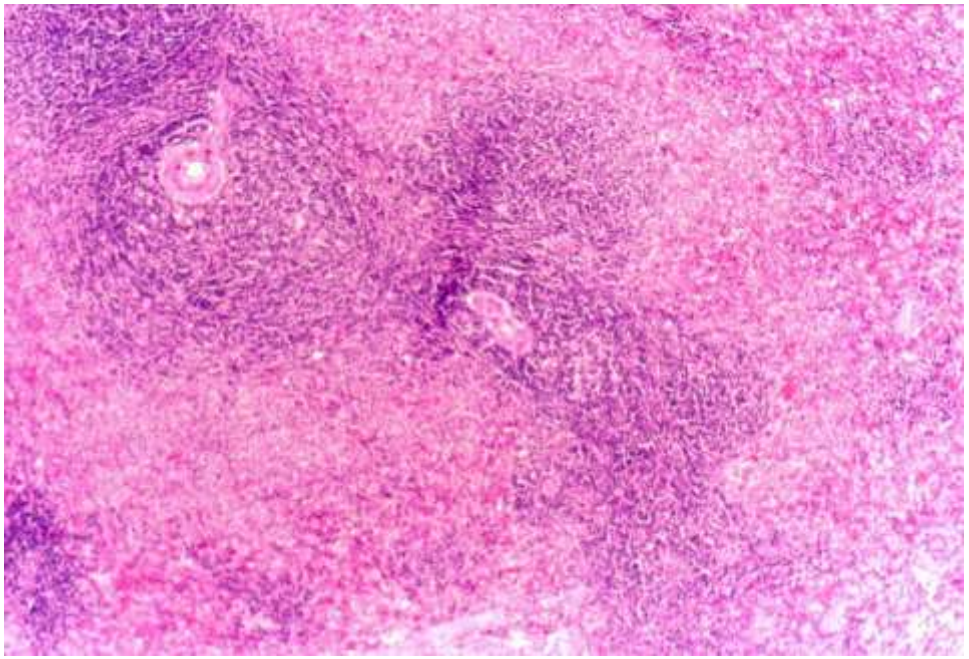

Spleen (H&E), rat, cyclosporin

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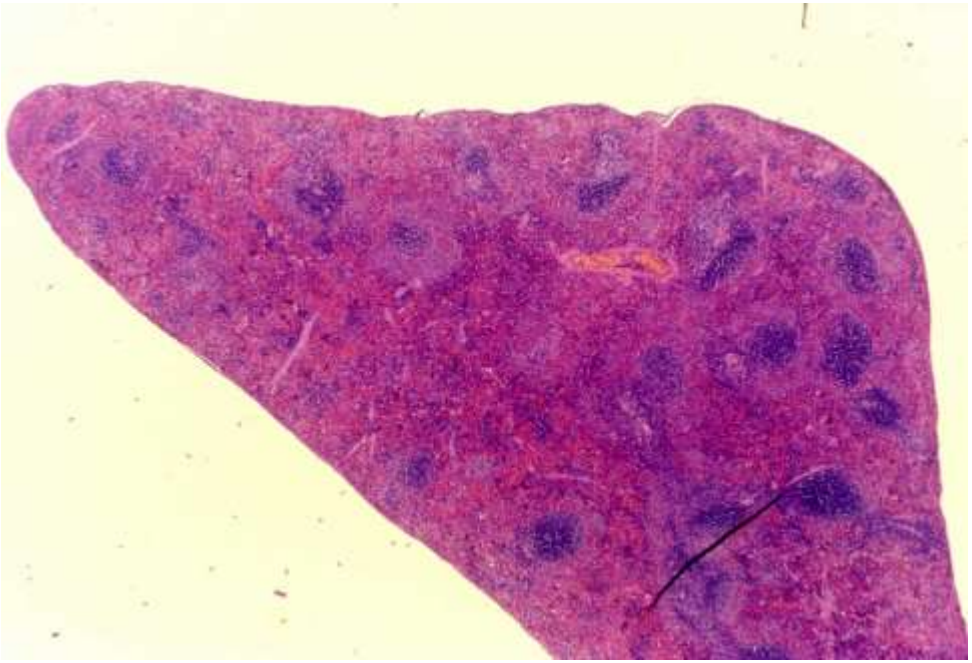
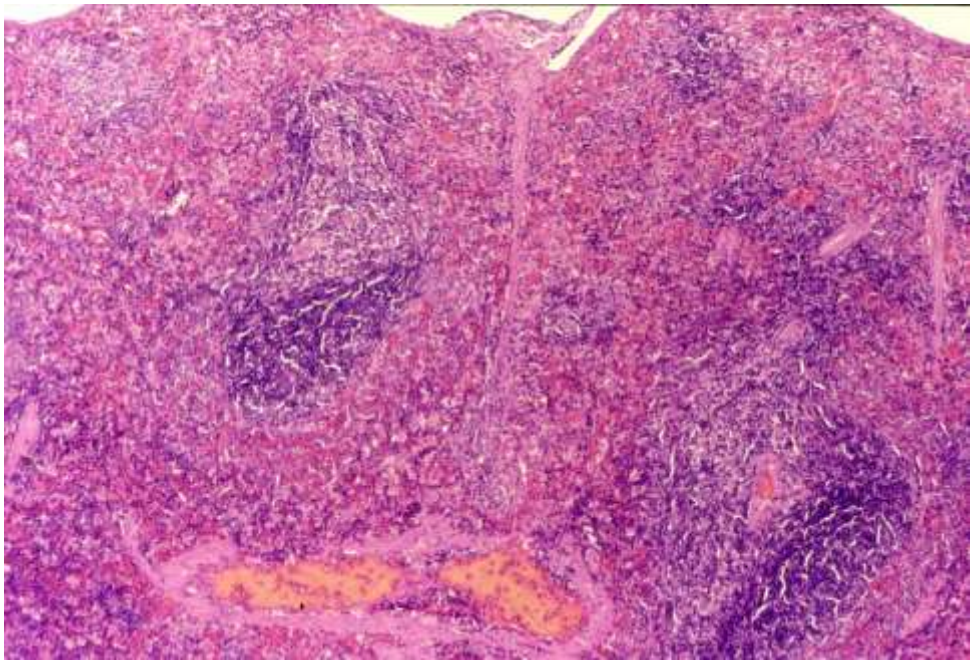
Note the marked reduction in cellularity of the PALS





Lymph node (H&E), rat, control animal

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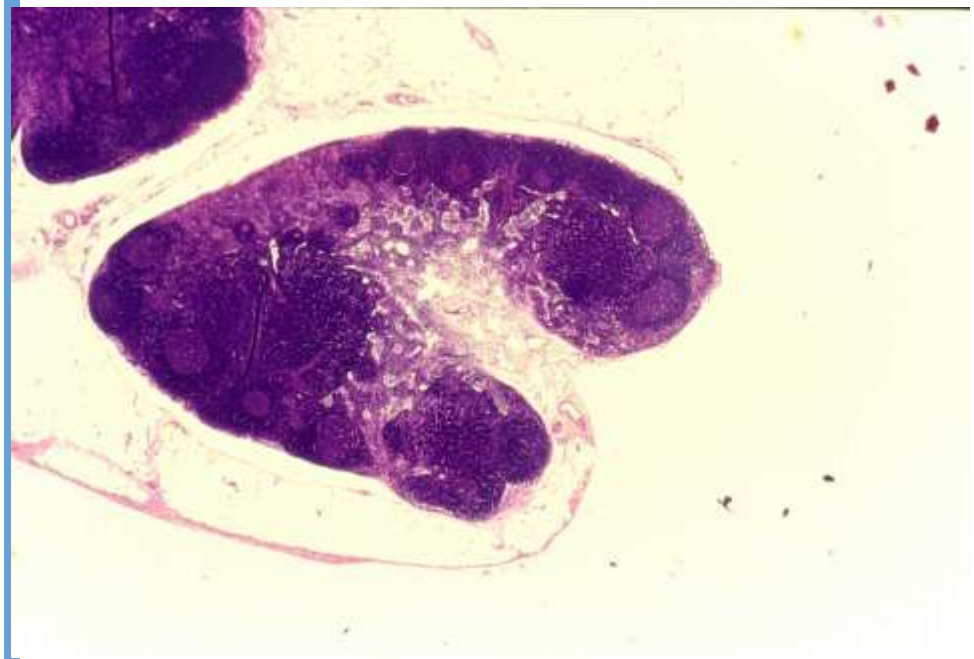

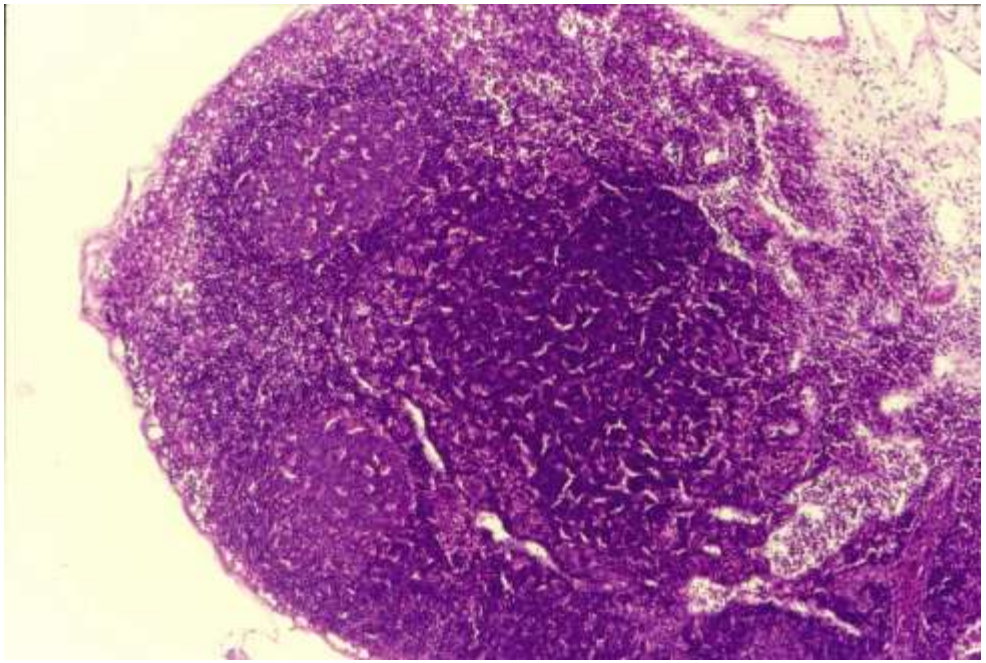

Lymph node (H&E), rat, cyclosporin

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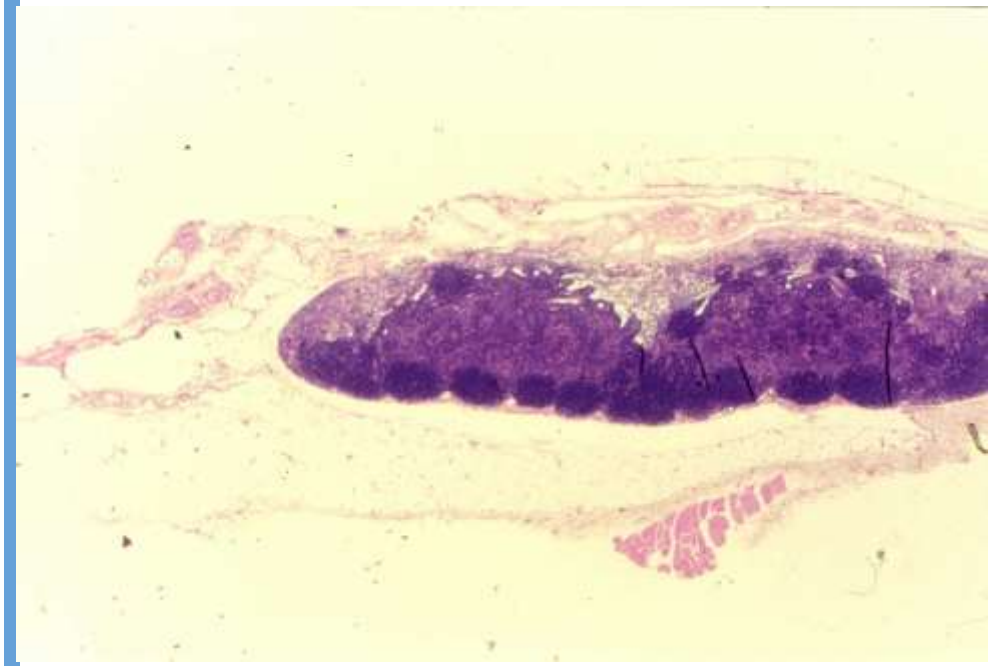
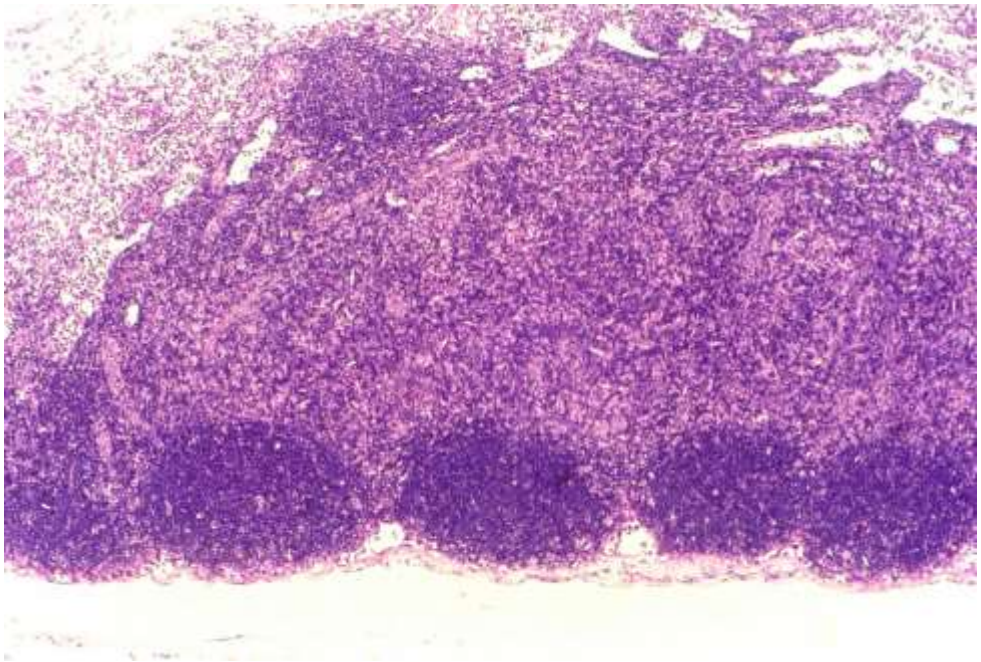
Note the reduced cellularity of the paracortex and and absence of follicular activity





Mesenteric Lymph node (H&E), dog, control animal

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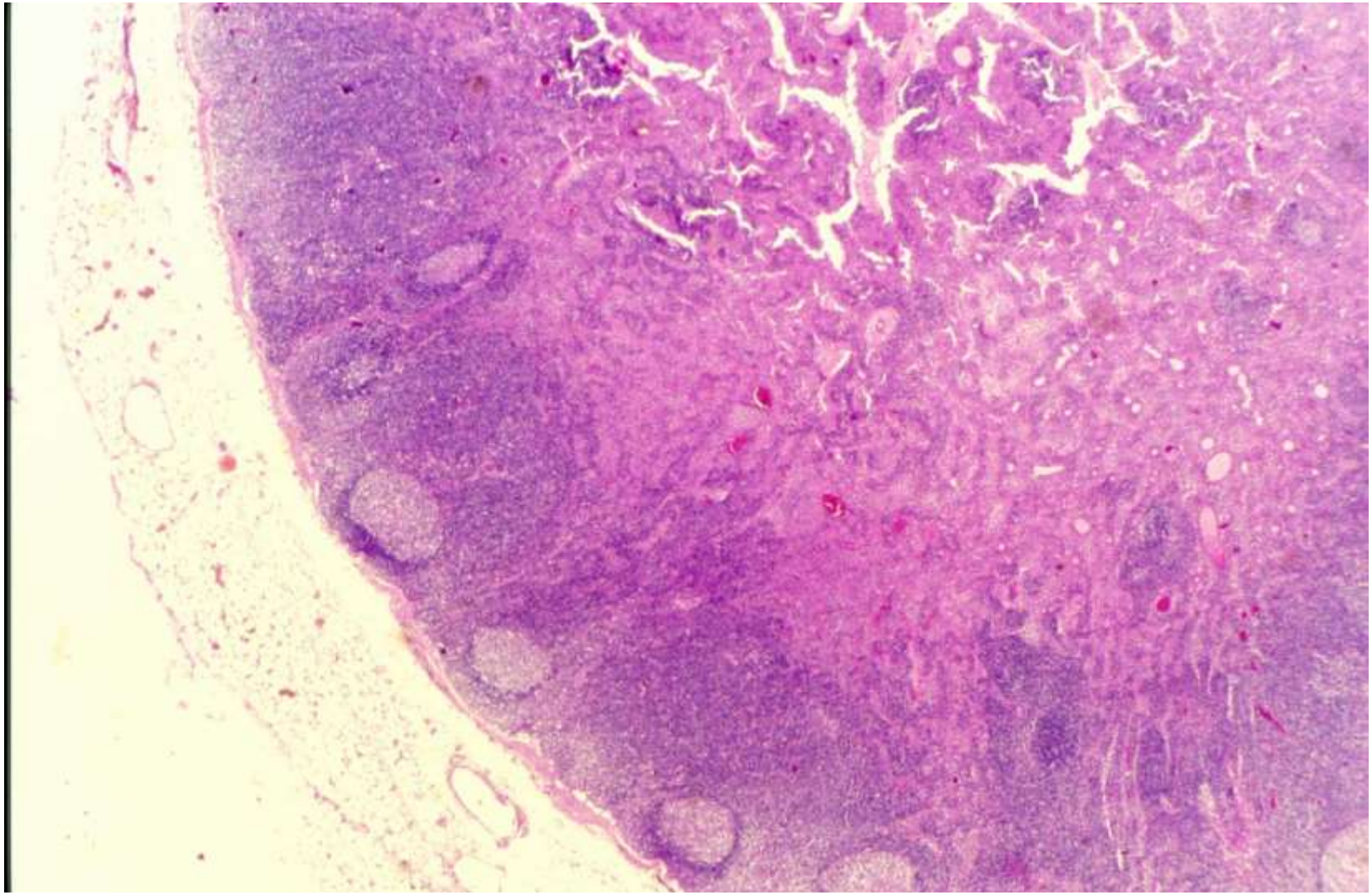

Mesenteric Lymph node (H&E), dog, high-dose, cytostatic

Note the "implosion" of follicular centers, reduced cellularity

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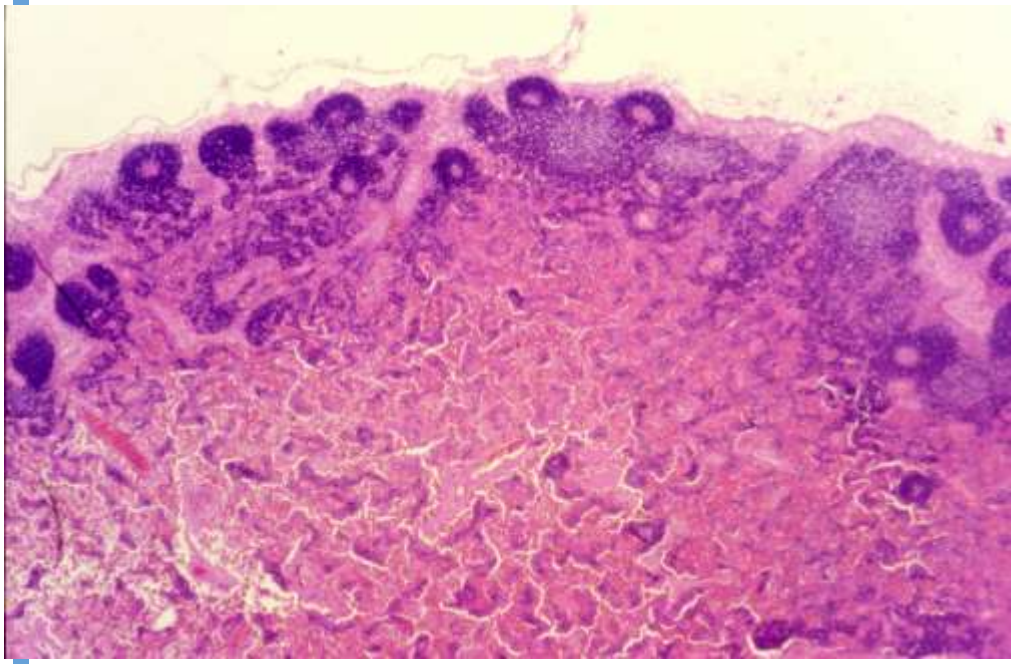

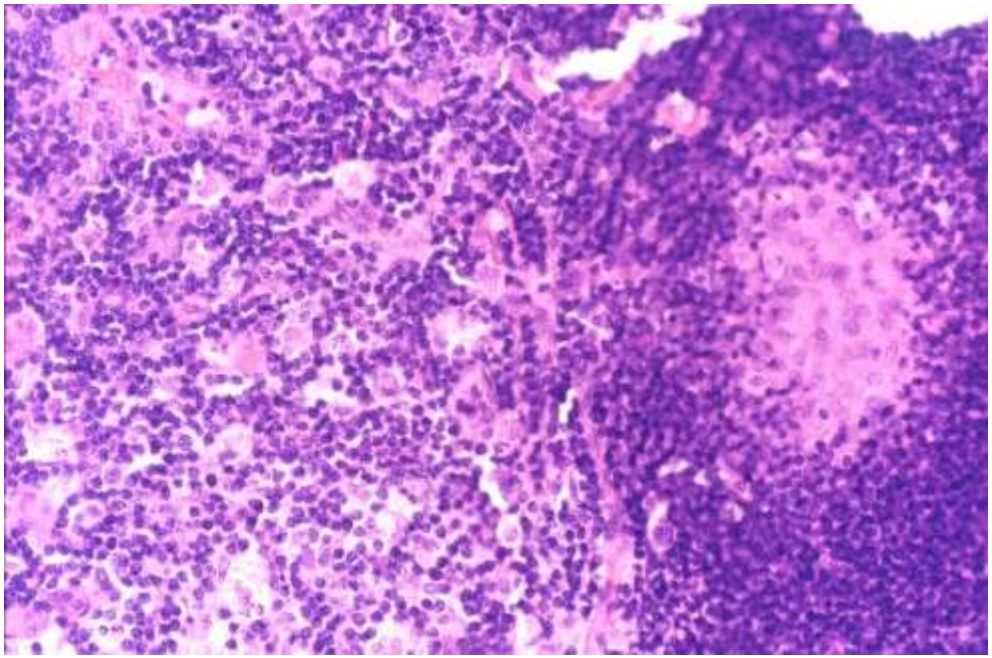

Spleen (H&E), dog, control animal and animal treated with a cytotoxic drug

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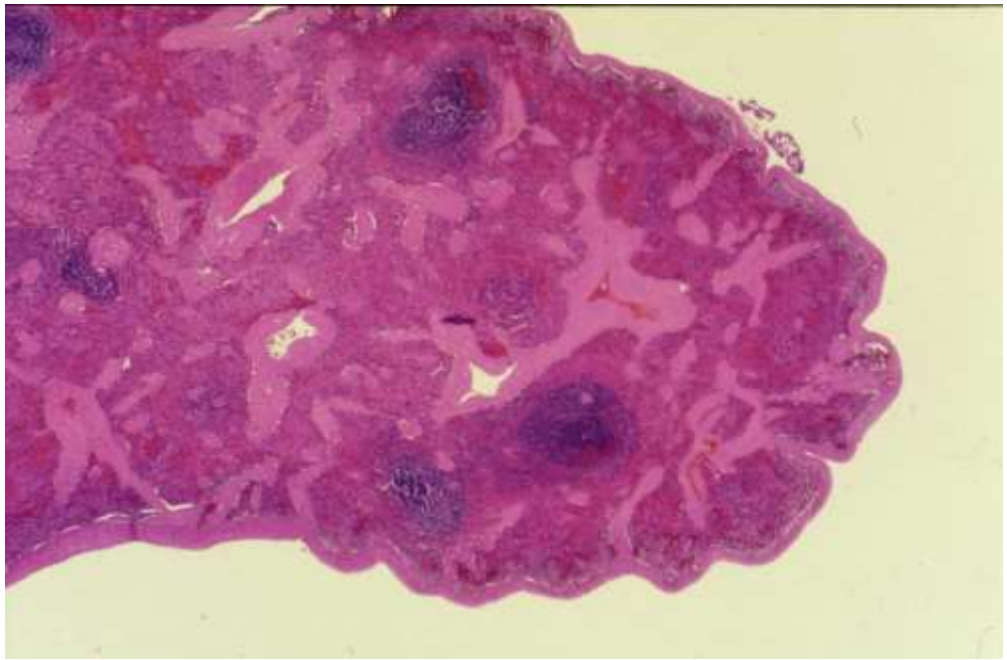
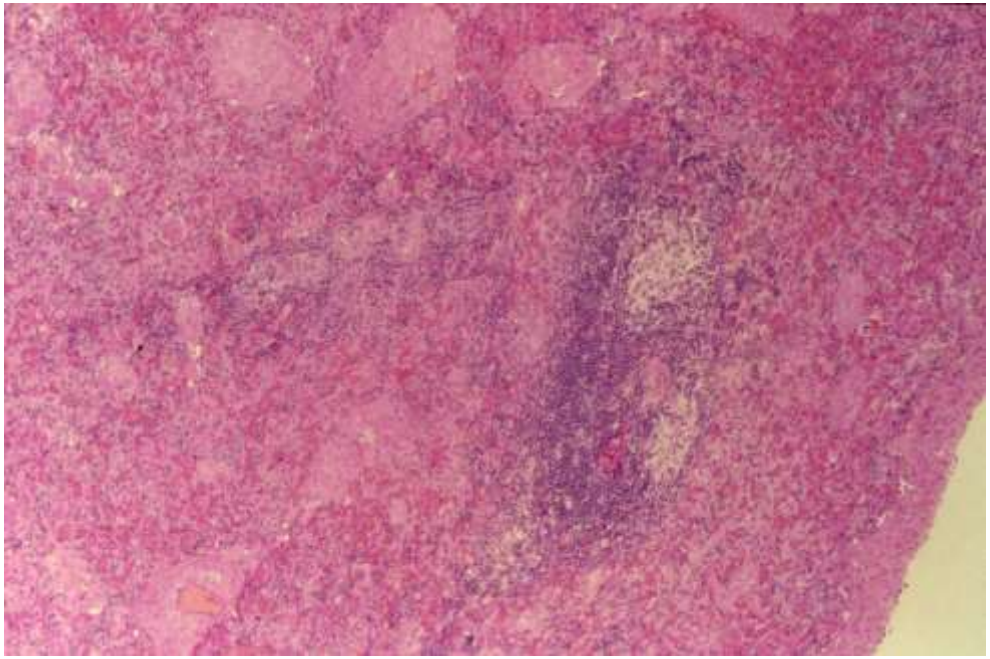


Control



Treated

Lymph node (H&E), rat, control animal (HCB study)

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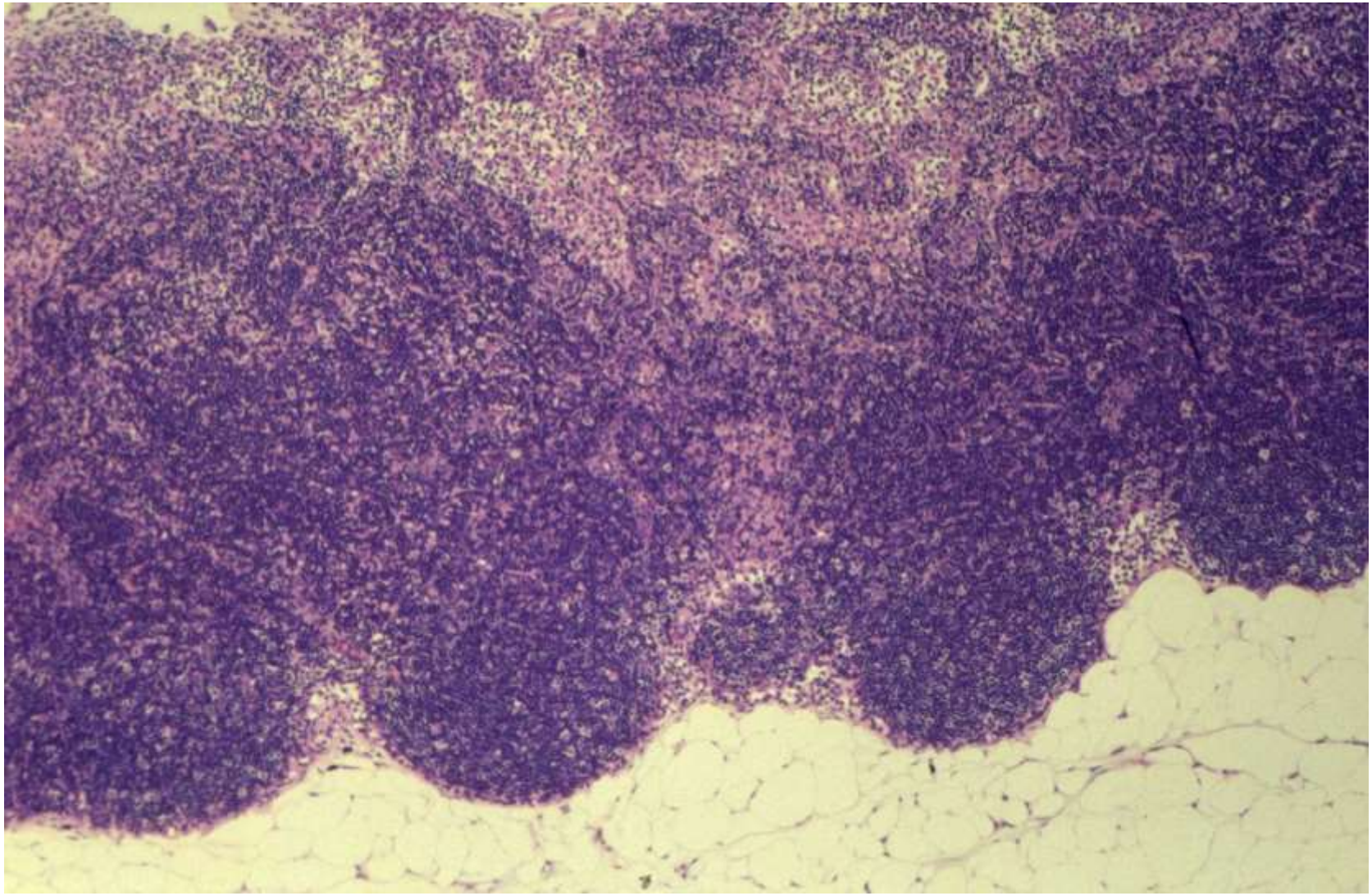

Lymph node (H&E), rat, HCB treated animal

Note the markedly enlarged prominent HEV

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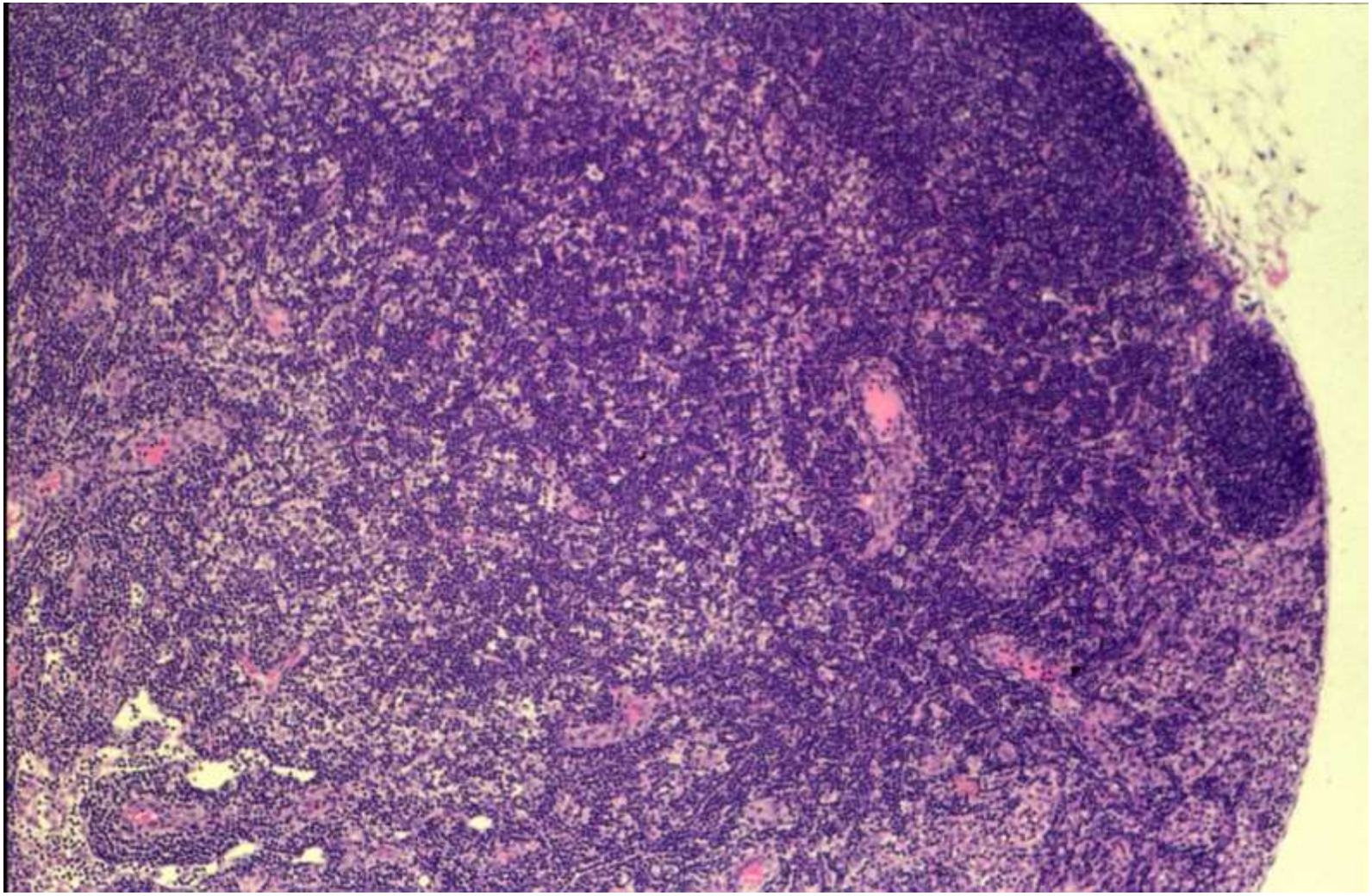

Pyers patch (H&E), rat, HCB treated animal

Note the markedly enlarged prominent HEV

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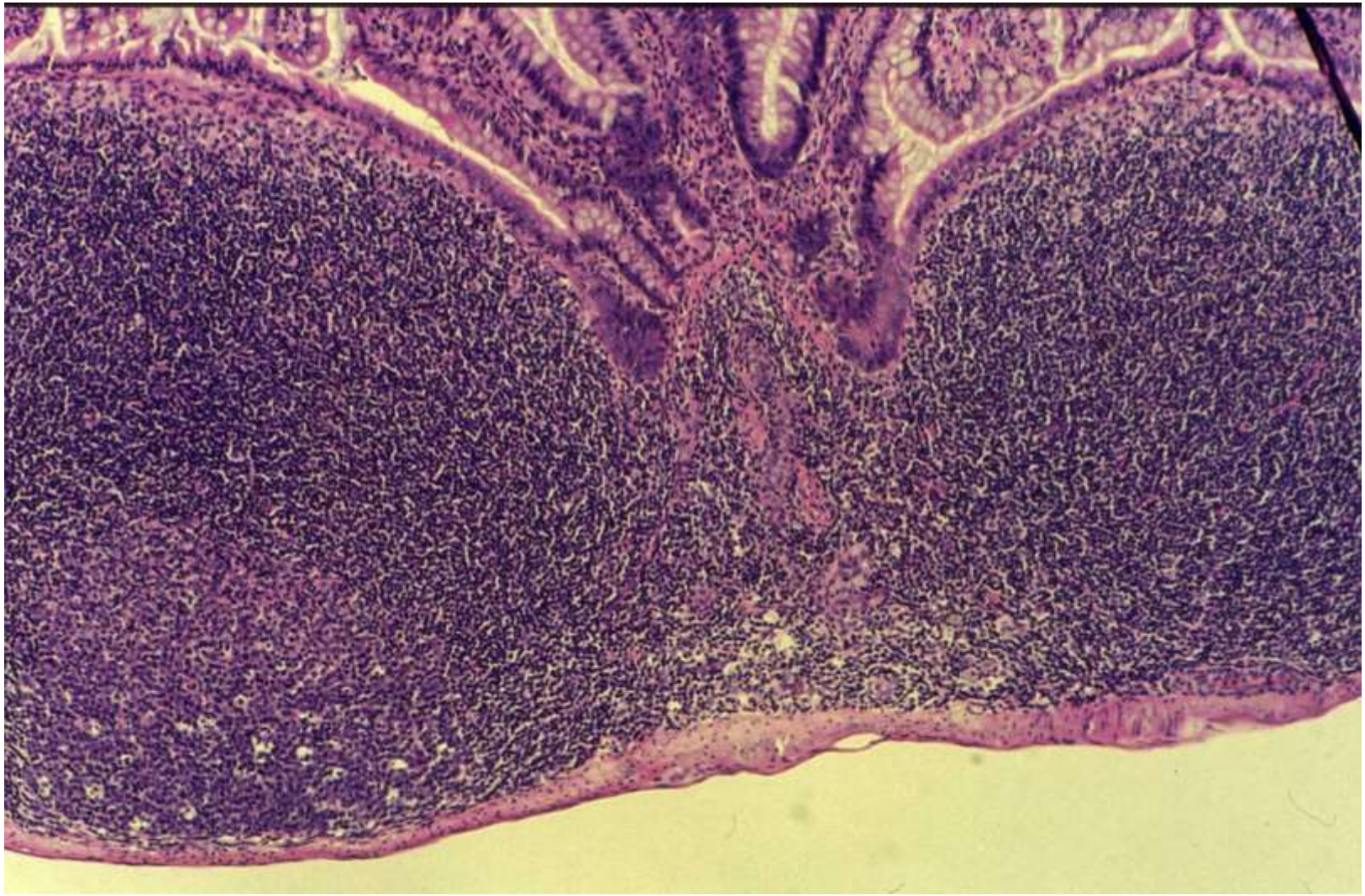

Spleen (H&E), dog, control animal and animal treated intravenously with oily vehicle (adjuvant effect)

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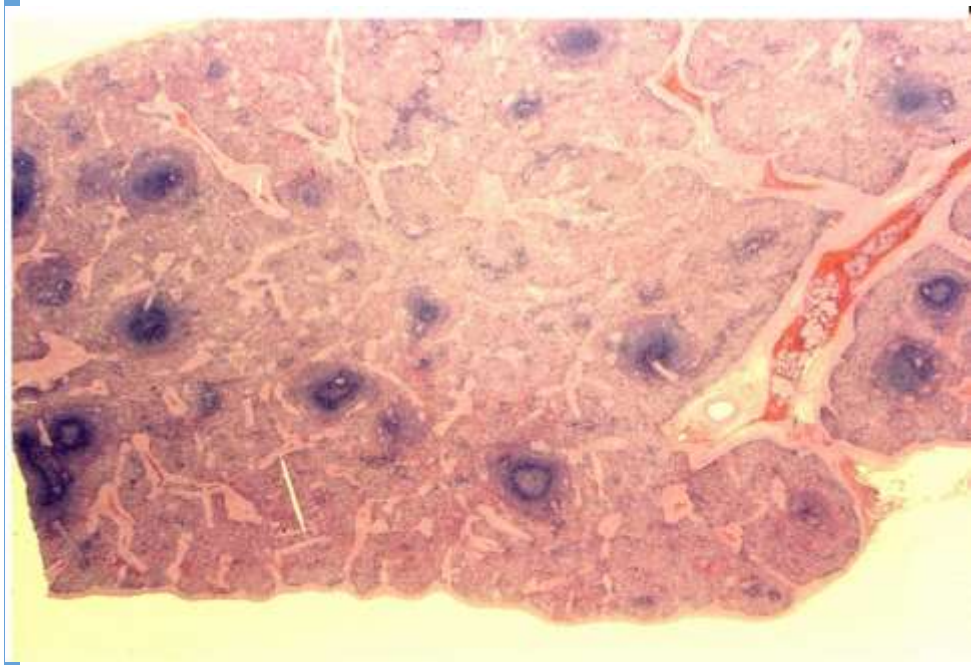
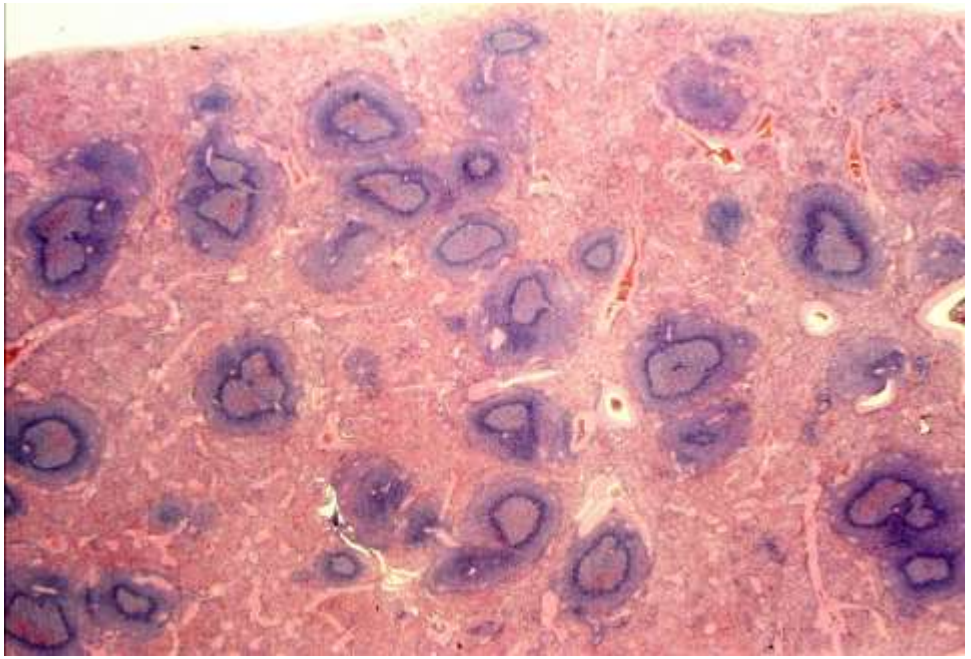
Note the increase in number and size of white pulp





Theoretical and actual immune modulatory effects (1)

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	Non-specific		Specific	
	I Inhibition	II Stimulation	III Stimulation	IV Inhibition (not rep.)
Clinical effect	Decreased resistance to infection	Increased antibody responses Increased incidence of background autoimmune diseases/allergy (non-specific)	Allergy Autoimmunity ANA Contact dermatitis Hemolytic anemia	Decreased resistance to specific infection agents (not reported)
	Increased rate of tumor (including lymphomas	Reduced incidence of tumors (except possibly lymphomas)		
Examples	Cyclosporin Steroids Antitumor drugs (alkylating substances)	Adjuvants used in immunizations, HCB	Heavy metal salts such as Au, Pt and Hg, Nickel, Penicillin	Experimental induction especially in neonates (teratology/reprotox

Theoretical and actual immune modulatory effects (2)

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	Non-specific		Specific	
	I Inhibition	II Stimulation	III Stimulation	IV Inhibition (not rep.)
Mechanism	**Inhibition / immunsuppression**	**Stimulation**	**Stimulation**	**Inhibition (not reported)/ tolerance**
Primary effect Detectable in safety studies	**+**	**+**	**-(?)**	**?**
Specialized studies (MK etc)			**+**	
Secondary clincal effects detectable in routine safety studies	**+**	**+**	**+**	**+**

Conclusions

Assessing Immunotoxicity - Histopathology

- Histopathology is able to “flag” non-specific inhibition and stimulation of the immune system
- The validation studies also showed that immune function tests are highly variable and difficult to standardize
- Specific stimulation (allergy) needs an induction phase and hence is generally not detected in 4-week studies

- …..and: Doses above MTD can lead to “false positive” effects.

Thymus (H&E) control animal

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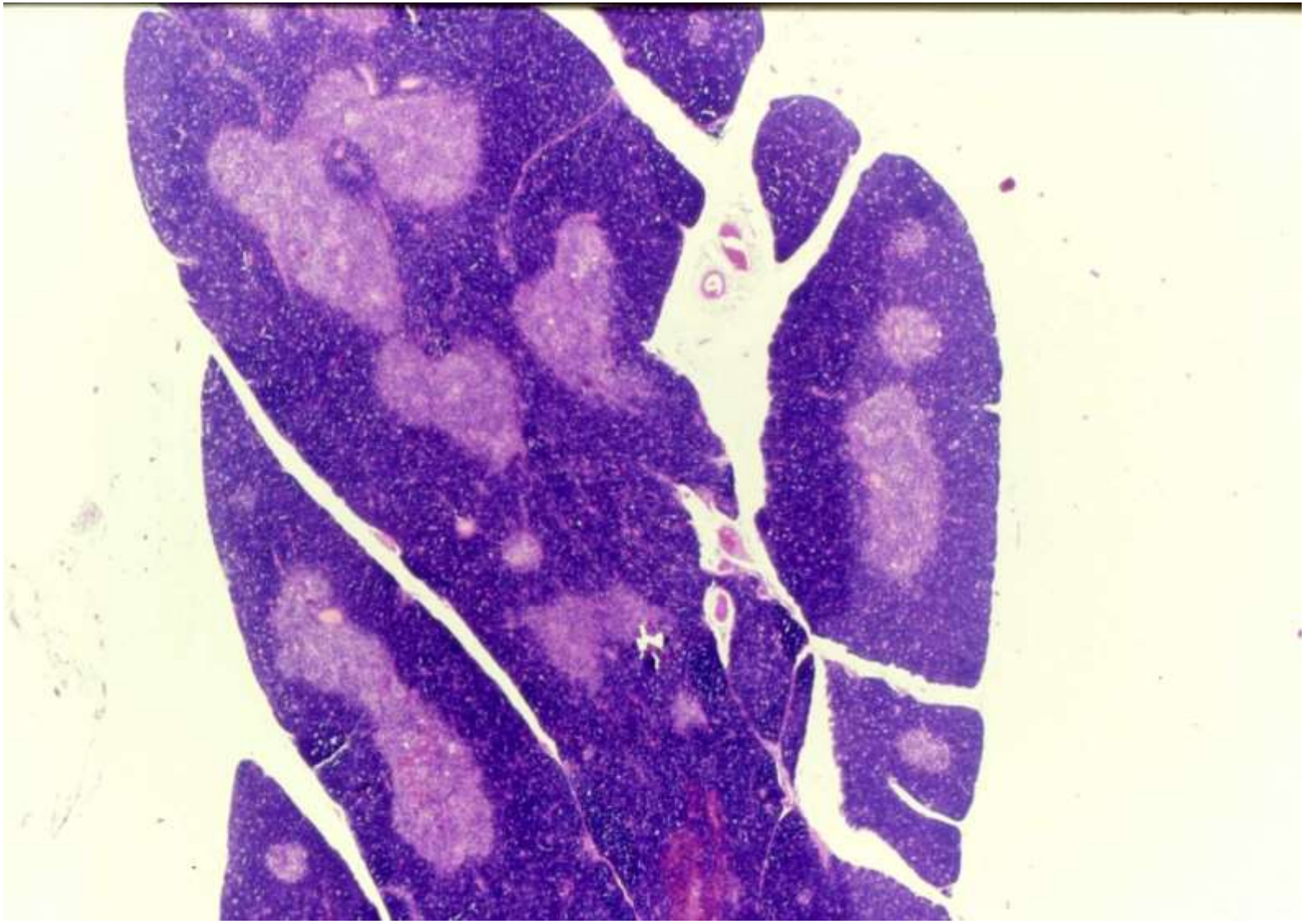

Thymus (H&E), acute toxicity study, intercurrent death

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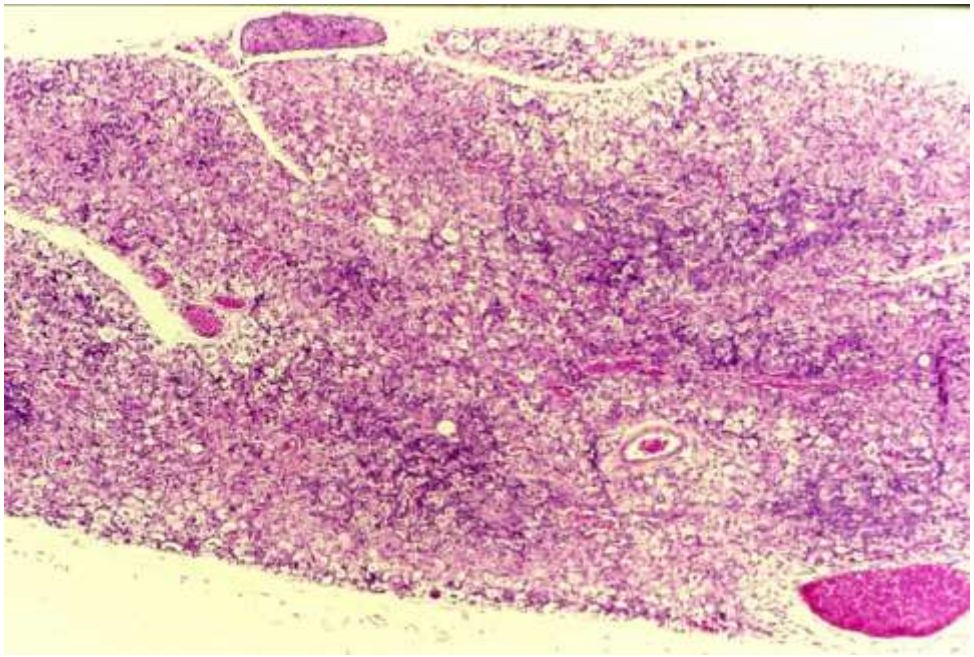

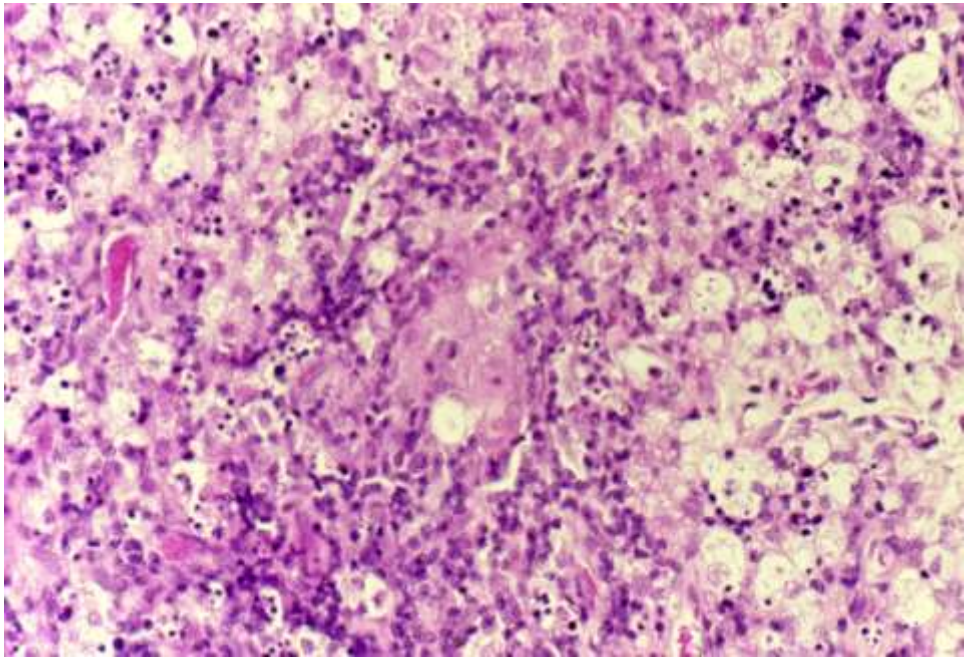

Lymph node of control and treated animal (acute toxicity, intercurrent death)

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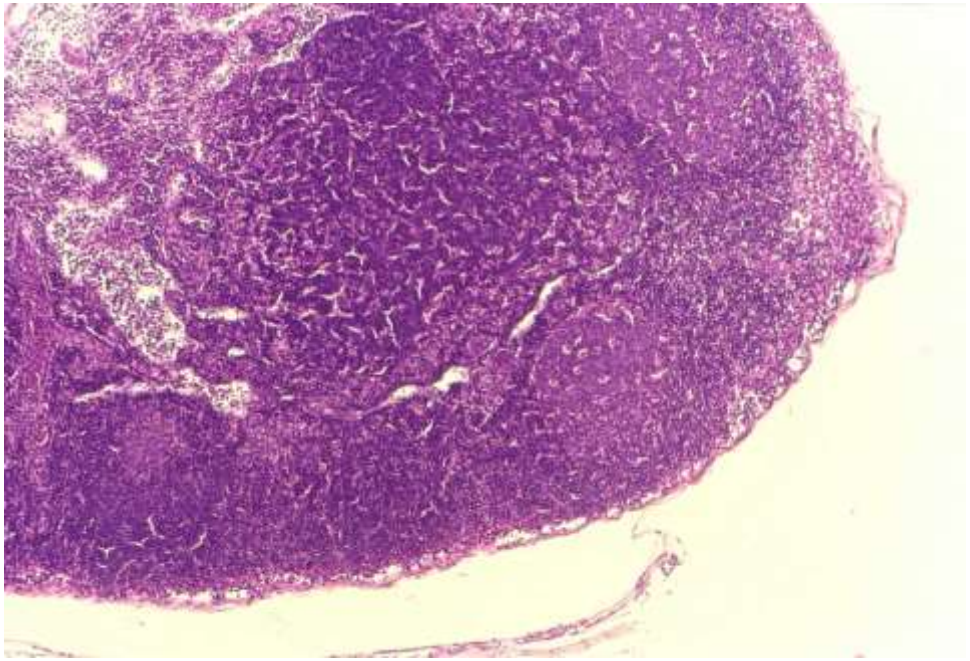
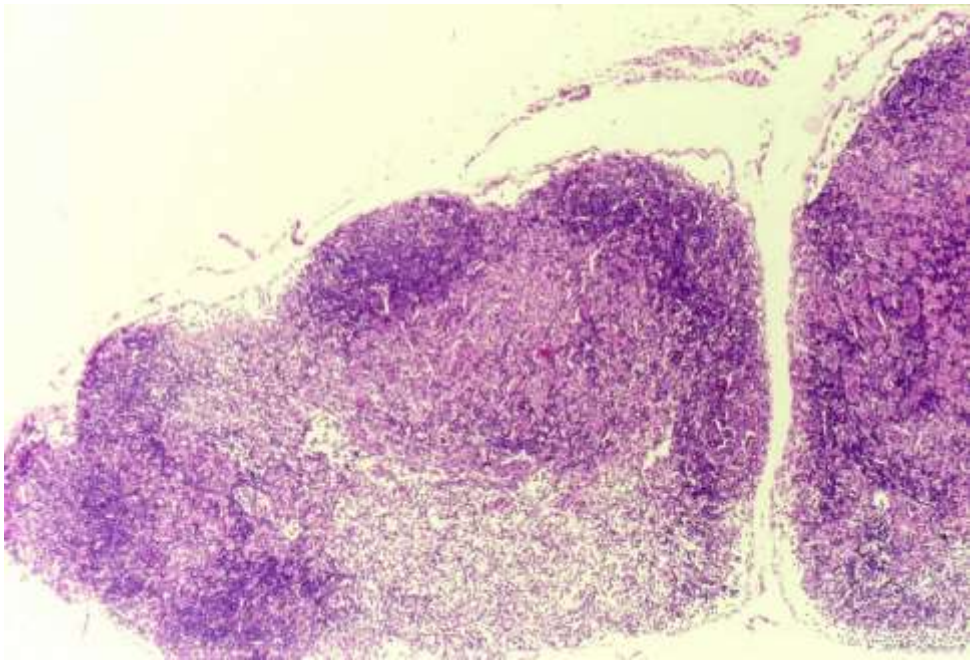


Control



Treated

Warning

By far the majority of the toxicologically induced lesions of the immune system that are detected by in vitro methods are functionally insignificant in the whole animal system and will have absolutely no effect on the health of the animals concerned. The reserve capacity of the mammalian immune system is enormous.

Immunotoxic changes that are seen by histopathological examination have already caused pertubations in cellular relationship and are of potential significance to the health of the animals concerned.

C Gopinath