Fundamental Concepts to Understand Adverse Effects in the Immune/Lymphoid System

Profa. Dra. Maria Lúcia Zaidan Dagli

FMVZ | USP

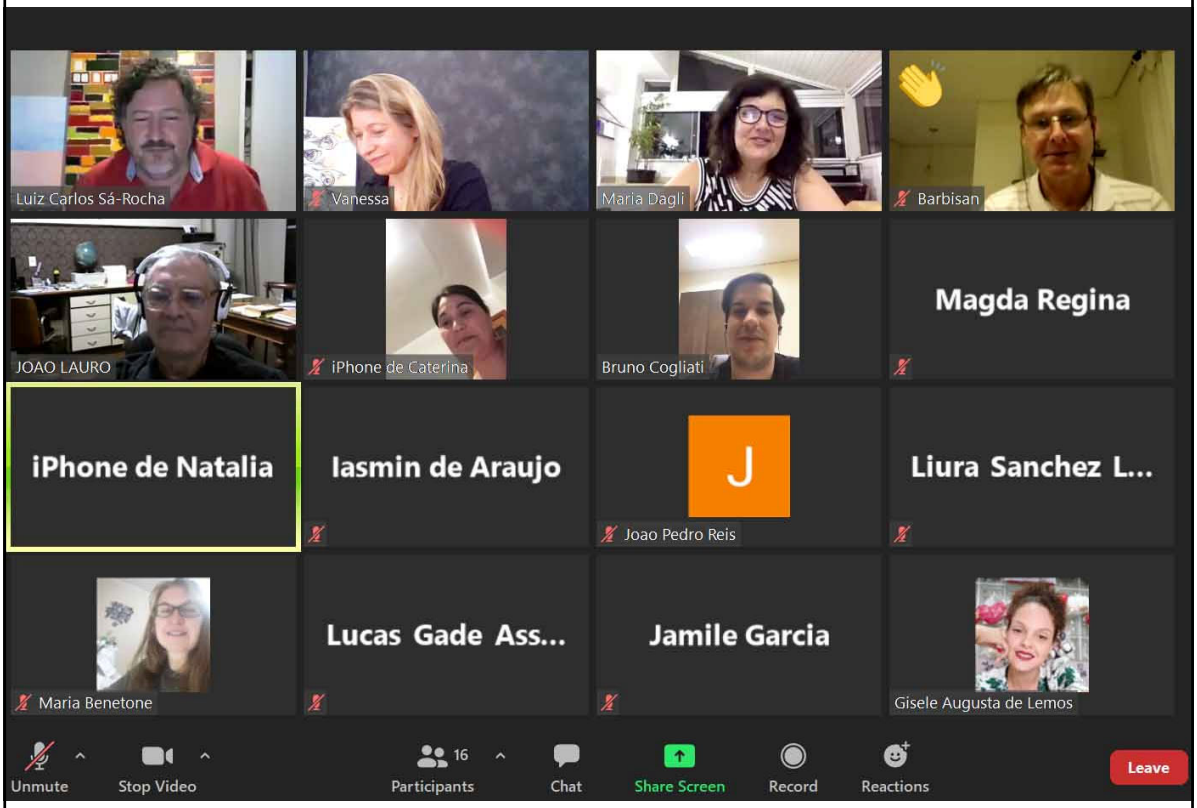
DOCs e “papers” de apoio

LIVE_01_2020_ALAPTE

01_OUTUBRO_2020

ASSOCIAÇÃO LATINO AMERICANA DE PATOLOGIA TOXICOLÓGICA E EXPERIMENTAL

LIVE_01_2020 | ALAPTE
01/10/2020 | 19:39HS
Professora Doutora Maria Lúcia Zaidan Dagli
Departamento de Patologia | FMVZ |USP



1

Fundamental Concepts to Understand Adverse Effects in the Immune/Lymphoid System

Maria Lucia Zaidan Dagli
School of Veterinary Medicine and Animal Science
University of Sao Paulo
Sao Paulo, SP, Brazil
+55 11 30917712
mlzdagli@usp.br

1

Outline of the Presentation

1. Introduction
2. Ontogeny and components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

2

Outline of the Presentation

1. Introduction
2. Ontogeny and components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

3

1. Introduction

- The **lymphoid system** consists of primary **lymphoid** organs, secondary **lymphoid** organs, and lymphatic vessels
- Associated organs that compose the **lymphoid tissue** are the sites of lymphocyte production.
- The **lymphoid system** enables lymphocytes to encounter antigens and it is here that **adaptive immune responses** are initiated.
- The bone marrow produces blood cells that are involved in **innate immune responses**.

4

1. Introduction
What is the importance of detecting adverse effects in the Lymphoid System?

- Regulatory agencies (US and EC) - immunotoxicity testing should be performed on all new investigational drugs and medicinal products.

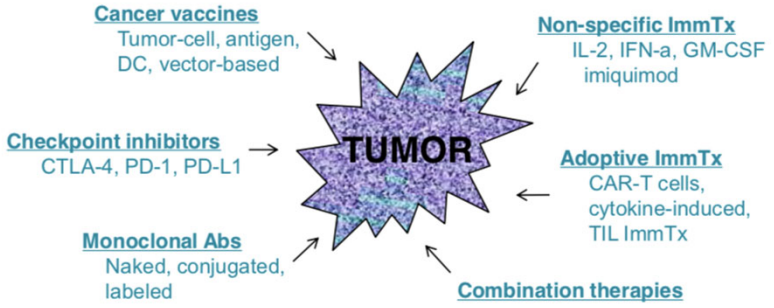
- Gross and histopathological examination of lymphoid tissues are necessary and pivotal steps in the assessment of new drugs for immunotoxic potential.

Haley et al, Toxicologic Pathology,33: 404-407, 2005

5

Types of Immune-modulating Therapies

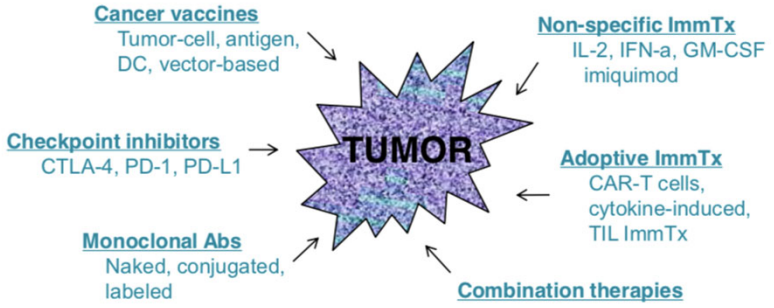
- Historical pharmacologic agents (e.g. steroids)
- Vaccines
- Hyposensitization/tolerance (e.g. allergy shots)
- Cancer immunotherapies



6

Types of Immune-modulating Therapies

- Historical pharmacologic agents (e.g. steroids)
- Vaccines
- Hyposensitization/tolerance (e.g. allergy shots)
- Cancer immunotherapies



7

Outline of the Presentation

1. Introduction
2. Ontogeny and components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

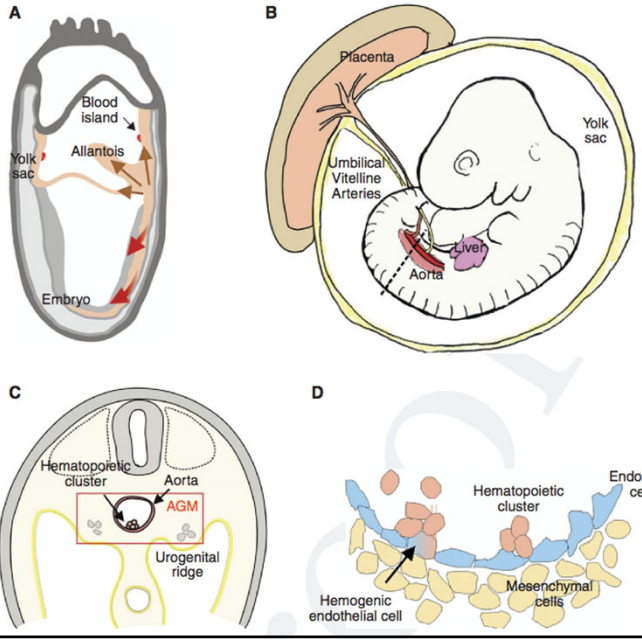
8

2. Ontogeny and Components of the Lymphoid System

- Bone marrow and blood
- Bursa of Fabricius (birds) or Bursa equivalent organ (others)
- Thymus
- Spleen
- Lymph nodes
- MALT – mucosa associated lymphoid tissues
- BALT – bronchus associated lymphoid tissue

9

Ontogeny of the Lymphoid System

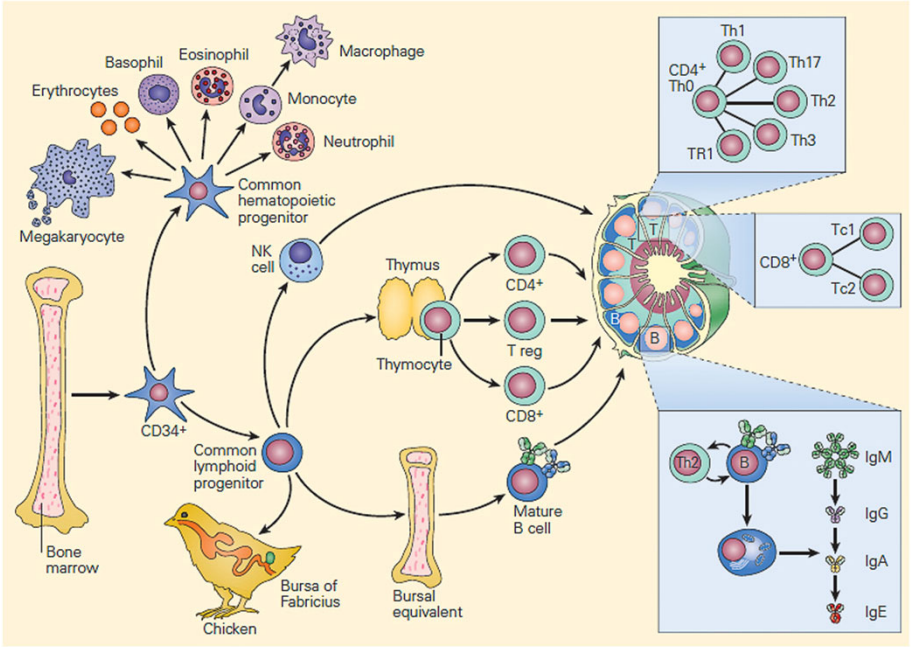


AGM : aorta-gonad-mesonephros
The **aorta-gonad-mesonephros** (**AGM**) is a region of embryonic mesoderm that develops during embryonic development from the para-aortic splanchnopleura in chick, mouse and human embryos. It has been suggested that this area, in particular the ventral wall of the dorsal aorta, is one of the primary origins of the definitive haematopoietic stem cell.

Dzierzak & Philipsen,
Cold Spring Harbor Perspectives in Medicine,
2013 (open access)

10

Ontogeny of the Lymphoid System



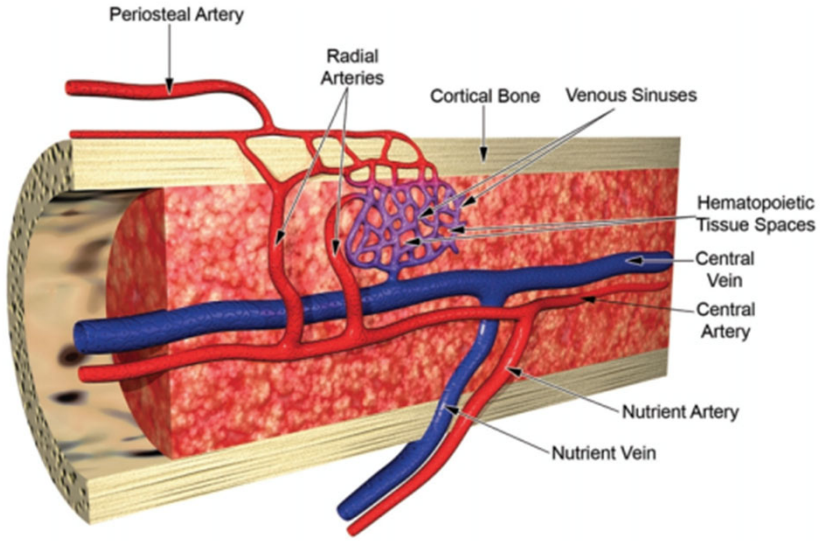
11

Components of the Lymphoid System

- Bone marrow (**primary**)
- Bursa of Fabricius (birds)/Bursa equivalent organ (others) (**primary**)
- Thymus (**primary**)
- Spleen (**secondary**)
- Lymph nodes (**secondary**)
- MALT (**secondary**)
- BALT (**secondary**)

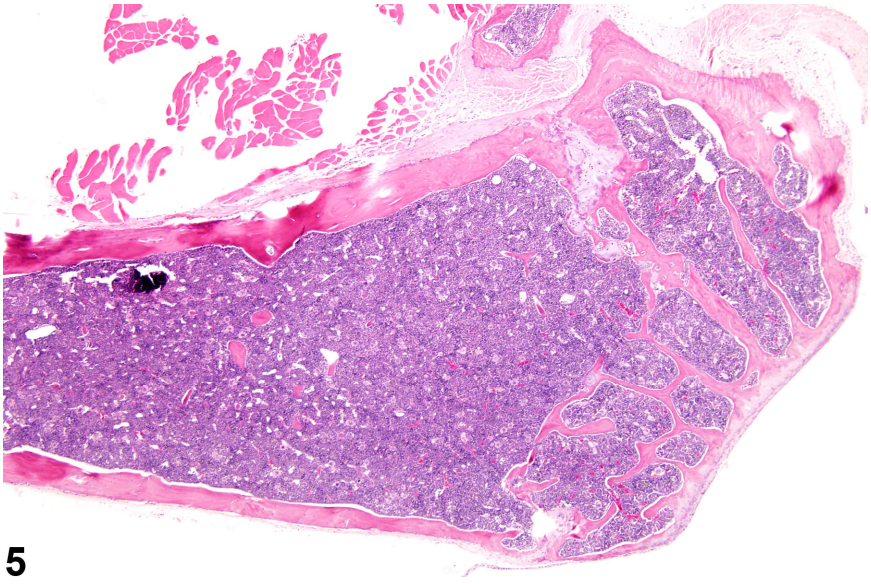
12

Histology of the Bone Marrow



13

Histology of the Bone Marrow

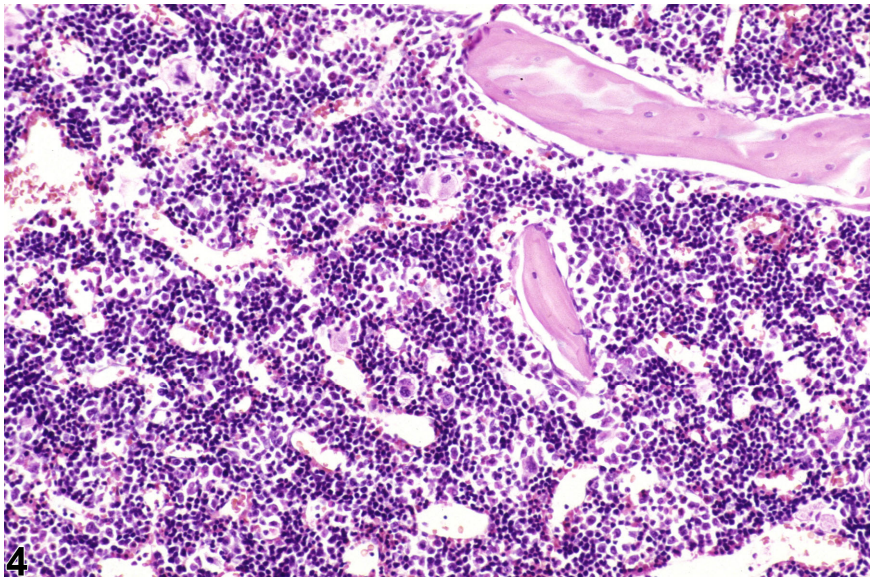


5

From: https://ntp.niehs.nih.gov/nnl/hematopoietic/index.htm

14

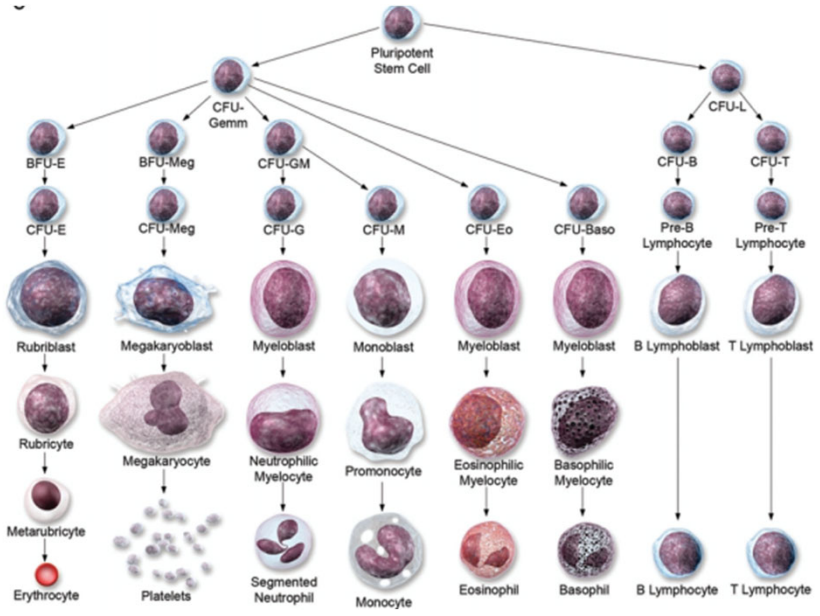
Histology of the Bone Marrow



From: https://ntp.niehs.nih.gov/nnl/hematopoietic/index.htm

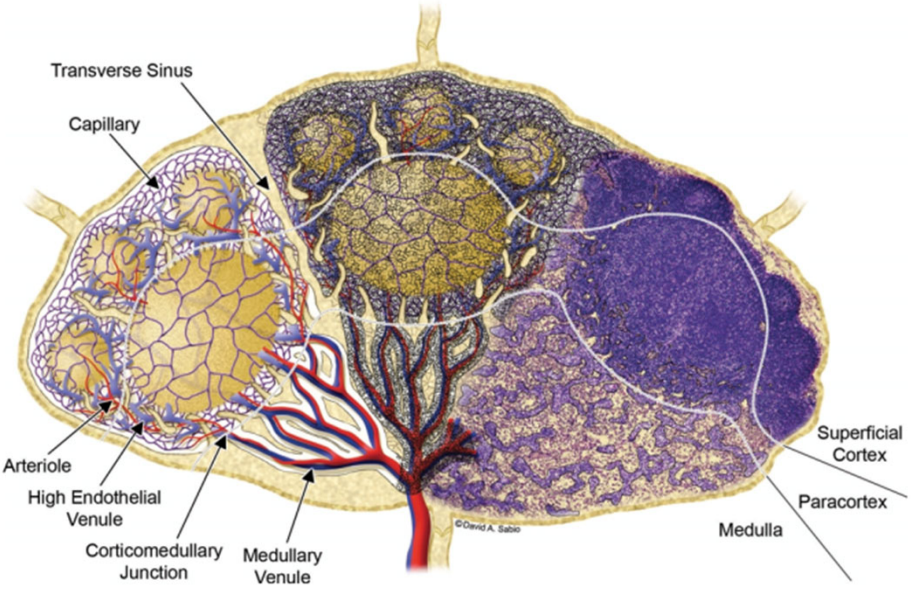
15

Histology of the Bone Marrow



16

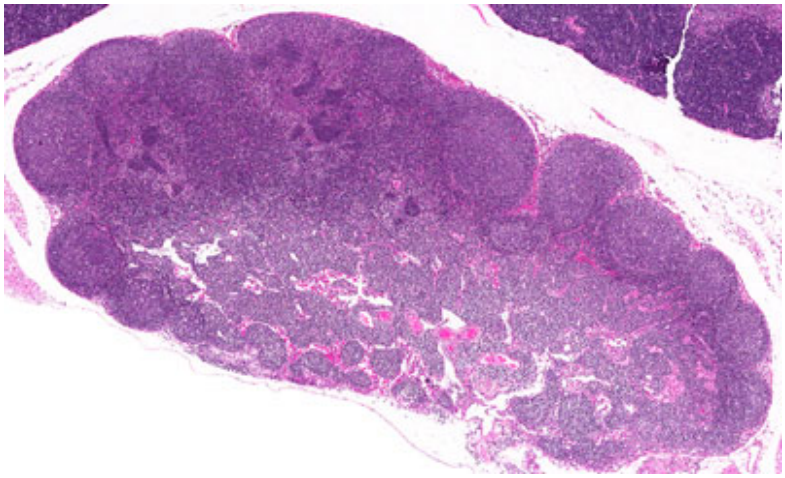
Histology of the Lymph node



Willard-Mack Tox Path 34(5), 2006

17

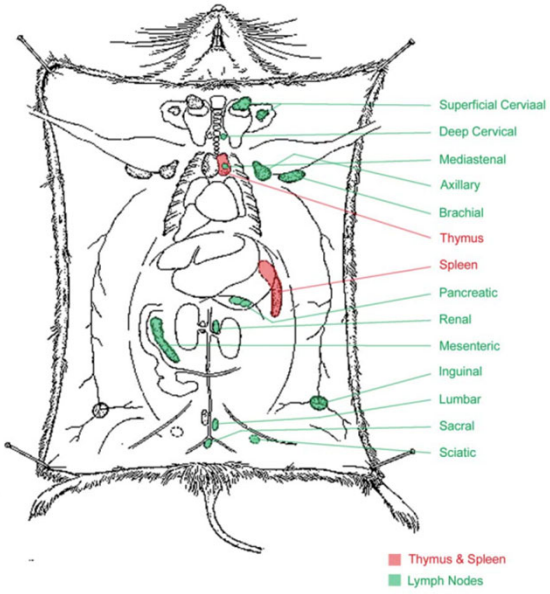
Histology of the Lymph node



From: https://ntp.niehs.nih.gov/nnl/immune/index.htm

18

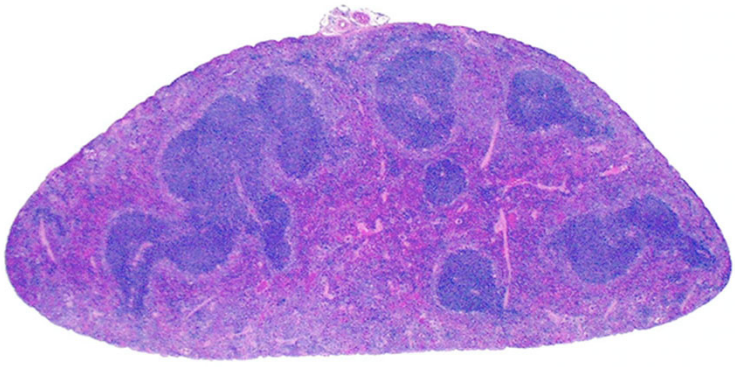
Distribution of Lymph nodes in the mouse

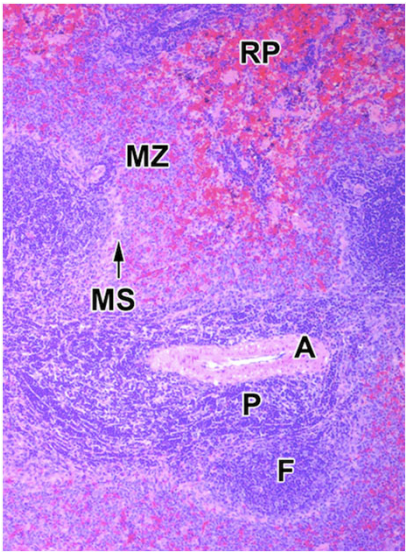


Mouse image from: The virtual mouse necropsy. (http://www.geocities.com/virtualbiology/necropsy.html)

19

Histology of the Spleen



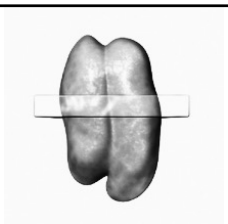


RP= red pulp, A= artery, MZ= marginal zone, MS= marginal sinus, P= PALS, F= follicle, P= periarteriolar sheath

ImageS from a CD provided with Toxicologic Pathology Volume 34, No. 5, 2006

20

Histology of the Thymus



Rat thymus

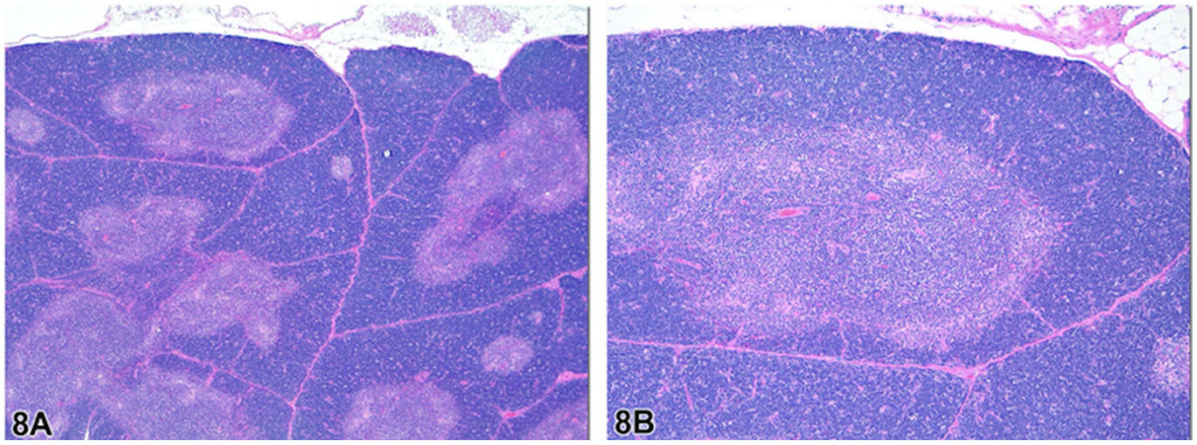
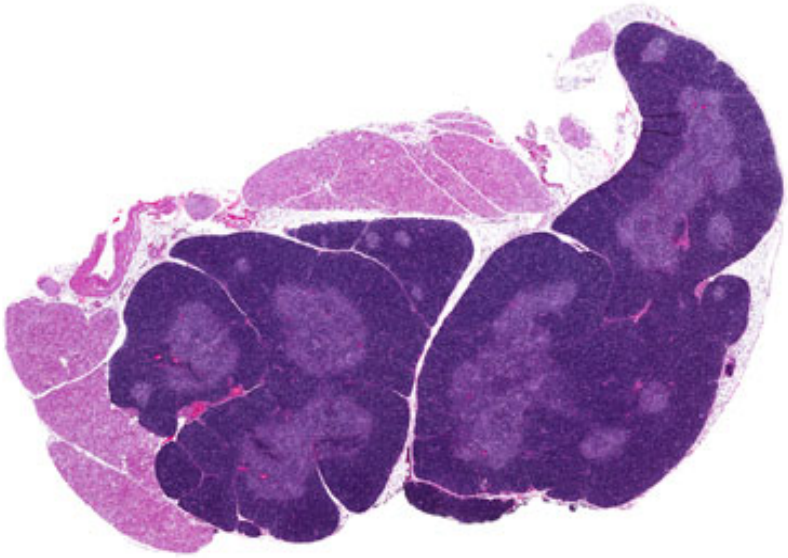


Image from a CD provided with Toxicologic Pathology Volume 34, No. 5, 2006

21

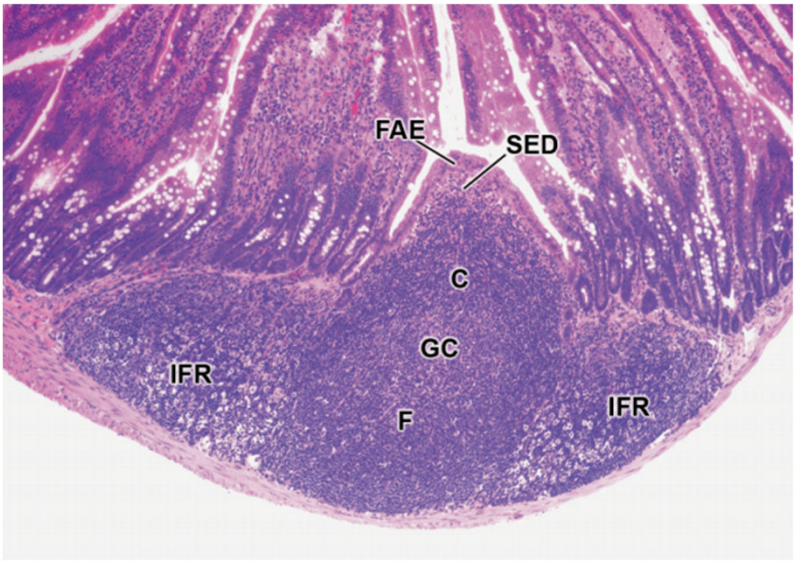
Histology of the Thymus



https://ntp.niehs.nih.gov/nnl/immune/thymus/index.htm

22

MALT – Mucosa associated lymphoid tissue



Cesta MF **Normal Structure, Function, and Histology of Mucosa-Associated Lymphoid Tissue. Tox Path** Volume 34 Issue 5, August 2006

23

Outline of the Presentation

1. Introduction
2. Ontogeny and Components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

24

3. Evaluation of the Lymphoid System

- Clinical pathology
- Lymphoid organ weights
- Histopathology - **compartments**!

Haley et al, Toxicologic Pathology,33: 404-407, 2005

25

Evaluation of the Lymphoid System

a. Is the lymphoid organ macroscopically increased or decreased in size?

b. Which compartment is specifically involved?

c. Is the change in size of the organ due to a change in components (e.g., cells, stroma, edema fluid) of a particular compartment?

d. Is this change in size due to a change in cell numbers in one or more compartments, i.e., microscopically increased or decreased number of cells, and if so which cells are involved ?

Haley et al, Toxicologic Pathology,33: 404-407, 2005

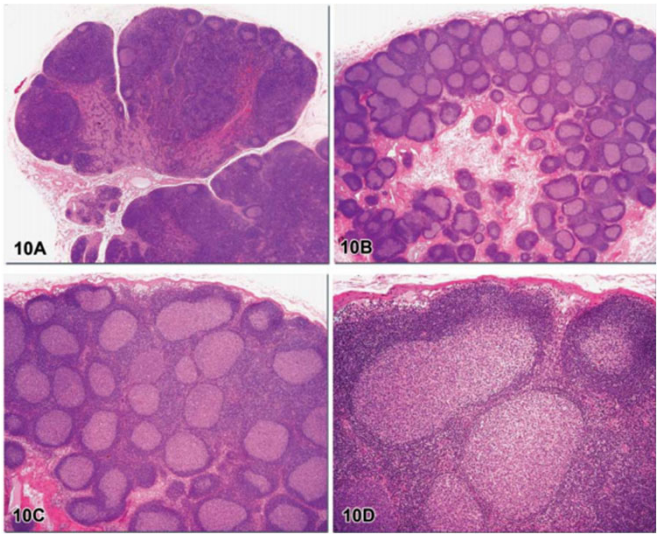
26

Evaluation of the Lymphoid System Enhanced histopathology approach

- Enhanced histopathology approach is based on overall evaluation of lymphoid organs/tissue and their compartments.
- It may involve semi quantitative analysis of the B, T and other compartments (cellular constituents, alterations in numbers, location, composition).
- Enhanced histopathology approach may use immunohistochemistry to detect specific lymphoid cell types

27

Evaluation of the Lymphoid System Enhanced histopathology approach

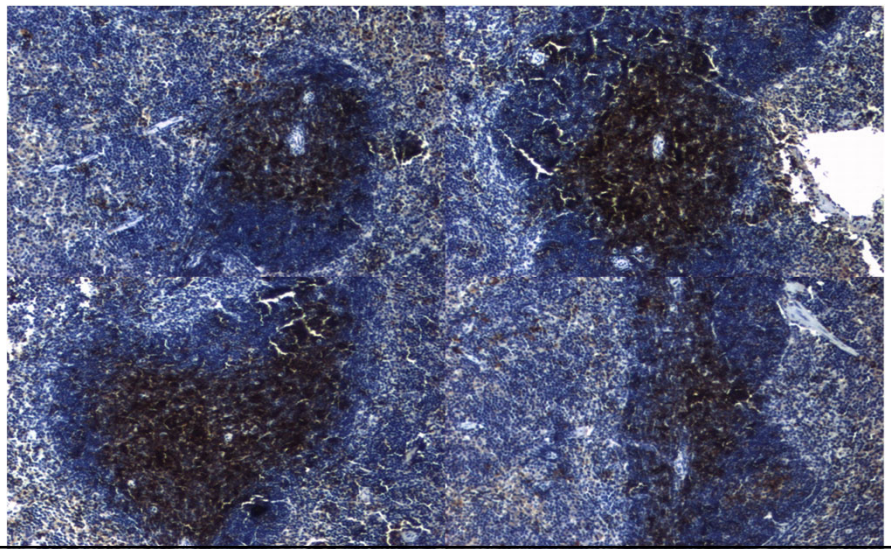


Elmore - Tox Path 34: 634-647, 2006

28

Evaluation of the Lymphoid System Enhanced histopathology approach

CD3+ cells



29

Evaluation of the Lymphoid System Enhanced histopathology approach example

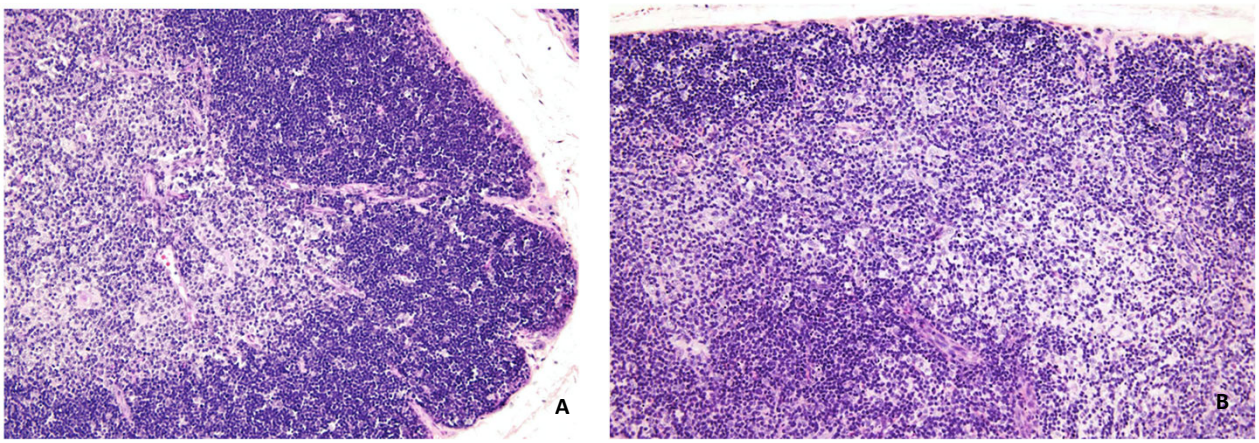
- Checklist for histopathological evaluation of the **thymus**
  - Cortex
  - Medulla
  - Cortex/medula ratio
  - Epithelium free areas (EFAs)
  - Other
    - Inflammation
    - Cysts
    - Pigments
    - Extramedullary hematopoiesis

- Obs. Answers must include information on:
  - increases/decreases
  - the severity/grades

Elmore SA 2006. Enhanced histopathology of the thymus Tox Path 34:656-665

30

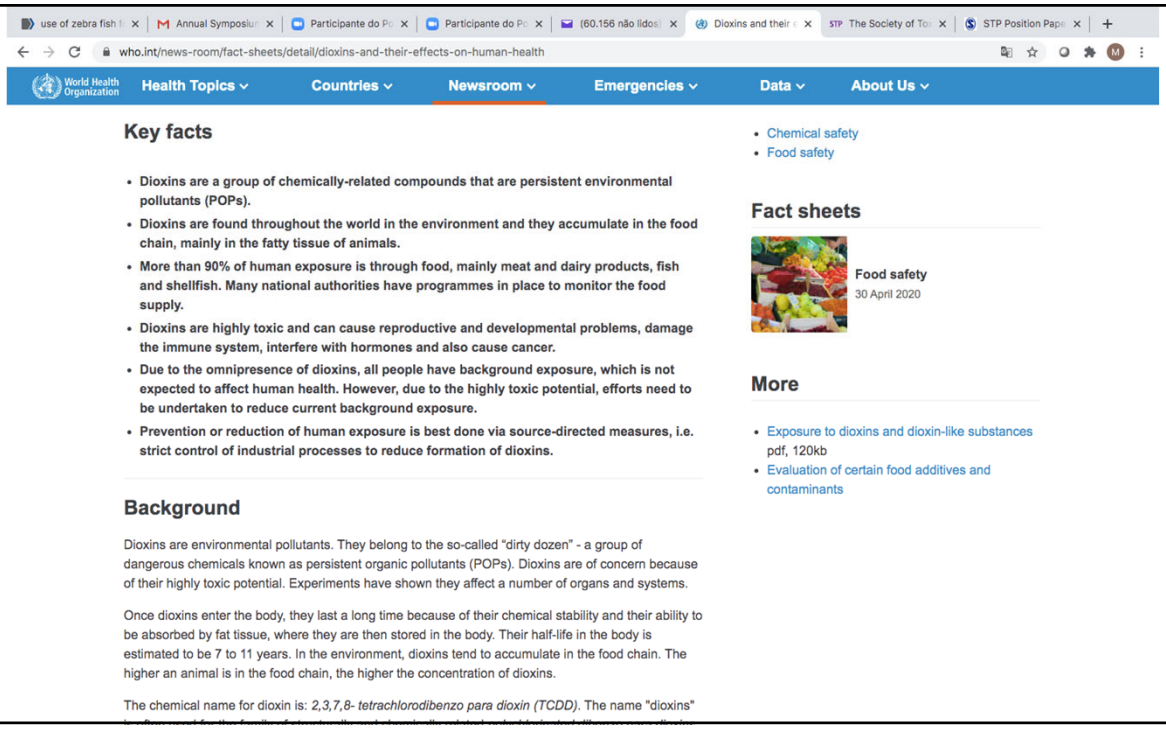
Evaluation of the Lymphoid System
Enhanced histopathology approach example



Sprague-Dawley rat thymus, treatment with Dioxin
A – control; B- treated

Elmore SA 2006. Enhanced histopathology of the thymus Tox Path 34:656-665

31



32

Outline of the Presentation

1. Introduction
2. Ontogeny and Components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

33

4. Immunotoxicity

- Definition:
- "any adverse effect on the components of and/or function of the immune system by a biological, chemical, or physical agent resulting from either direct or indirect actions and reflecting either permanent or reversible toxicity."

Hinton, Tox Path 28 (3) 467-478, 2000

34

Immunotoxicity – adverse effects
Five Areas of adverse effect categories defined by FDA for the immunotoxicology evaluation of new drugs

a. **Immunosuppression**: Effects on the immune system that result in decreased immune function

b. **Immunogenicity**: Immune reactions elicited by a drug and/or its metabolites

c. **Hypersensitivity**: Immunological sensitization due to a drug and/or its metabolites

d. **Autoimmunity**: Immune reactions to self-antigens

e. **Adverse Immunostimulation**: Activation of immune system effector mechanisms

FDA 2002 – Guidance document.

35

How to characterize immunotoxicity?

- Two levels of testing are defined:
- **Level I** tests do not require further perturbation of the test animal (eg, by injection of test antigen) and can be done with the same animals used in a standard toxicity study (acute, subchronic, and reproduction).
- **Level II** tests are defined as functional tests and usually require a concurrent satellite group of test animals or an additional follow-up study to evaluate immunologic function.

Hinton, Tox Path 28 (3) 467-478, 2000

36

How to characterize immunotoxicity?

- Hematology – including differential cell counting
- Total serum immunoglobulin level
- Weights of lymphoid organs (thymus, spleen, lymph nodes, mesenteric and popliteal)
- Histopathology of lymphoid tissues (thymus, spleen, lymph nodes, mucosa associated lymphoid tissue)
- Immunohistochemistry of lymphoid tissues
- Flow cytometry of lymphoid suspensions

Van Loveren et al., Therap Innov and Reg Sci, 1996

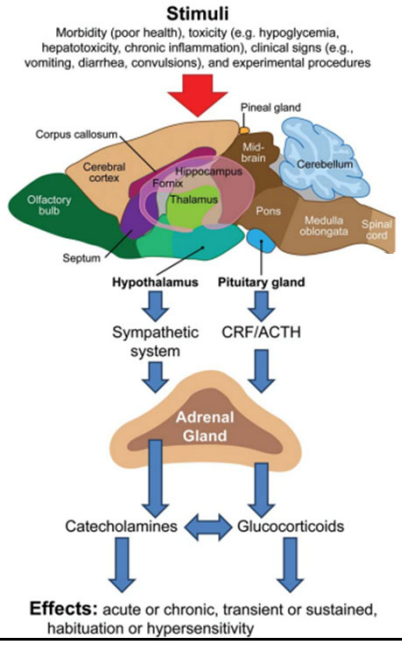
37

Outline of the Presentation

1. Introduction
2. Ontogeny and Components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

38

Stress and the Lymphoid System



Systems affected: In-life parameters, hematology, organ weights, immune system, endocrine, reproductive, neural, clinical signs

Everds et al., ToxPath 41: 560-614, 2013

39

Stress and the Lymphoid System

- Glucocorticoids induce the **apoptosis of lymphocytes** and alter leukocyte migration and redistribution;
- A major component of their action is the **inhibition of cytokines**, resulting in a decreased release of interleukins (IL), interferons (IFN) and tumor necrosis factor (TNF), such as IL-2, IL-6, IFN-γ and TNF-α.

Meier, CA, https://doi.org/10.1530/eje.0.1340050

40

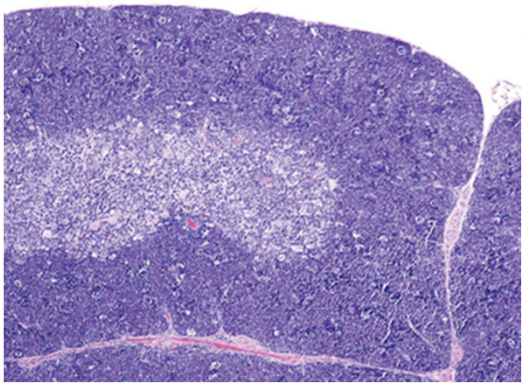
Stress and the Lymphoid System

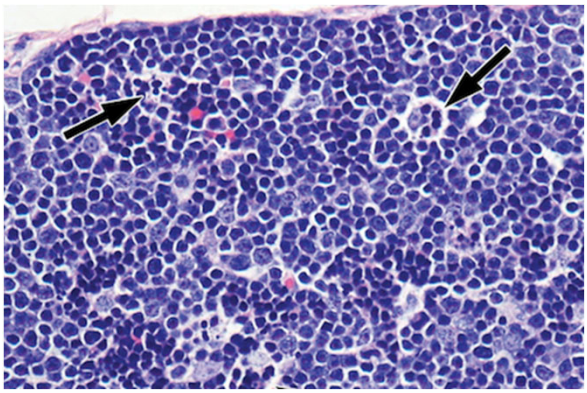
- Stress effects on organ weights :
  - Thymus weight decreased
  - Spleen weight decreased
- Stress induced alterations in histology :
  - Thymic cellularity decreased
  - Spleen cellularity decreased.

Everds et al., ToxPath 41: 560-614, 2013

41

Stress and the Thymus





Pearse, Tox Path 34(5), 2006

42

Outline of the Presentation

1. Introduction
2. Ontogeny and Components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

43

Importance of Lymphoid System in juvenile studies

- A number of regulatory guidelines have emerged to address the safety of drugs intended for pediatric indications.
- Developmental immunotoxicity (DIT) testing - exposure to immunotoxicants early in development may result in **enhanced susceptibility of, or unique or more persistent effects on, the immune system, in comparison to adult exposure.**
- The best approach to DIT is to address the possible impacts of exposure during all of the critical windows of development

HOLSAPPLE AND O'LONE, Toxicologic Pathology, 40: 248-254, 2012

44

Importance of Lymphoid System in juvenile studies

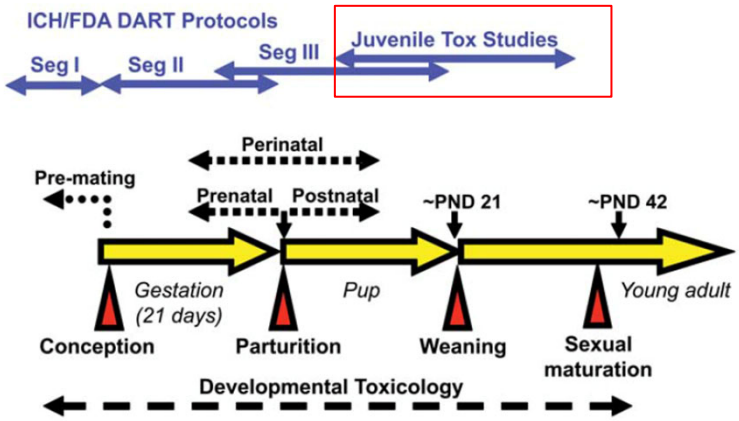


FIGURE 1.—Developmental toxicology timeline in the rat.

HOLSAPPLE AND O'LONE, Toxicologic Pathology, 40: 248-254, 2012

45

Importance of Lymphoid System in juvenile studies

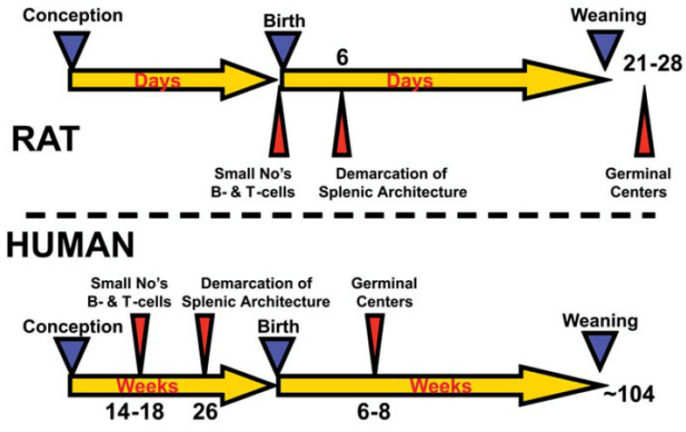


FIGURE 2.—Comparison of the development of the rat and human immune systems.

HOLSAPPLE AND O'LONE, Toxicologic Pathology, 40: 248-254, 2012

46

Importance of Lymphoid System in juvenile studies

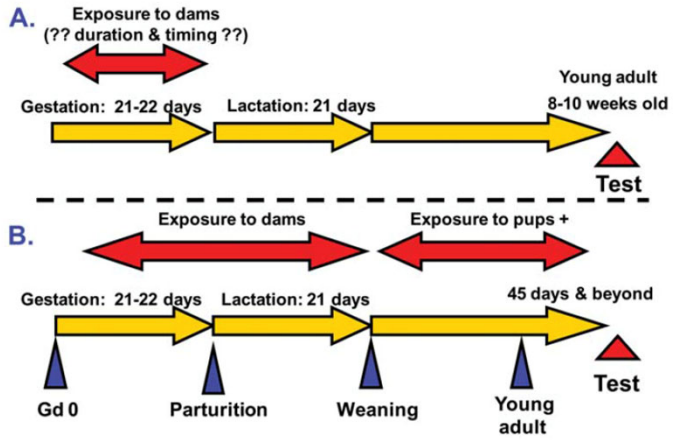
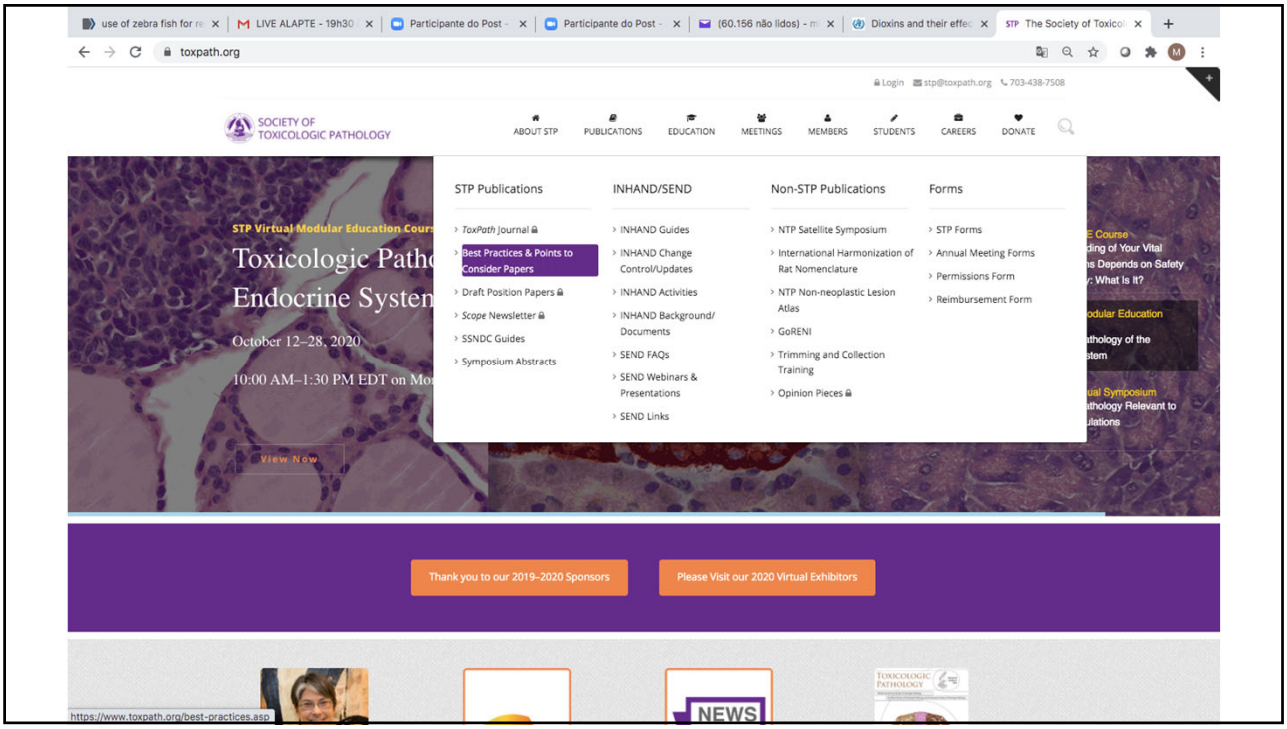


FIGURE 3.—Comparison of DIT protocols.

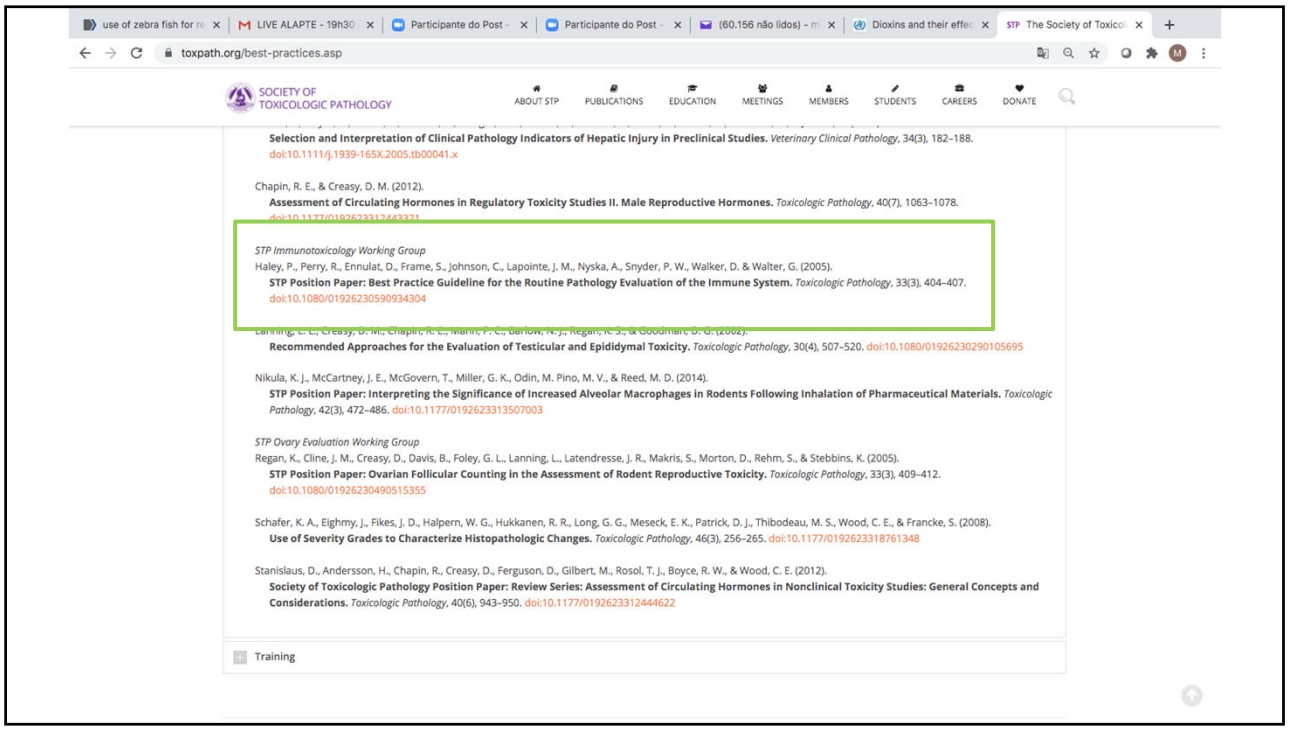
DIT = development immunotoxicity

HOLSAPPLE AND O'LONE, Toxicologic Pathology, 40: 248-254, 2012

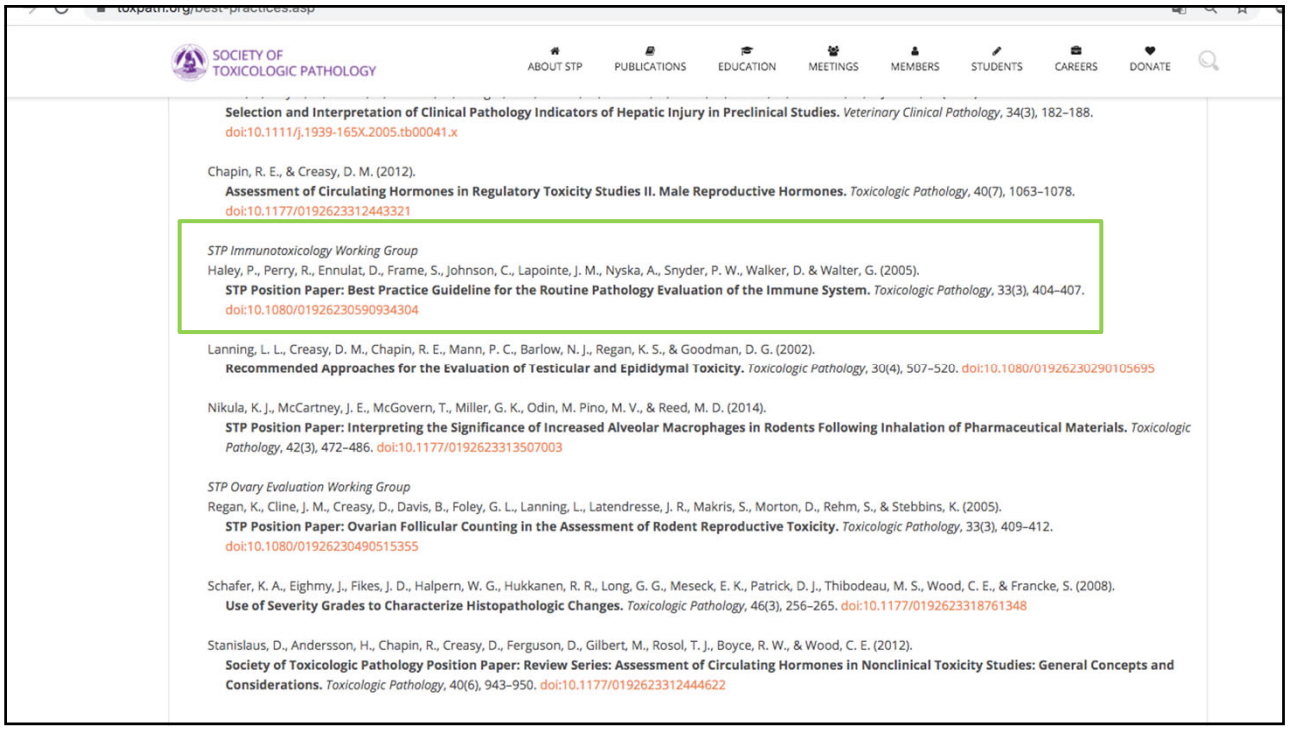
47



48


toxpath.org/best-practices.asp
SOCIETY OF TOXICOLOGIC PATHOLOGY
ABOUT STP
PUBLICATIONS
EDUCATION
MEETINGS
MEMBERS
STUDENTS
CAREERS
DONATE
Selection and Interpretation of Clinical Pathology Indicators of Hepatic Injury in Preclinical Studies. Veterinary Clinical Pathology, 34(3), 182–188. doi:10.1111/j.1939-165X.2005.tb00041.x
Chapin, R. E., & Creasy, D. M. (2012).
Assessment of Circulating Hormones in Regulatory Toxicity Studies II. Male Reproductive Hormones. Toxicologic Pathology, 40(7), 1063–1078.
STP Immunotoxicology Working Group
Haley, P., Perry, R., Ennulat, D., Frame, S., Johnson, C., Lapointe, J. M., Nyska, A., Snyder, P. W., Walker, D. & Walter, G. (2005).
STP Position Paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System. Toxicologic Pathology, 33(3), 404–407. doi:10.1080/01926230590934304
Recommended Approaches for the Evaluation of Testicular and Epididymal Toxicity. Toxicologic Pathology, 30(4), 507–520. doi:10.1080/01926230290105695
Nikula, K. J., McCartney, J. E., McGovern, T., Miller, G. K., Odin, M. Pino, M. V., & Reed, M. D. (2014).
STP Position Paper: Interpreting the Significance of Increased Alveolar Macrophages in Rodents Following Inhalation of Pharmaceutical Materials. Toxicologic Pathology, 42(3), 472–486. doi:10.1177/0192623313507003
STP Ovary Evaluation Working Group
Regan, K., Cline, J. M., Creasy, D., Davis, B., Foley, G. L., Lanning, L., Latendresse, J. R., Makris, S., Morton, D., Rehm, S., & Stebbins, K. (2005).
STP Position Paper: Ovarian Follicular Counting in the Assessment of Rodent Reproductive Toxicity. Toxicologic Pathology, 33(3), 409–412. doi:10.1080/01926230490515355
Schafer, K. A., Eighmy, J., Fikes, J. D., Halpern, W. G., Hukkanen, R. R., Long, G. G., Meseck, E. K., Patrick, D. J., Thibodeau, M. S., Wood, C. E., & Francke, S. (2008).
Use of Severity Grades to Characterize Histopathologic Changes. Toxicologic Pathology, 46(3), 256–265. doi:10.1177/0192623318761348
Stanislaus, D., Andersson, H., Chapin, R., Creasy, D., Ferguson, D., Gilbert, M., Rosol, T. J., Boyce, R. W., & Wood, C. E. (2012).
Society of Toxicologic Pathology Position Paper: Review Series: Assessment of Circulating Hormones in Nonclinical Toxicity Studies: General Concepts and Considerations. Toxicologic Pathology, 40(6), 943–950. doi:10.1177/0192623312444622
Training

49


SOCIETY OF TOXICOLOGIC PATHOLOGY
ABOUT STP
PUBLICATIONS
EDUCATION
MEETINGS
MEMBERS
STUDENTS
CAREERS
DONATE
Selection and Interpretation of Clinical Pathology Indicators of Hepatic Injury in Preclinical Studies. Veterinary Clinical Pathology, 34(3), 182–188. doi:10.1111/j.1939-165X.2005.tb00041.x
Chapin, R. E., & Creasy, D. M. (2012).
Assessment of Circulating Hormones in Regulatory Toxicity Studies II. Male Reproductive Hormones. Toxicologic Pathology, 40(7), 1063–1078. doi:10.1177/0192623312443321
STP Immunotoxicology Working Group
Haley, P., Perry, R., Ennulat, D., Frame, S., Johnson, C., Lapointe, J. M., Nyska, A., Snyder, P. W., Walker, D. & Walter, G. (2005).
STP Position Paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System. Toxicologic Pathology, 33(3), 404–407. doi:10.1080/01926230590934304
Lanning, L. L., Creasy, D. M., Chapin, R. E., Mann, P. C., Barlow, N. J., Regan, K. S., & Goodman, D. G. (2002).
Recommended Approaches for the Evaluation of Testicular and Epididymal Toxicity. Toxicologic Pathology, 30(4), 507–520. doi:10.1080/01926230290105695
Nikula, K. J., McCartney, J. E., McGovern, T., Miller, G. K., Odin, M. Pino, M. V., & Reed, M. D. (2014).
STP Position Paper: Interpreting the Significance of Increased Alveolar Macrophages in Rodents Following Inhalation of Pharmaceutical Materials. Toxicologic Pathology, 42(3), 472–486. doi:10.1177/0192623313507003
STP Ovary Evaluation Working Group
Regan, K., Cline, J. M., Creasy, D., Davis, B., Foley, G. L., Lanning, L., Latendresse, J. R., Makris, S., Morton, D., Rehm, S., & Stebbins, K. (2005).
STP Position Paper: Ovarian Follicular Counting in the Assessment of Rodent Reproductive Toxicity. Toxicologic Pathology, 33(3), 409–412. doi:10.1080/01926230490515355
Schafer, K. A., Eighmy, J., Fikes, J. D., Halpern, W. G., Hukkanen, R. R., Long, G. G., Meseck, E. K., Patrick, D. J., Thibodeau, M. S., Wood, C. E., & Francke, S. (2008).
Use of Severity Grades to Characterize Histopathologic Changes. Toxicologic Pathology, 46(3), 256–265. doi:10.1177/0192623318761348
Stanislaus, D., Andersson, H., Chapin, R., Creasy, D., Ferguson, D., Gilbert, M., Rosol, T. J., Boyce, R. W., & Wood, C. E. (2012).
Society of Toxicologic Pathology Position Paper: Review Series: Assessment of Circulating Hormones in Nonclinical Toxicity Studies: General Concepts and Considerations. Toxicologic Pathology, 40(6), 943–950. doi:10.1177/0192623312444622

50

Outline of the Presentation

1. Introduction
2. Ontogeny and Components of the lymphoid system
3. Evaluation of the lymphoid system
4. Immunotoxicity
5. Stress and the lymphoid system
6. The lymphoid system and juvenile studies.
7. Summary and conclusions

51

7. Summary and Conclusions

- Lymphoid / immune system should be analyzed in standard toxicity studies (acute, subchronic, and DART).
- If required, lymphoid/immune system should be analyzed specifically with functional tests to evaluate immunologic function.
- Assessment of immunotoxicity is highly important in juvenile studies.

52

References

- Cesta MF **Normal Structure, Function, and Histology of Mucosa-Associated Lymphoid Tissue. Tox Path** Volume 34 Issue 5, August 2006
- Dzierzak E, Philipsen S. Erythropoiesis: development and differentiation. Cold Spring Harb Perspect Med. 2013 Apr 1;3(4):a011601. doi:10.1101/cshperspect.a011601.
- Everds NE, Snyder PW, Bailey KL, Bolon B, Creasy DM, Foley GL, Rosol TJ, Sellers T. Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. Toxicol Pathol. 2013;41(4):560-614.
- FDA Guidance Document on Immunotoxicology Evaluation of Investigational New Drugs, 2002.
- Haley P, Perry R, Ennulat D, Frame S, Johnson C, Lapointe JM, Nyska A, Snyder P, Walker D, Walter G; STP Immunotoxicology Working Group. STP position paper: best practice guideline for the routine pathology evaluation of the immune system. Toxicol Pathol. 2005;33(3):404-7
- Hinton DM. US FDA "Redbook II" Immunotoxicity Testing Guidelines and Research in Immunotoxicity Evaluations of Food Chemicals and New Food Proteins. Toxicologic Pathology 28 (3): 467-478, 2000.
- Holsapple and O'Lone. Juvenile Immunotoxicology. Toxicologic Pathology, 40: 248-254, 2012
- Meier CA. Mechanisms of immunosuppression by glucocorticoids. https://doi.org/10.1530/eje.0.1340050
- Osmond DG. The ontogeny and organization of the lymphoid system. J Invest Dermatol. 1985 Jul;85(1 Suppl):2s-9s.
- Toxicologic Pathology 2006;34(5) – all articles
- Van Loveren et al., Testing immunotoxicity of chemicals as a guide for testing approaches for pharmaceuticals. Drug Information Journal, Vol. 30, pp. 275-219. 1996
- http://www.immunopaedia.org.za

53

Toxicologic Pathology, 33:404–407, 2005

ISSN: 0192-6233 print / 1533-1601 online
DOI: 10.1080/01926230590934304

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

STP IMMUNOTOXICOLOGY WORKING GROUP,
P. HALEY,[1] (CHAIR), R. PERRY,[2] (COCHAIR), D. ENNULAT,[3] S. FRAME,[4] C. JOHNSON,[5] J-M LAPOINTE,[6] A. NYSKA,[7]
P. W. SNYDER,[8] D. WALKER,[2] AND G. WALTER[9]

[1]*AstraZeneca, Wilmington, DE 19850-5437*
[2]*Wyeth Research, Chazy, NY 12921*
[3]*GlaxoSmithKline, King of Prussia, PA 19406*
[4]*DuPont Company, Haskell Lab for Environmental Sciences Toxicology and Industrial Medicine, Newark, DE 19714-0050*
[5]*Amgen, Inc., Thousand Oaks, CA 91320*
[6]*Pfizer Global Research & Development, Groton, CT 06340*
[7]*National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709*
[8]*Purdue University School of Veterinary Medicine, West Lafayette, IN 47907-2027*
[9]*Independent Consultant, Kalamazoo, MI 49001*

INTRODUCTION

The European and United States regulatory agencies have recently finalized guidance documents that either include a section referring to immunotoxicity (*CPMP: Note for Guidance on Repeated Dose Toxicity*) or pertain directly to immunotoxicity testing (*FDA: Guidance for Industry, Immunotoxicology Evaluation of Investigational New Drugs*). Both documents state that immunotoxicity testing should be performed on all new investigational drugs or medicinal products. In addition, both documents clearly identify gross and microscopic examinations of lymphoid tissues as necessary and pivotal first steps in the assessment of new drugs for immunotoxic potential. The recommendations contained herein focus primarily on, but are not restricted to, subchronic toxicology studies utilizing rodents. The Society of Toxicologic Pathology (STP), as one of the leading professional organizations devoted to the preclinical safety assessment of new drugs, chemicals, and biologicals, and toxicity studies in general, authorized the formation of the STP Immunotoxicity Screening Working Group (STP IWG) to prepare "best practice" recommendations concerning the collection, interpretation and reporting of organ weights, gross and microscopic observations, and other pathology data relevant to the immune system. The STP IWG consists of anatomic and clinical pathologists from industry, academia, and the National Toxicology Program who specifically identified pathology methods and standardized terminology most appropriate for the detection and reporting of alterations of the immune system. It is the goal of the STP IWG to provide a scientifically sound and well-considered guidance document for routine pathology evaluation of the immune system.

While this document focuses primarily on collection and evaluation of lymphoid tissues from specific pathogen free rodents, many techniques and approaches presented herein can be considered appropriate for use in studies involving other species, such as dog and monkey. However, whenever non-rodent species are used, it is necessary to be aware of variability of lymphoid organ weight, and gross and microscopic morphology that is associated with age, sex and husbandry of such outbred species. In addition, complete exsanguination of dogs and nonhuman primates is necessary to minimize the variations of spleen weights.

Assessment of Clinical Pathology

This document focuses on anatomic pathology parameters. An in-depth description of clinical pathology evaluation for detection of immunotoxicity is beyond its intended purpose. However, any initial evaluation of immunotoxicity should include routine analysis of hematology and clinical chemistry parameters. Assessment of individual globulin components, i.e., protein electrophoresis, is not recommended as standard procedure, but should be considered if an initial evaluation of other endpoints indicates that a change has occurred. Bone marrow cytology may also be indicated based on observations from bone marrow histopathology and/or hematology results. In particular, bone marrow cytology may be useful if there is a need to differentiate between lymphoid, myeloid and erythroid elements, which can be difficult to distinguish in routine H&E marrow sections. However, cytology for the purpose of determining precise percentages of specific cell types within the bone marrow is often of limited diganostic value.

Collection and Weighing of Lymphoid Tissue

Pathology practices that include recording and evaluating thymic and splenic weights are supported in the current guidances and should be continued. However, interpretation of these organ weights should only be done in the context of all other clinical, histopathology, and clinical pathology data from the study. Based on general laboratory experience and a review of the literature, the STP IWG contends that alterations of spleen and thymus weights (considered in

Address correspondence to: James Klaunig, Editor-in-Chief, Toxicologic Pathology Editorial Office, 199 Grassmur Turn, Pine Hill, NJ 08021.

conjunction with histopathology of these tissues) are reasonable indicators of systemic immunotoxicity and are likely to be more reliable indicators than are changes in the weight of peripheral lymph nodes (The ICICIS Group Investigators, 1998; Schulte et al., 2002).

Routine Best Practice for Histopathologic Examination of Lymphoid Tissues as Indicators of Systemic Immunotoxicity

Each animal should receive a thorough macroscopic examination of the lymphoid system and any changes in the spleen, thymus and lymph nodes should be noted. The STP IWG agrees that thymus, spleen, draining lymph nodes, bone marrow in situ, and any gross lesions of a lymphoid organ represent the minimum of tissues for routine evaluation of the lymphoid system, and that microscopic evaluation of thymus, spleen, and bone marrow is a reliable indicator for systemic immunotoxicity. Although bone marrow is considered to be a lymphoid tissue in this document, the STP recognizes that bone marrow is not strictly a lymphoid organ; examination of this tissue is also appropriate to evaluate for potential toxicity to nonlymphoid elements of the immune system and functions not directly associated with immune tissues.

The STP IWG also believes that the most proximal regional lymphoid tissues that drain the drug application site can and should be examined microscopically. Generally, draining lymphoid tissues affected by orally administered drugs are Peyer's patches and mesenteric lymph nodes and should be examined by light microscopy using standard 'best practice' for histopathologic examination in studies involving oral dosing. However, while histopathological examination of the most proximal draining peripheral lymph nodes is appropriate in cases of cutaneous, subcutaneous, or intradermal application of xenobiotic, a thorough understanding of the number and distribution of the targeted nodes must first be established. Additional references concerning lymphatic drainage patterns, and the actual number of nodes involved, in the rat may be necessary and the reader is strongly encouraged to refer to the work of N. Tilney (*J. Anat*. 109:369–383, 1971).

It is the position of the STP IWG that the normal histology of peripheral lymph nodes (i.e., popliteal, auricular, axillary, etc.) that do not drain the site of xenobiotic application can be highly variable, often overlaps with that of altered node morphology, and cannot be unequivocally used as an indicator of systemic immunotoxicity. Moreover, minor differences in collection, embedding, and sectioning combined with high intrinsic variability can seriously limit the value of small lymph nodes for the identification of immunotoxicity. Because of these limitations, collection and examination of peripheral lymph nodes that do not drain the site of xenobiotic application is not recommended for routine assessment of systemic immunotoxicity. In contrast, the normal morphology of the spleen, thymus, and bone marrow is considered more consistent and less subject to differences resulting from collection and processing. Thus, the STP IWG contends that alterations of spleen, thymus, and bone marrow histology are likely to be more reliable indicators of systemic immunotoxicity than are changes in distal peripheral lymph nodes.

Semiquantitative Description of Lymphoid Tissue Changes

Central to many discussions concerning histopathologic examination of lymphoid tissues is the suggestion that semiquantitative descriptive techniques may significantly improve the ability to identify potential immunotoxicants early in xenobiotic discovery or development. In fact, the FDA Guidance specifically comments that: "Methods to enhance detection of immunosuppression in standard toxicology studies have been described, including exact tissues that should be examined and effects that should be noted" (Kuper et al., 1995, 2000). Further: "To better characterize such (histologic) changes, a more quantitative histopathological assessment of lymphoid organs as well as immunohistochemical techniques might be useful" (Ward et al., 1993; Kuper et al., 1995; Mitsumori et al., 1996); additional discussion of such approaches is presented by Kuper et al. (2000, 2002) and the reader is encouraged to review these documents. The recommendations presented herein by the STP IWG have used some of the approaches described by these authors as a starting point.

Based on a review of relevant literature and discussions at international meetings the STP IWG endorses the concept that "best practice" for lymphoid tissue microscopic examination involves "a semiquantitative description of changes in compartments and/or microenvironments of specified lymphoid organs."

Three primary points are emphasized by this approach: (1) each lymphoid organ has separate compartments that support specific immune functions, (2) these compartments can and should be evaluated individually for changes, and (3) descriptive, rather than interpretative terminology, should be used to characterize changes within these compartments.

Such "best practice" does not mandate specialized techniques such as lymphoid tissue immunohistochemistry, blind scoring, morphometry, or flow cytometric evaluation of cell suspensions. These specialized techniques, which may be valuable for clarifying specific lymphoid tissue alterations, should be considered after lymphoid tissue change has been identified and should be directed at answering a specific scientific question; they should not be used as routine screening tools.

Examination of Lymphoid Tissue by Compartments

Current "best practice" for histopathologic examination of any tissue requires that the anatomic pathologist identify and examine all compartments of all tissues, including lymphoid tissue, but only make entries for specific compartments if an abnormality is identified within that compartment. If all tissue compartments demonstrate changes considered to be within normal ranges of variability for that organ, an entry of "No Abnormality Determined (NAD)" or other such term can be entered for the entire organ. This document reiterates that such "best practice" is appropriate for the histologic evaluation of lymphoid tissues. The pathologist should carefully and systematically examine each compartment within each tissue and note any abnormality using appropriate terminology. The use of this approach will add precision to histopathology data tables concerning the relevant lymphoid changes. Nevertheless, the recording of incidental background changes may be performed at the discretion of the investigator(s).

Because such "best practices" are not readily apparent to the nonpathologist, it is recommended that inclusion of a definitive statement regarding the procedures for histopathologic examination of all tissues, including lymphoid/immune system tissues be included within appropriate SOPs. The statement might read as follows: "Histopathologic examination of all tissues will include a detailed examination of all tissue compartments and histopathologic abnormalities will be recorded according to the compartment in which they are identified."

TERMINOLOGY

In order to achieve an accurate, consistent and useful "semiquantitative description" it is necessary to develop consensus on consistent terminology used in characterization of lymphoid tissue changes. Whenever possible, semiquantitative/descriptive terms (i.e., reduced numbers of lymphocytes) rather than interpretative terms (i.e., lymphoid atrophy) for registering lymphoid tissue changes is recommended. To illustrate this point further, consider immunotoxicological changes of the thymus; a semiquantitative description such as: "thymus, cortex, decreased lymphocytes, marked" would be preferable to "thymic involution."

Terms such as atrophy, hypoplasia, hypertrophy, and hyperplasia can be applied to either macroscopic or microscopic changes. However, in many cases selection of a more descriptive term, i.e., increased or decreased cells (specify), will provide a more objective semiquantitative description for that change, and is encouraged. In addition, because the term "altered cellularity" can indicate *altered* size of the compartment *or altered* cell density, it is suggested herein that the term "*altered* cellularity" not be used. As it is likely that either increased or decreased cellularity (and the type of cell that is increased or decreased) will be identifiable thereby allowing one to eliminate the ambiguity of the term "*altered*." A more unequivocal description of the change can be achieved by clearly stating which cell type, i.e., lymphocyte, macrophage, mast cell, etc. is either increased or decreased in number. Nevertheless, there may be cases in which the terms atrophy, hypoplasia, hypertrophy and/or hyperplasia remain the most appropriate microscopic descriptors, for example in carcinogenicity studies. The use of such terms should include appropriate indicators of severity so as to maximize their descriptive value.

Thus, one may evaluate lymphoid tissues by asking such questions as:

1. Is the lymphoid organ grossly larger or smaller than normal: macroscopically *increased or decreased in size*?
2. Which compartment is specifically involved?
3. Is the change in size of the organ due to a change in components (e.g., cells, stroma, edema fluid) of a particular compartment?
4. Is this change in size due to a change in cell numbers in one or more compartments, i.e., microscopically *increased or decreased number of cells, and if so which cells are involved* (lymphocytes, macrophages, stromal cells, etc.)?
5. Are the changes due to resident cells within the compartment, or due to passenger cells migrating through the compartment?
6. Does the weight, color or texture differ from normal background and/or control group organs (macroscopy)?

This approach emphasizes the use of descriptive terms in the recording of observations as compared to interpretative terms or diagnoses.

Recording of Lymphoid Tissue Changes

In routine evaluation of lymphoid tissues, it is recommended that the pathologist evaluate the individual compartments separately and in the context of the entire organ or tissue. Because the immune system is anatomically and functionally complex and dynamic, it is beyond the scope of this document to provide a comprehensive scheme for each lymphoid organ or tissue. However, a foundation or basis for evaluating major compartments and components in the routine evaluation of these tissues is presented in Tables 1 and 2. The STP IWG has attempted to provide examples of terminology for commonly observed changes for each lymphoid tissue.

The use of descriptive terms should not prevent or interfere with the pathologist's responsibility to interpret the constellation of changes in a meaningful pathobiologic context. However, interpretation is best reserved for the Discussion section of a pathology report rather than the Results section.

TABLE 1.—Compartments of lymphoid tissues.[1]

Thymus	Spleen	Lymph node	Bone marrow
Cortex	White pulp • PALS • Lymphoid follicles • Germinal centers	Cortex • Subcapsular sinus • Follicles • Germinal centers • High endothelial venules	Erythroid component
Medulla		Paracortex	Granulocytic component
Cortex-medulla ratio[2]		Medulla • Medullary cords • Medullary sinuses	Fat Lymphoid component
	Marginal zone Red pulp		Stroma Megakaryocyte Other cells

Note: The table shows only illustrations as to how one might approach the collection of data. It is *not recommended* that these tables be included as part of the data tables or part of the final report.

[1]Compartmental descriptors can also be applied to the Peyer's Patches.

[2]Not a compartment per se.

TABLE 2.—Possible findings associated with lymphoid tissue alterations.[1]

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

Note: The table shows only illustrations as to how one might approach the collection of data. It is *not recommended* that these tables be included as part of the data tables or part of the final report.

[1]This is a nonexhaustive and nonprioritized list and represents only some of the more commonly encountered findings.

Moreover, final determination of the most appropriate terminology to be used in a study is the shared prerogative and responsibility of the Study Pathologist and the Peer Review Pathologist.

CONCLUSION

Recently, there has been an increased focus on ensuring consistency in the evaluation of xenobiotics for immunotoxicity. New regulatory guidance documents concerning testing of new agents for potential immunotoxicity recognize the importance that histopathology of lymphoid tissue plays in identification of immunotoxic effects. However, the guidances suggest that current histopathology methods could be enhanced by the application of improved techniques, semiquantitative terminology, and additional training. Adoption of these procedures will not only ensure that compartments of lymphoid tissues are routinely examined and that abnormalities are recorded with consistent terminology but also will facilitate compliance with these new guidances and help ensure that immunotoxic compounds are identified. Specialized techniques such as lymphoid tissue immunohistochemistry, blind scoring, morphometry, or flow cytometric evaluation of cell suspensions, may be valuable for clarifying specific lymphoid tissue alterations after the initial identification of such changes and should be directed at answering a specific scientific question; they should not be used as routine screening tools. All changes observed for a given lymphoid organ should be interpreted in the context of the complex interaction of different lymphoid organs and/or other organ systems, as well as hematologic and clinical chemistry data, so as to place the changes in the appropriate toxicologic and pathologic perspective.

ACKNOWLEDGMENT

The STP Immunotoxicity Working Group would like to extend their sincere thanks and appreciation to Drs. Frieke Kuper, Jerry Ward, George Parker, Stuart Levin, and Robert Maranpot for their thorough and scholarly review of this document. The Working Group also thanks all of the ACVP and STP membership who provided rigorous and scientifically sound critque of this effort.

REFERENCES

Gopinath, C. (1996). Pathology of toxic effects on the immune system. *Inflamm Res* **45**, S74–S78.

Kuper, C. F., deHeer, E., Van Loveren, H., and Vos, J. G. (2002). Chapter 39, Immune System, In Handbook of Toxicologic Pathology, 2nd ed. (W. Haschek, C. G. Rousseaux, M. A. Wallig, eds.), Vol. 2, pp. 585–646. Academic Press, San Diego.

Kuper, C. F., Harleman, J. H., Richter-Reichelm, H. B., and Vos, J. G. (2000). Histopathologic approaches to detect changes indicative of immunotoxicity. *Toxicol Path* **28**, 454–66.

Kuper, C. F., Schuurman, H.-J., and Vos, J. G. (1995). Pathology in immunotoxicology. In Methods in Immunotoxicology (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Vol. 1, pp. 397–436. Wiley-Liss, New York.

Mitsumori, K., Takegawa, K., Shimo, T., Onodera, H., Yasuhara, K., and Takahashi, M. (1996). Morphometric and immunohistochemical studies on atrophic changes in lympho-hematopoietic organs of rats treated with piperonyl butoxide or subjected to dietary restriction. *Arch Toxicol* **70**, 809–14.

Schulte, A., Althoff, J., Ewe, S., Richter-Reichhelm, H.-B., BGVV Group Investigators. (2002). Two immunotoxicity ring studies according to OECD TG-407-comparison of data on cyclosporin a and hexachlorobenzene. *Reg Toxico Pharm* **36**, 12–21.

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

Tilney, N. (1971). Patterns of lymphatic drainage in the adult laboratory rat. *J Anat* **109**, 369–83.

Ward, J. M., Uno, H., and Frith, C. H. (1993). Immunohistochemistry and morphology of reactive lesions I. lymph nodes and spleen from rats and mice. *Toxicol Pathol* **21**, 199–205.

Frith CH, Ward JM, Chandra M, Losco P (2000). Non-proliferative Lesions of the Hematopoietic System in Rats. HL-1. In: **Guides for Toxicologic Pathology**. STP/ARP/AFIP, Washington, DC.

Non-proliferative Lesions of the Hematopoietic System in Rats

C.H. Frith[1], J.M. Ward[2], M. Chandra[3] and P.E. Losco[4]

[1]Toxicology Pathology Associates, Little Rock, Arkansas
[2]National Cancer Institute, Frederick, Maryland
[3]Pathology Toxicology Consultant, Paramus, New Jersey
[4]Nycomed Amersham Imaging, Wayne, Pennsylvania

INTRODUCTION

Terminology used to describe non-proliferative lesions of the hematopoietic system in rats is quite varied. Since this species is utilized in both research and testing, proper diagnosis and characterization of non-proliferative hematopoietic lesions in this species is important. This guide presents a biologically accurate, morphologic classification of non-proliferative lesions of the hematopoietic system in rats. The authors have placed each of the non-proliferative lesions into one of the following classifications: congenital, disturbances of growth, degenerative, vascular, inflammatory and miscellaneous.

LYMPH NODES

Degenerative Changes

Lymphoid Necrosis (Figure 1)

Lymphoid necrosis can be multifocal or diffuse and can involve T cell areas, B cell areas, or both. It is characterized by fragmentation of cells with pyknotic or karyorrhectic nuclei, often accompanied by phagocytic macrophages. Individual cell necrosis may be present within the germinal centers of antigen-stimulated nodes undergoing rapid lymphocyte proliferation. Diffuse necrosis of both T and B cell areas has been observed after irradiation (11), exposure to viruses (10), injection of bacterial endotoxin (2), and exposure to certain chemotherapeutic or immunosuppressant drugs (15). Lymphoid necrosis can also occur as an agonal event associated with hypoxia and stress, presumably due to the release of endogenous glucocorticoids (3).

Infarction (Figure 2)

Lymph node infarctions are uncommon, and are generally observed secondary to a predisposing condition, such as polyarteritis nodosa. Infarctions are characterized by diffuse coagulation necrosis of the tissue with loss of nodal architecture, although a peripheral rim of viable lymphocytes may survive. Consolidation of the node, with replacement of the parenchyma by collagen and mineralization of necrotic tissue, may be present, depending on the chronicity of the lesion. Abdominal lymph nodes are more likely to be affected due to their proximity to diseased blood vessels.

Mineralization (Figure 3)

Mineralization is a secondary effect of tissue damage observed infrequently in lymph nodes. It appears as basophilic crystalline mineral deposits within necrotic or degenerating tissue, and is most likely to be associated with an infarction or neoplasm. Mineralization of arteriolar

Address correspondence to: Society of Toxicologic Pathologists, 19 Mantua Road, Mt. Royal, NJ, 08061.

walls may occur occasionally in the paracortical region.

Fibrosis (Figure 4)

Fibrosis is characterized by an increase in collagenous stroma with resulting distortion of normal tissue architecture. It invariably occurs as a sequela to inflammation, necrosis, or neoplasia. It may involve only the capsular surface in an animal with peritonitis, or it may affect localized or diffuse portions of the parenchyma such as fibrotic encapsulation of an abscess, granulation tissue around a hematoma, or fibrosis within an infarcted neoplasm.

Pigmentation (Figures 5, 6)

Hemosiderin is the most common pigment found in lymph nodes. It consists of brown granular material within the cytoplasm of macrophages. It is most likely to be found within the medullary cords and lymphatic sinuses of nodes exhibiting sinus erythrocytosis. Macrophages containing hemosiderin pigment stain iron-positive and PAS-positive (20). Other pigments, that are not iron or PAS-positive, may also be found within macrophages and reticular cells lining sinuses. These substances are usually of finer grain than hemosiderin and range from pink to pale tan in color. Their origin is not certain. They may represent metabolic breakdown products such as lipofuscin, or they may represent insoluble material cleared from the blood or respiratory tract. A number of inhaled, ingested, and injected chemicals induce sinus histiocytosis in the lymph nodes in which macrophages contain inert or insoluble pigmented test substance (3).

Lymphoid Atrophy/Depletion (Figure 7)

Lymphoid atrophy, or depletion, is observed as a sequelae to any disease or toxic condition that causes lymphoid necrosis. It is also seen as a spontaneous finding in aged rats where it may affect B cells and/or T cells. Follicular atrophy is characterized either by the presence of a few follicles, small follicles, or no follicles. Paracortical, or T cell atrophy, is perhaps more common in aging rats and may parallel involution of the thymus. In paracortical atrophy, lymphoid follicles are present, but parenchymal areas around the follicles are hypocellular. Plasmacytosis may be prominent in animals with T cell atrophy.

Vascular Changes

Sinus Erythrocytosis/Erythrophagocytosis (Figures 8, 9)

Sinus erythrocytosis, characterized by the presence of free red blood cells within the lymphatic sinuses, generally occurs as a result of hemorrhage in organs or tissues drained by the affected node. In acute lesions, there is no cellular reaction. In chronic lesions, there may be erythrophagocytosis by macrophages, crystallization of hemoglobin from degenerate red blood cells, and accumulation of hemosiderin pigment within lymphatic sinuses.

Hemal lymph nodes, which resemble normal nodes except that some of their sinuses contain blood instead of lymph, are found occasionally in rats in the perirenal area (8).

Lymphatic Ectasia (Figure 10)

Synonyms: lymphatic sinus ectasia, lymphangiectasis, lymphangiectasia, cystic ectasia, lymphatic cysts

Dilated or cystic sinuses are common spontaneous findings in the lymph nodes of aging rats, particularly in the mesenteric, mediastinal, and paralumbar nodes. Diffuse ectasia tends to be associated with lymphoid atrophy and usually involves the medullary portion of the node, although subcapsular lymphatic dilation can also be present. Cystic lymphatic ectasia occurs less frequently and may be found in any node. Cysts range from microscopic size to 8 mm in diameter. They contain a few cells, including erythrocytes, and a pale, pink homogenous fluid (1). They are lined by endothelial cells. Incidences of lymphatic ectasia in an aged rat population vary from 5 to 26% (8).

Vascular Sinus Ectasia (Figure 11)

Synonyms: angiectasis, vascular sinus dilation, peliosis, telangiectasis

Vascular sinus ectasia is similar, but less common than lymphatic ectasia. It is characterized by dilation of thin vascular channels (sinuses), located within the medullary cords. It should be distinguished from hematoma or early hemangioma. It is distinguished from the former by the presence of endothelial lining cells, and from the latter by hypertrophic and/or hyperplastic vascular endothelium and in some cases, the absence of fibrosis and the regular distribution of dilated vessels within the medullary cords. Collagenous stroma is minimal.

Thrombosis (Figure 12)

Thrombosis is observed infrequently in the lymph nodes of rats. It is characterized by the formation of a solid mass within the lumen of a blood vessel. It is composed largely of fibrin and platelets and may contain a small number of trapped red blood cells.

Inflammatory Changes

Abscess (Figure 13)

Abscesses may be acute or chronic. They are generally the result of an infectious or antigenic substance reaching the lymph nodes via the bloodstream or lymphatics. Abscesses are characterized by a central area of necrosis in which neutrophils are the predominant inflammatory cells. The abscesses make up the eosinohilic portion of a hemotoxalin and eosin (H&E) section. They are surrounded by variable amounts of fibrous

connective tissue whose density is dependent on the chronicity of the lesion. Acute abscesses have a soft to liquefied central area due to the high enzyme content of neutrophils. Chronic abscesses are surrounded by fibrous connective tissue and may have solid caseous central cores. Certain infectious agents, particularly poorly degradable substances with a high lipid content of their capsule or cell wall, are more likely to produce caseous necrosis. Abscesses were more common in rats prior to the development of specific pathogen free (SPF) animals. Corynebacteria species, cultured from submandibular lymph node abscesses, were one of the more commonly isolated etiologic agents.

Acute Inflammation, (Figure 14)
Synonym: acute lymphadenitis

Aggregates of inflammatory cells are occasionally seen in lymph nodes without necrosis or abscess formation in the node. Such infiltrates usually occur in lymph nodes that are draining acute or chronic ulcerative lesions of the skin or intestinal tract, including ulcerated neoplasms. They are most likely to be found in superficial nodes draining the skin and in the cecal or mesenteric nodes draining the intestines (17). Inflammatory cells, primarily neutrophils and macrophages, may be present in the capsule, subcapsular sinuses, and medullary cords. Inflammatory infiltrates must be distinguished from extramedullary hematopoiesis and granulocytic leukemia. Extramedullary hematopoiesis usually has megakaryocytes and other hematopoietic elements intermixed. Granulocytic leukemia has a high proportion of immature myeloid cells as well as multiple organ involvement.

Chronic Inflammation, (Figure 15)
Synonym: chronic lymphadenitis

The term "chronic lymphadenitis" is generally applied to animals exhibiting chronic abscesses, as described above, in which the normal lymphoid architecture may be partially or completely obliterated by the destructive process. "Lymphadenitis" should not be used to describe lymph nodes that are subjected to chronic antigenic stimulation. In the latter case, there is generally a secondary immune response characterized by hyperplasia of the lymphoid follicles, germinal center formation, and plasmacytosis of the medullary cords and paracortical region. In some cases, lymphoid atrophy, rather than hyperplasia, is seen along with plasmacytosis of the medullary cords, particularly in older animals. Chronic antigenic stimulation of a node may also result in hyperplasia of the follicular dendritic cells that trap and present antigen to the lymphocytes. Dendritic cell hyperplasia is characterized by a diffusely cellular node in which tingible body macrophages with large irregular nuclei and pale cytoplasm are scattered throughout the tissue producing the "starry sky" appearance. Sinus histiocytosis may occur as a component of chronic inflammation. Histiocytes are normally present within the medullary sinuses where they trap and remove exogenous and endogenous pigments, erythrocytes, micro-organisms, and insoluble foreign materials such as particles derived from food, air, and drugs (17). Any disease or condition that causes an increase in absorption of such materials may result in sinus histiocytosis of the associated lymph nodes. The mesenteric and peribronchial lymph nodes are most likely to be affected.

Granulomatous Inflammation, (Figure 16)
Synonym: granulomatous lymphadenitis

Granuloma formation within the paracortical and medullary cord regions is a common finding in rats, particularly of mesenteric lymph nodes. Affected lymph nodes show multiple small aggregates of macrophages with abundant pink to tan colored (on H&E staining) cytoplasm (1, 8). Hemosiderin or lipofuscin pigment may be present in the granulomas (20), or they may contain poorly degradable substances, possibly from the feed. Such granulomas rarely show degenerative changes and are not associated with other forms of inflammation.

SPLEEN

Congenital Changes

Accessory Spleen (Figure 17)

Small pieces of splenic tissue (capsule, red and white pulp, trabeculae) may rarely be found in the mesentery or omentum, and attached to visceral organs of rats. The accessory spleen can be seen grossly as a small red nodular lesion in these tissues. Histologically, all normal components of the spleen may be seen (capsule, red and white pulp, trabeculae). The etiology may be congenital or related to previous trauma to the spleen.

Disturbances of Growth

Abnormal growth of the spleen is rare in rats. Enlarged spleens are usually due to those processes described in other sections.

Capsular Cyst (Figure 18)

Capsular cysts occur as small or large cysts on the capsular surface of the spleen of rats. The cysts are lined by endothelial cells and are filled with eosinophilic fluid. They may be of lymphatic origin or from previous trauma.

Degenerative changes

Degenerative changes may be found in any of the cellular elements in the spleen in its various anatomical regions. For examples, lymphocytes in red and white pulp may show vacuolated and other degenerative changes after exposure to toxins. Focal and diffuse changes in

capsule components can be seen after intraperitoneal injection and induction of peritonitis.

Lymphoid Hypoplasia (Figure 19)

Lymphoid hypoplasia is found in nude rats. There are few or no small lymphocytes in T cell zones (PALS) and the white pulp is smaller than normal. The spleen may be smaller than normal in size and weight. Loss of lymphocytes from B or T cell zones is often seen after necrosis in chemical toxicity (16, 18), viral infections and after irradiation.

Lymphoid Atrophy/Depletion

Loss of lymphocytes from B or T cell zones is often seen after necrosis in chemical toxicity, viral infections and after irradiation.

Pigmentation (Figure 20)

Pigments of various types (most often hemosiderin and lipofuscin) are found in aging rats and after chemical exposure. Differentiating features of pigments requires histochemical procedures (20).

Hemosiderosis. Hemosiderosis is an age-related lesion in rats. Hemosiderosis may also be related to old hemorrhage. Accumulation of iron-positive pigment is found in the red pulp of aging rats. The presence of a small amount of hemosiderin pigment in the spleen is considered normal. Special stains are sometimes required to determine if the pigment is hemosiderin. It may arise from normal hemoglobin breakdown or to chemically-induced methemoglobinemia or autoimmune hemolytic anemia, as seen in LGL lymphoma (leukemia).

Lipofuscin. Lipofuscin is an acid fast pigment, probably from oxidative breakdown of lipids, that can be found in aging rats.

Lipid Accumulation (Figure 21)

Lipid accumulation occurs as either a focal or diffuse accumulation and is a rare spontaneous lesion in rat spleen. It has been reported in rats exposed to some aromatic amines (aniline, para-chloroaniline). These lesions are usually associated with focal fibrosis and sarcoma development. The lipid accumulation may be focal or almost nodular. The lesion may appear similar to angiolipoma of humans.

Mineralization

Mineralization is often found after necrosis induced by chemical agents. It is not found as an aging lesion in rat spleen. Capsular mineralization may be found. Mineralization may also be observed in old areas of hemorrhage. Mineralization may also rarely be observed in the walls of vessels.

Fibrosis (Figure 22)

Fibrosis may involve both the capsular surface, as well as parenchymal tissue.

Fibrosis, Capsular. Focal or diffuse fibrosis of the splenic capsule can be seen in rats with peritoneal metastatic tumors and in rats exposed to some aromatic amines. Fibrosis may be associated with chronic inflammation.

Fibrosis, Parenchymal. Parenchymal fibrosis of the spleen occurs as a focal or diffuse parenchymal (red pulp) lesion and can be seen as rare aging lesions in rats but is more commonly induced by some aromatic amines (aniline, para-chloroaniline). It is also sometimes associated with LGL leukemia. Focal areas of the red pulp become fibrotic with loose or dense collagenous tissue. Hemangiomas, hemangiosarcomas and other sarcomas may arise within areas of fibrosis. Grossly, scars may be seen.

Cell Death

Individual cell death (apoptosis, or individual cell necrosis) in splenic cell components, as well as those in other tissues, often occurs after exposure to toxins. The differentiation of apoptosis from cell necrosis may be difficult.

Apoptosis. Apoptosis is a distinct mode of cell death that is responsible for deletion of cells in normal tissues (6,7). The process can be quantitated and may be increased or decreased by xenobiotics. Markers for apoptotic cells are used for tissues. Normal age-matched controls are important for determining levels of apoptosis. Individual cell necrosis, not a component of normal apoptosis, may be found after low doses of toxins.

Necrosis. Necrosis may affect white pulp, red pulp, trabeculae or the capsule. Necrosis of white pulp may include necrosis of marginal zone cells, B cell zone lymphocytes, or T cell zone lymphocytes. Red pulp necrosis may be of hematopoietic or vascular cells, or trabeculae. Necrosis of the capsule can be seen in various conditions. Chemicals, viruses and irradiation are etiologies of splenic necrosis.

Vascular Changes

Hemorrhage (Figure 23)

Hemorrhage occurs after chemical exposure, irradiation and some viral infections. It may be focal or diffuse. Congestion is a common finding in the rat spleen. The method of euthanasia is often a cause.

Periarteritis

Periarteritis was found more frequently in aging rats in past years than it is today. It may involve trabecular arteries and is similar to that seen in other tissues.

Infarction

Infarction of the spleen has been seen in rats with LGL and other leukemias. Sometimes vascular lesions are seen also. Grossly, these areas appear as scars.

Inflammatory changes

Inflammation in the spleen may be acute or chronic, suppurative, fibrinous, or granulomatous. Lesions are usually seen within the red pulp. However, splenic inflammation is not common in rats as a spontaneous lesion or after experimental procedures. Infectious agents may cause these lesions.

Inflammation, Acute/Chronic (Figure 24)

Severe suppurative inflammation in associated tissues may result in suppurative splenic inflammation. Chronic inflammation is rare.

Inflammation, Granulomatous (Figure 25)

Injection of granuloma-inducing agents (e.g., BCG) or infection with granuloma-causing agents may produce granulomas in the spleen. Also, a rare spontaneous lesion in F344 rat spleens is characterized by foci of macrophages forming small nodules.

Miscellaneous Changes

Mesothelial Hypertrophy (Figure 26)

The mesothelium can be focally or diffusely hypertrophied. Mesothelial hypertrophy can be seen on the splenic capsular surface in rats with peritoneal metastatic tumors, peritonitis and other conditions.

THYMUS

CONGENITAL CHANGES

Ectopic Parathyroid/Thymus (Figure 27)

Synonym: aberrant parathyroid

Aberrant or ectopic thymic tissue is sometimes seen in the parathyroid gland and aberrant or ectopic parathyroid is sometimes seen in the thymus of rats. At approximately day 13 of gestation in the rat, the thymus and parathyroid migrate caudally. The organs separate from each other on day 15 when the thymus moves down into the thorax. Thymic tissue becomes thin in the neck and breaks up into small fragments. These fragments may be found in the thorax close to or embedded in the thyroid gland (3). It is necessary to identify the presence of pale staining clusters of thymic epithelial cells and/or Hassall's corpuscles within these foci in order to be certain that they represent ectopic thymic tissue. Ectopic thymic tissue must be differentiated from simple lymphoid aggregates. In addition, fragments of ectopic thyroid and/or parathyroid tissue, of normal histolic appearance, may be found adjacent to or embedded within the thymus. These findings are not common, and no toxicological significance has been attached to them.

Disturbances of Growth

Physiological Involution (Figure 28)

Synonym: atrophy

The thymus of the rat reaches its maximum size in young adult rats and then begins to slowly decrease in size with age beginning at sexual maturity. This normal decrease in size is commonly referred to as physiological involution. Both lymphocytic and epithelial components decrease in size in normal involution, although atrophy is more pronounced in the cortex than in the medulla. The thymus of a two year old rat is quite small as a result of a decrease in size in both the cortex and medulla. As involution progresses with age, cuboidal to columnar epithelial cells become more prominent in the medulla and may form cysts. In advanced cases of involution, infiltration of adipose tissue is pronounced in the cortex.

Some pathologists record this finding in older rats as either atrophy or physiological involution. Others regard this finding as a normal change and do not record it as a lesion. The benefit of recording this finding is that some toxicants can cause an atrophy of the thymus. Although the age-related reduction of thymic tissue is a normal process and not a pathologic lesion, it can be useful in toxicologic studies to grade the degree of aging in order to investigate the influence of test substances on the rate of aging (7).

Degenerative Lesions

Lymphoid Necrosis (Figure 29)

Necrosis of the cortical lymphocytes may occur as a direct effect of toxicants or secondary to debilitation and stress as a result of an elevated level of endogenous corticoids (13, 16). It may be especially prominent in rats sacrificed in a moribund condition.

Necrosis may consist of either individual cell necrosis or more advanced necrosis characterized by large clumps of nuclear debris.

It has been reported that cytostatic chemicals result in atrophy and necrosis of the thymus of rats (4). Imai reported a decrease in the number of thymic lymphocytes, as well as abundant pyknotic thymic lymphocytes, cellular debris, and hemorrhage (4).

Fibrosis (Figure 30)

Fibrosis is an uncommon lesion of the thymus of rats, but it is sometimes seen as a secondary to chronic inflammation that may occur as a result of a gavage injury.

Mineralization

Mineralization is an uncommon finding in the thymus of rats. It may be observed in old areas of hemorrhage.

Vascular Lesions

Hemorrhage (Figure 31)

Focal or multifocal hemorrhage may be seen in the thymus and is more commonly seen in the medulla than the cortex. It is usually more common in sacrificed animals and may be observed as an agonal lesion in rats anesthetized with CO_2.

Stefanski et al (13) state that in the absence of necrosis or other lesions, these areas of extravascular erythrocytes are generally attributed to necropsy technique or dissection-induced artifact and are not considered to be a vascular lesion. Vitamin K deficiency in rats may cause thymic hemorrhage.

Inflammatory Changes

Inflammation (Figures 32, 33)

Primary inflammatory changes in the thymus of rats are rare. Inflammation may occur as a result of extension of inflammation from other tissues, particularly as a result of gavage injury.

miscellaneous changes

Epithelial Cysts (Figure 34)

Epithelial cysts are common findings in the involuted thymus of aged rats. These cysts are believed to be either remnants of the thymopharyngeal duct or are believed to develop as a result of dilatation of thymic tubular structures (13). They are often associated with epithelial glandular hyperplasia (1).

BONE MARROW

disturbances of growth

Atrophy (Figure 35)

Synonym: hypocellularity

Bone marrow atrophy may be focal, multifocal or diffuse. It is characterized by well demarcated areas with a decrease in the number of hematopoietic cells, an increase in the number of fat cells and an increased prominence of reticular stroma. Diffuse atrophy is usually observed in old rats, especially those sacrificed in a moribund condition. In young rats it is associated with decreased weight gain or reduced weight. It may be associated with chemical administration. Focal or multifocal hypocellularity is rarely observed and is more common in female rats. The aging process results in fatty replacement of bone marrow in long bones.

Degenerative changes

Fibrosis (Figure 36)

Synonym: myelofibrosis

Myelofibrosis is characterized by an increase in reticulum and collagenous fibers with decreased number of hematopoietic cells. This lesion must be distinguished from focal atrophy, fibrous osteodystrophy and stromal hyperplasia. Focal fibrosis is usually observed in young rats and it may occur secondarily as a result of injury, inflammation, or necrosis.

Necrosis (Figure 37)

Bone marrow necrosis is characterized by focal or diffuse nuclear pyknosis, karyorrhexis, cytoplasmic vacuolization, and lysis. It may result from infections, toxins or inflammatory processes. Bone marrow necrosis can also be induced with chemicals. It may also be associated with malignancies, leukemia, lymphoma, metastases, vascular obstruction (thrombosis) or hemorrhage in the acute stage due to the vascularity of the tissue.

Infarction

Occlusion of blood vessels that supply blood to the bone marrow may lead to infarction. It may also be associated with infection, growing malignancies, metastases or vascular obstruction (thrombosis).

Pigmentation (Figure 38)

Hemosiderin pigment is sometimes found in the bone marrow. It is present in macrophages and may be associated with old hemorrhage. An iron stain such as Prussian blue may be helpful in determining hemosiderin pigment.

Vascular Lesions

Hemorrhage (Figure 39)

Escape of blood from the vessels in the bone marrow is referred as hemorrhage. In old hemorrhage where brown pigment (iron positive) accumulation is observed in the endothelial cells, the lesion should be referred to as hemosiderosis. In some cases these lesions may be associated with bone marrow necrosis.

Inflammatory Lesions

Inflammation (Figure 40)

Inflammatory lesions of rat bone marrow are rare. Focal granulomatous lesions have been reported and are more common in females than in males (9). They are characterized by accumulation of macrophages, having oval, elongated or fusiform nuclei and abundant pale eosinophilic cytoplasm. These lesions can be induced by

some chemicals. Bacterial sepsis with acute inflammation has rarely been observed.

Miscellaneous Lesions

Lymphoid Follicles

Lymphocytes are found in the normal marrow population and are dispersed singly among hematopoietic and fat cells. Lymphoid cells may also be present in aggregates or lymphoid follicles with well developed germinal centers but are not usually found in untreated rats.

DISCUSSION

The previously described lesions may be found in both control and treated rats. The use of immunocytochemistry can further define the nature of these lesions.[14]

Hematopoietic tissues are commonly fixed in 10% neutral buffered formalin along with the remaining tissues of the animals. This is satisfactory for light microscopy. However, if the investigator anticipates the use of immunocytochemistry on hematopoietic tissues, special procedures or fixatives may be needed. Cell surface antigens and immunoglobulins may require frozen sections or Bouin's, B-5 or Zenker's fixation. Predigestion with trypsin or another protease may enhance immunoreactivity of immunoglobulins in formalin-fixed rodent tissue. Frozen sections are required for many cell surface antigens found on hematopoietic cells. Bouin's fixative is a good fixative for demonstrating immunoglobulins in cells in paraffin-embedded tissue sections.

Immunocytochemistry can serve as a valuable adjunct in diagnosing non-proliferative hematopoietic lesions in rats, especially in paraffin-embedded tissues. Specific antigens may be localized in cells and tissues, and the immunoreactivity of polyclonal and monoclonal antibodies to these antigens provide a more accurate basis in the diagnosis and also aid in understanding the pathogenesis of these lesions. Both specific and nonspecific staining patterns may be focal or diffuse and may be categorized by localization as tissue, nuclear, cell membrane, nuclear membrane, cytoplasmic-diffuse, cytoplasmic-focal, or granular, whole cell (nucleus and cytoplasm and extracellular) (19).

Many techniques are available and many commercial kits can be obtained (e.g., ABC, PAP, immunogold). Antigen retrieval methods using antigen retrieval solutions and microwave pretreatment of tissue sections may allow detection of some cell surface antigens in paraffin embedded sections (12).

Many rat leukocyte antigens can be detected using frozen sections of rat tissues. A thorough evaluation of them for use in paraffin sections, especially after antigen retrieval has not been made. The following table lists a number of antibodies along with their source.

RECOMMENDED NOMENCLATURE AND DIAGNOSTIC CRITERIA

LYMPH NODE

Degenerative Changes

Lymphoid Necrosis

1. Focal, multifocal or diffuse
2. Fragmentation of cells with pyknotic or karyorrhectic nuclei
3. Often accompanied by phagocytic macrophages

Infarction

1. Uncommon finding
2. May be secondary to polyarteritis nodosa

Table 1 - Selected Rat Hematopoietic Antibodies Immunoreactive on Paraffin-Embedded Sections and Frozen Sections.

Antibody	CD Number	Commercial Source	Cells Expressed in	Optimal fixative
Lysozyme	N/A	DAKO	macrophages/histiocytes	formalin
ED-1	N/A	Chemicon, Harlan	macrophages/histocytes	formalin
Ig Kappa, IgG, IgA, IgM	N/A	Harlan	B lymphocytes	Bouin's Zenker's, B-5, frozen
OX33	CD45RA	Harlan	B cells	frozen
OX-8	CD8	Harlan	cytotoxic/suppressor T lymphocytes/LGLs	frozen, Bouin's, B-5, Zenker's, formalin (with trypsin digestion)
W3/25	CD4	Harlan	Helper T Macrophages	frozen

3. Diffuse coagulation necrosis with loss of nodal architecture
4. Peripheral rim of viable lymphocytes may survive
5. Replacement of parenchyma with collagen and mineralization

Mineralization

1. Secondary effect of tissue damage
2. Basophilic crystalline mineral deposits within necrotic or degenerating tissue
3. Most likely to be associated with infarction or neoplasm

Fibrosis

1. Increase in collagenous stroma with resulting distortion of normal tissue architecture
2. Occurs as a sequelae to inflammation, necrosis or neoplasia

Pigmentation

1. Deposition of pigment
2. Present in cytoplasm of macrophages
3. Most likely to be present in medullary cords and lymphatic sinuses
4. Hemosiderin is most common
5. Other pigments may be found within macrophages and reticular cells lining sinuses
6. Special stains may be required for differentiation

Lymphoid Atrophy/Depletion

1. Sequelae to disease or toxin resulting in necrosis
2. May affect B cells or T cells
3. Follicular atrophy characterized by presence of a few, small or no follicles
4. Paracortical or T cell atrophy characterized by the presence of lymphoid follicles, but parenchymal areas around follicles are hypocellular

Vascular Changes

Sinus Erythrocytosis/Hemorrhage

1. Presence of free red blood cells within lymphatic sinuses
2. Usually occurs as a result of hemorrhage in organs or tissues drained by affected node
3. In chronic lesions, accompanied by macrophages, crystallization of hemoglobin from degenerate red blood cells, erythrophagocytosis, and accumulation of hemosiderin pigment

Lymphatic Ectasia

1. Dilated or cystic lymphatic sinuses
2. Spaces are lined by endothelial cells
3. Dilated sinuses contain a few cells, including erythrocytes, and a pale, pink homogeneous fluid
4. Most common in mesenteric and mediastinal lymph nodes

Vascular Sinus Ectasia

1. Dilatation of vascular channels, sinuses within medullary cords or subcapsular sinuses
2. Sinuses lined by endothelial cells
3. Sinuses contain erythrocytes

Thrombosis

1. Solid mass within lumen of blood vessel
2. Composed of fibrin, platelets and may contain trapped red blood cells

Inflammatory Changes

Abscess

1. May be acute or chronic
2. Generally result of infections or antigenic substance
3. Central area of necrosis in which neutrophils are predominant inflammatory cell
4. Surrounded by variable amounts of fibrous connective tissue

Acute Inflammation

1. Aggregates of neutrophils without necrosis or abscess formation
2. Commonly found in superficial nodes draining skin or mesenteric nodes draining intestinal tract
3. Must be distinguished from extramedullary hematopoiesis and granulocytic leukemia

Chronic Inflammation

1. Associated with chronic abscesses
2. Normal lymphoid architecture may be partially or completely obliterated

Granulomatous Inflammation

1. Multiple small aggregates of macrophages/ histiocytes
2. Hemosiderin or lipofuscin pigment may be present
3. Most commonly found in mesenteric lymph nodes

SPLEEN

Congenital Changes

Accessory Spleen

1. Small pieces of splenic tissue present separate from spleen in mesentery, omentum or exocrine pancreas

Disturbance of Growth

Capsular Cyst

1. Cyst-like structures on capsular surface of spleen
2. Lined by endothelial cells
3. Filled with homogeneous eosinophilic fluid
4. May be of lymphatic origin

Degenerative Changes

Lymphoid Hypoplasia

1. Found in nude rats
2. Few or no small lymphocytes in T cell zones (PALS)
3. Decrease in white pulp

Lymphoid Atrophy/Depletion

1. Loss of lymphocytes of B or T cell zones
2. Often seen after necrosis in chemical toxicity, viral infections or after irradiation

Pigmentation

1. Pigment may be either hemosiderin or lipofuscin
2. Both pigments are more common in aging rats
3. Small amount of hemosiderin considered normal
4. Hemosiderin is iron positive
5. Lipofuscin is acid fast

Lipid Accumulation

1. Focal or diffuse accumulation
2. Characterized by presence of empty clear vacuoles

Fibrosis

1. May be focal, multifocal or diffuse
2. May involve capsule or parenchymal tissue
3. Capsular involvement may be due to peritoneal metastatic tumors or peritonitis
4. Parenchymal fibrosis occurs as fibrotic areas with loose or dense collagen in the red pulp

Mineralization

1. May occur in capsule or parenchyma
2. May be observed in areas of old hemorrhage
3. Basophilic crystalline mineral deposits within necrotic or degenerating tissue

Apoptosis

1. Distinct mode of cell death responsible for deletion of cells in normal tissues
2. Not associated with inflammation
3. Characteristic biochemical feature is cleavage of nuclear DNA
4. Presence of apoptotic bodies

Necrosis

1. Cell death characterized by nuclear pyknosis, karyorrhexis or karyolysis associated with increased cytoplasmic eosinophilia
2. May affect white pulp, red pulp, trabeculae or capsule
3. Necrosis of white pulp may include marginal zone cells, B cell zone lymphocytes or T cell zone lymphocytes
4. Red pulp necrosis may be of hematopoietic or vascular cells or trabeculae

Vascular Changes

Hemorrhage

1. Presence of erythrocytes outside of vascular channels
2. May be focal, multifocal or diffuse
3. May follow chemical exposure, irradiation and viral infections

Periarteritis

1. Accumulation of inflammatory cells around periphery of vessels and within vessel walls
2. Small muscular arteries commonly affected
3. Varies from an acute inflammation with neutrophils and fibrinoid necrosis of vessel walls to a chronic inflammation with infiltration of lymphocytes

Infarction

1. Cell death as result of vascular occlusion
2. Occlusion often not identified microscopically
3. Secondary hemorrhage

Inflammatory Changes

Acute Inflammation

1. Infiltration of neutrophils
2. Usually associated with inflammation in adjacent tissues

Granulomatous Inflammation

1. Foci of macrophages forming small granulomas

Miscellaneous

Mesothelial Hypertrophy

1. Focal or Diffuse
2. Increase in size and prominence of mesothelial cells on capsular surface of spleen
3. Usually secondary to peritoneal metastatic neoplasms, peritonitis and related conditions

THYMUS

Congenital Changes

Ectopic Parathyroid/Thymus

1. Aberrant or ectopic thymic tissue in parathyroid gland
2. Ectopic thymic tissue contains pale clusters of thymic epithelial cells and/or Hassall's corpuscles
3. Aberrant or ectopic parathyroid tissue in thymus
4. Ectopic parathyroid tissue usually embedded in thymus

Disturbances of Growth

Involution/Atrophy

1. Normal physiological change

2. Begins at sexual maturity
3. Decrease in both lymphocytic and epithelial components but more pronounced in cortex than medulla
4. Some toxicants may increase or enhance this process

Degenerative Changes

Necrosis

1. Consists of either individual cell necrosis or more advanced necrosis with prominent nuclear debris

Mineralization

1. Uncommon finding
2. May be found in areas of old hemorrhage
3. Basophilic crystalline mineral deposits within necrotic or degenerating tissue

Vascular Changes

Hemorrhage

1. Presence of erythrocytes outside of vascular channels
2. Focal, multifocal or diffuse
3. More common in medulla

Miscellaneous Changes

Epithelial Cysts

1. Dilatation of thymic tubular structures
2. Common in involuted thymus
3. Often associated with glandular hyperplasia

BONE MARROW

Disturbances of Growth

Atrophy/Hypocellularity

1. Well demarcated areas containing a decrease in the number of hematopoietic cells
2. Focal, multifocal or diffuse

Degenerative Changes

Myelofibrosis

1. Increase in reticulum and collagenous fibers with decreased number of hematopoietic cells
2. May occur as a result of injury, inflammation, or necrosis

Hematopoietic Cell Necrosis

1. Cell death characterized by nuclear pyknosis, karyorrhexis cytoplasmic vacuolization and lysis
2. May be associated with malignancies, leukemia, metastases or vascular obstruction

Infarction

1. Cell death as a result of vascular occlusion
2. Occlusion usually not visible microscopically
3. May be associated with malignancies and metastases

Pigmentation

1. Brown granular pigment in cytoplasm of macrophages
2. Most commonly hemosiderin
3. May be associated with old hemorrhage
4. Can be confirmed with special stains for iron

Vascular Changes

Hemorrhage

1. Presence of free red blood cells outside of vascular channels
2. May be associated with necrosis
3. Hemosiderin pigment may be present in old hemorrhages

Inflammatory Changes

Granulomatous Inflammation

1. Focal granulomatous lesions characterized by accumulations of macrophages with oval, elongated or fusiform nuclei and abundant eosinophilic cytoplasm

Miscellaneous Changes

Lymphoid Follicles

1. Aggregates of lymphocytes that appear normal within marrow
2. Germinal centers are prominent

References

1. Burek JD. *Pathology of Aging Rats*. Boca Raton, Fla: CRC Press, Inc; 1978.
2. Dardick I, Sinnott N, Hall R, Bajenko-Carr T, and Setterfield G. Nuclear morphology and morphometry of B-lymphocyte transformation. *Am J Pathol.* 1983;111:35-49.
3. Gopinath C, Prentice DE, Lewis DJ. *Atlas of Experimental Toxicological Pathology*. Boston, Mass: MTP Press Limited; 1987.
4. Imai K . Atrophy of thymus induced by cytostatic chemicals, rat. In: Jones TC, Ward JM, Mohr U, Hunt RD, eds. *Hematopoietic System*. Berlin Heidelberg New York: Springer-Verlag; 1990:293-295.
5. Jones TC, Ward JM, Mohr U and Hunt RD. Monographs on Pathology of Laboratory Animals. In: Jones TC, Ward JM, Mohr U and Hunt RD, eds. *Hematopoietic System.* Berlin Heidelberg New York:Springer-Verlag; 1990:1-336.
6. Kerr FR, Winterford CM, Harmon, BV. Apoptosis: Its significance in cancer and cancer therapy. *Cancer.* 1994;73: 2013-2026.
7. Kuper CF, Beems RB, Hollanders VMH. Development and aging, thymus, rat. In: Jones TC, Ward JM, Mohr U, Hunt RD, eds. *Hematopoietic System*. Berlin, Germany: Springer-Verlag; 1990:257-263.
8. Losco P, Harleman H. Normal development, growth, and aging of the lymph node. In: Mohr U, Dungworth D and Capen C, eds. *Pathobiology of the Aging Rat*. Volume I. Washington, DC: ILSI Press; 1992:50-57.
9. MacKenzie WF, Eustis SL. Bone marrow. In: Boorman GA, Eustis SL, Elwell MR, Montgomery CA Jr. and MacKenzie WF, eds. *Pathology of the Fischer Rat*. San Diego, CA: Academic Press; 1990:395-403.
10. Mims C, Gould J. Splenic necrosis in mice affected with cytomegalovirus. *J Infect Dis.* 1978;137:587-591.
11. Papilian V, Carpen E, Muresan D, Olinic A, Gocan M, Radu-Toader M. Spleen and lymph node changes in rats irradiated with x-rays and treated with testerone. *Exp Pathol.* 1979;25:163-166.
12. Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem.* 1991;39:741-748.
13. Stefanski AS, Elwell MR, Stromberg PC. Spleen, lymph nodes and thymus. In: Boorman GA, Eustis SL, Elwell MR, Montgomery CA Jr. and MacKenzie WF, eds. *Pathology of the Fischer Rat*. San Diego, CA: Academic Press; 1990: 369-393.
14. Taylor CL, Cote RJ . *Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist.* 2nd ed. Philadelphia, Pa: WB Saunders Co; 1994.
15. Turk J, Poulter L. Selective depletion of lymphoid tissue by cyclophosphamide. *Clin Exp Immunol.* 1972;10:285-296.
16. Vos JG, Kraknc-Franken MAM. Toxic effects on the immune system, rat. In: Jones T, Ward J, Mohr U and Hunt R. eds. *Hematopoietic System*. Berlin Heidelberg New York: Springer-Verlag; 1990:168-181.
17. Ward J. Classification of reactive lesions of lymph nodes. In: Jones T, Ward J, Mohr U and Hunt R. eds. *Hematopoietic System*. Berlin Heidelberg New York: Springer-Verlag; 1990a:155-161.
18. Ward J. Classification of reactive lesions, spleen. In: Jones T, Ward J, Mohr U and Hunt R. eds. *Hematopoietic System*. Berlin Heidelberg New York: Springer-Verlag; 1990b:220-226.
19. Ward JM, Rehm S. Applications of immuno-histochemistry in rodent tumor pathology. *Exp Pathol.* 1990;40:301-312.
20. Ward JM, Reznik-Schuller H. Morphological and histochemical characteristics of pigments in aging F344 rats. *Vet Pathol.* 1980;17:678-685.

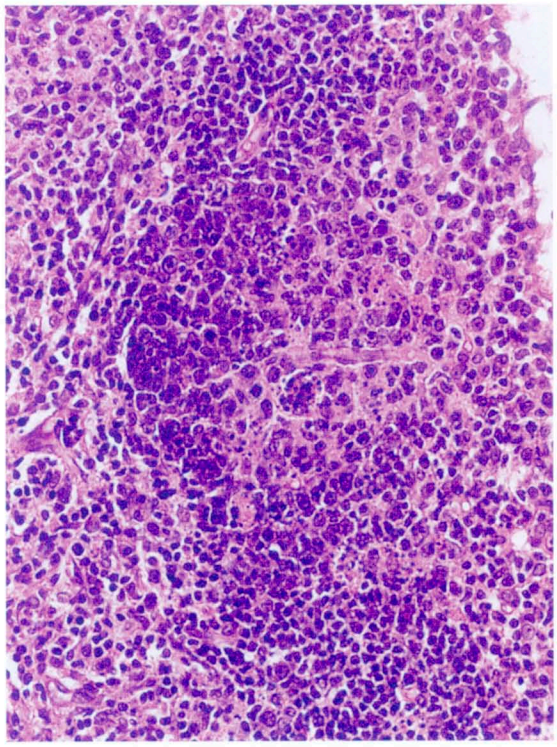


Fig. 1 *- Necrosis, lymph node from a rat administered E. coli toxin.*

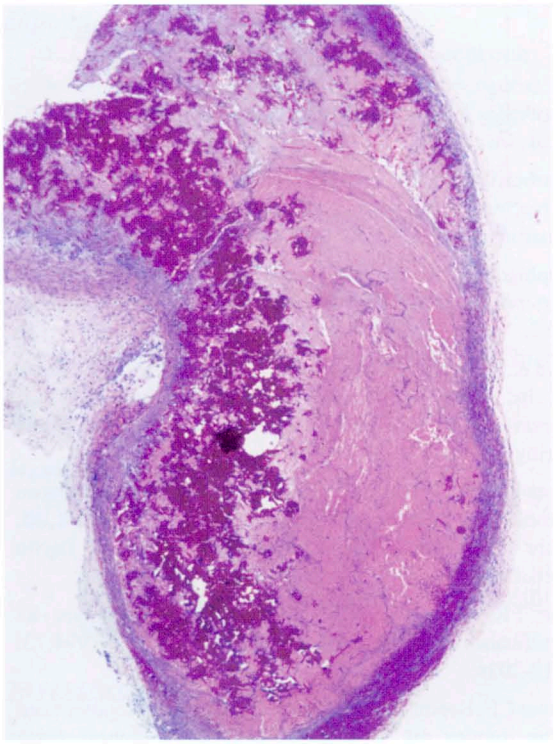


Fig. 2 *- Infarcted lymph node.*

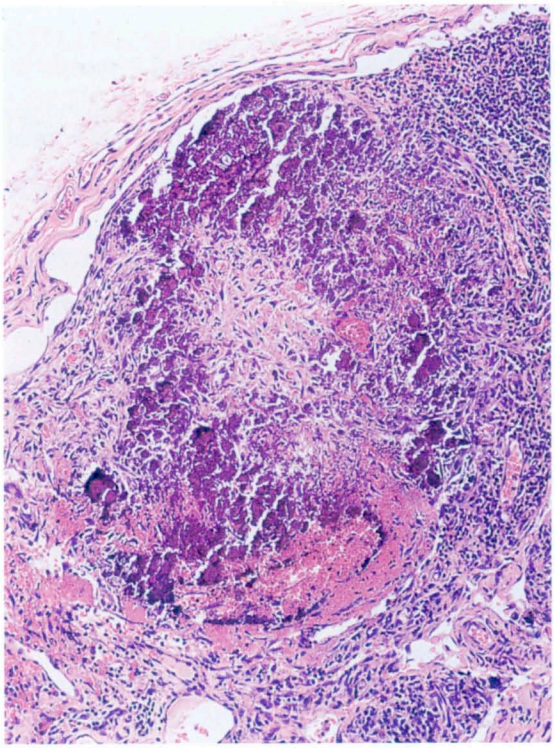


Fig. 3 *- Mineralization, lymph node.*

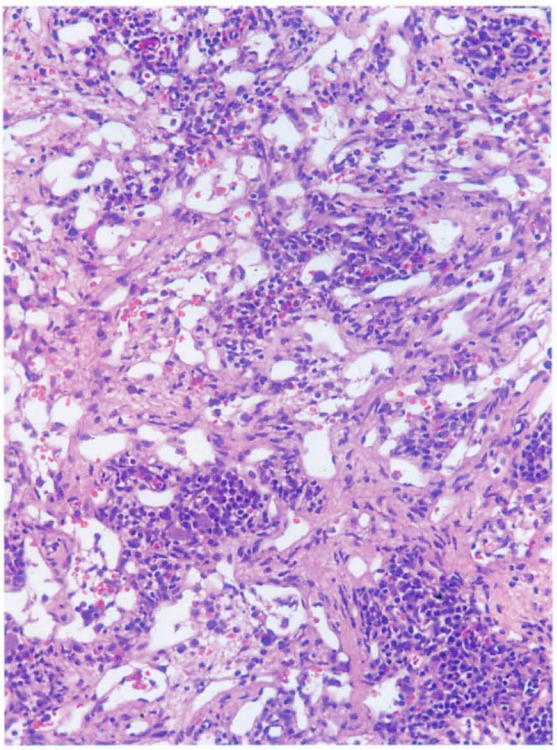


Fig. 4 *- Fibrosis, lymph node.*

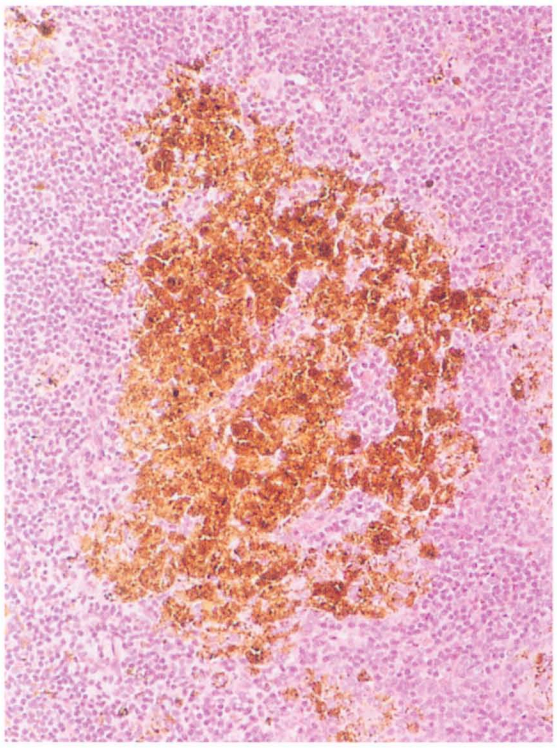


Fig. 5 *- Hemosiderin pigment, lymph node.*

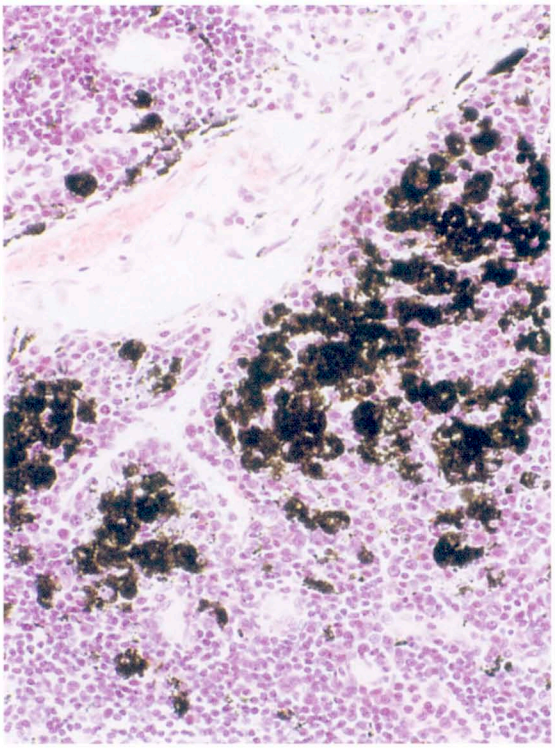


Fig. 6 *- Black treatment-related pigment , lymph node.*

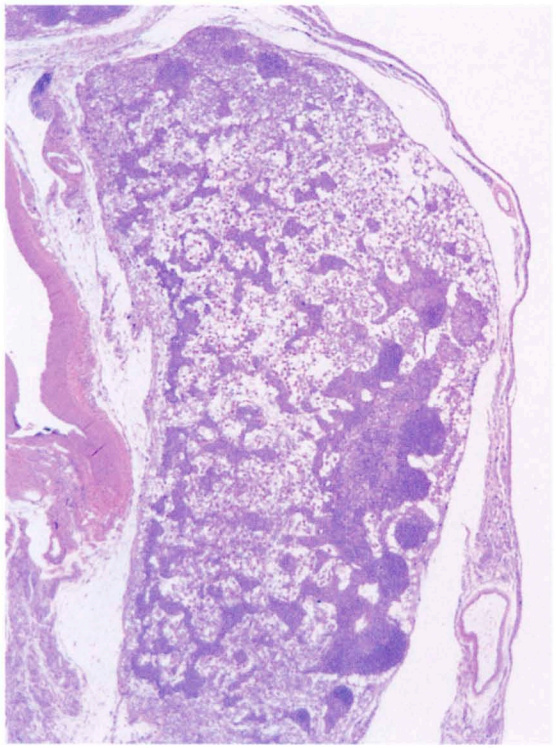


Fig. 7 *- Generalized atrophy, lymph node.*

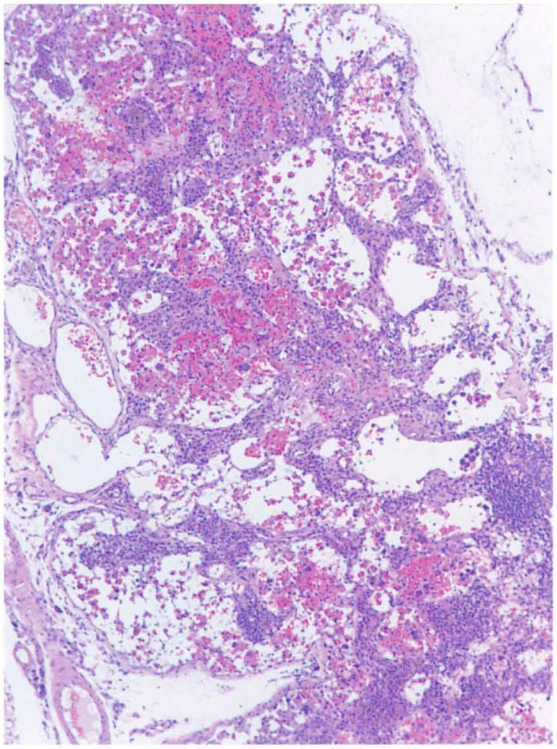


Fig. 8 *- Sinus erythrocytosis, lymph node.*

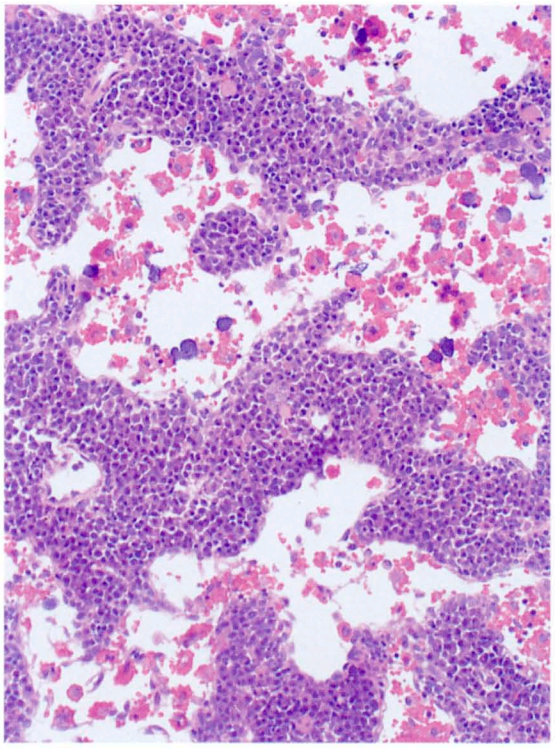


Fig. 9 - *Erythrophagocytosis, lymph node.*

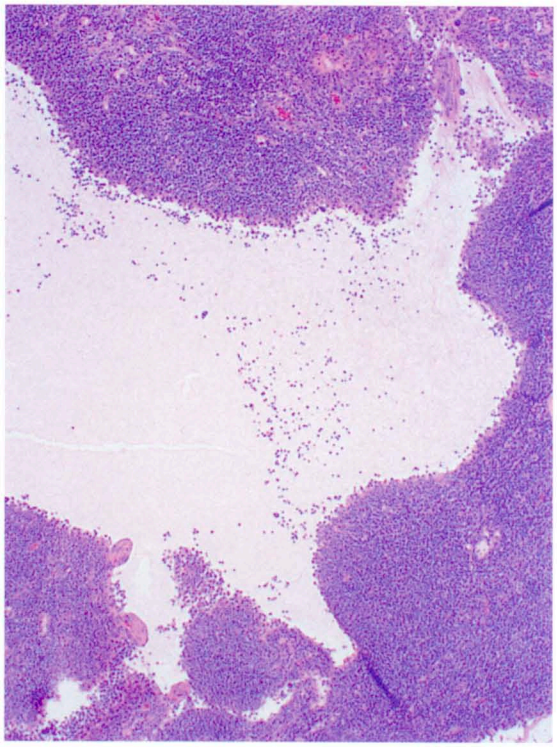


Fig. 10 - *Lymphatic ectasia, lymph node. Note eosinophilic fluid.*

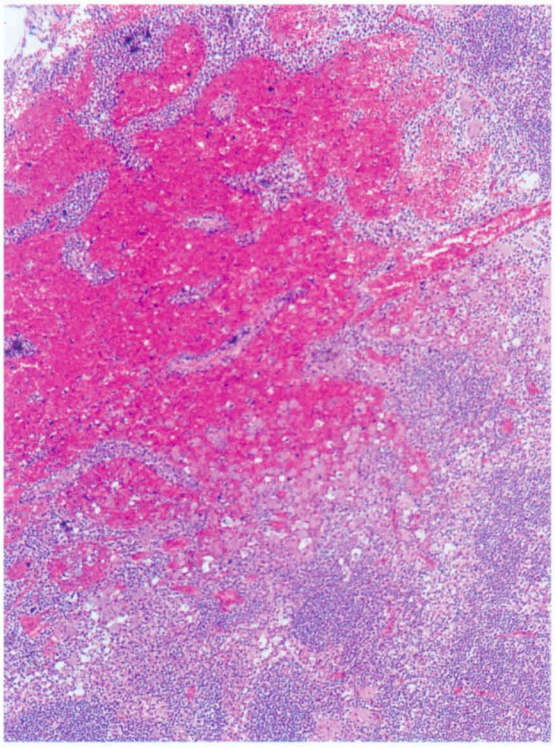


Fig. 11 - *Vascular ectasia, lymph node.*

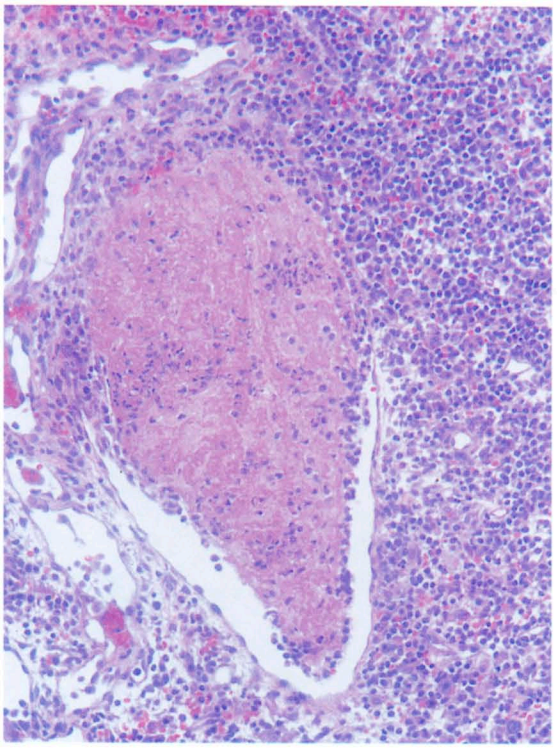


Fig. 12 - *Thrombosis, lymph node.*

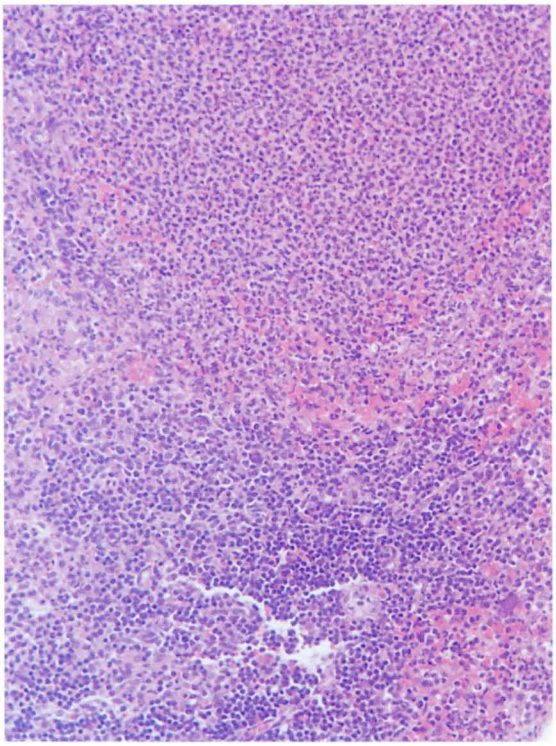


Fig. 13 - *Abscess, lymph node.*

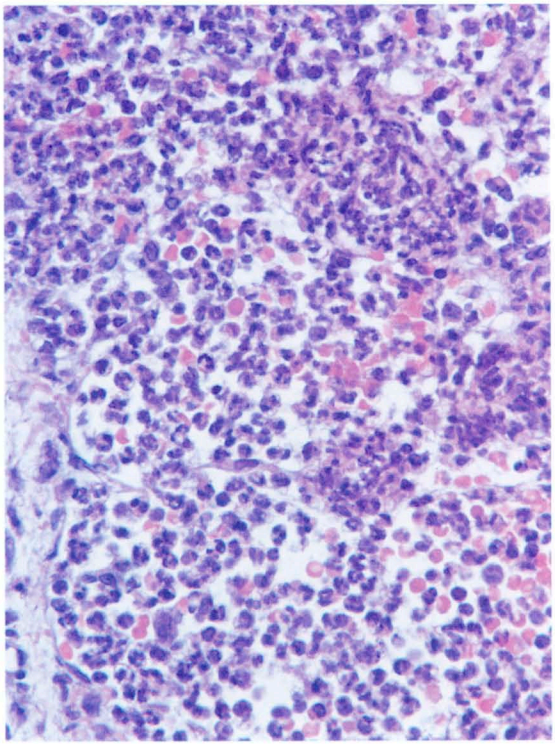


Fig. 14 - *Acute inflammation, lymph node. Note mature neutrophils.*

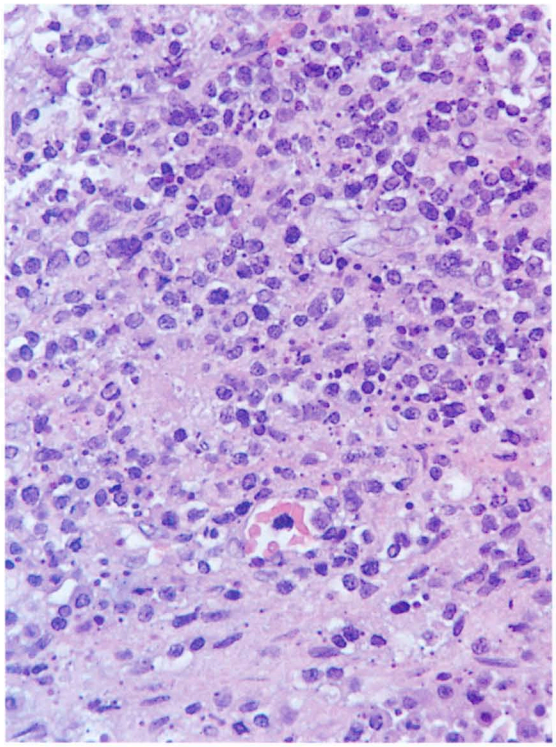


Fig. 15 - *Chronic inflammation, lymph node.*

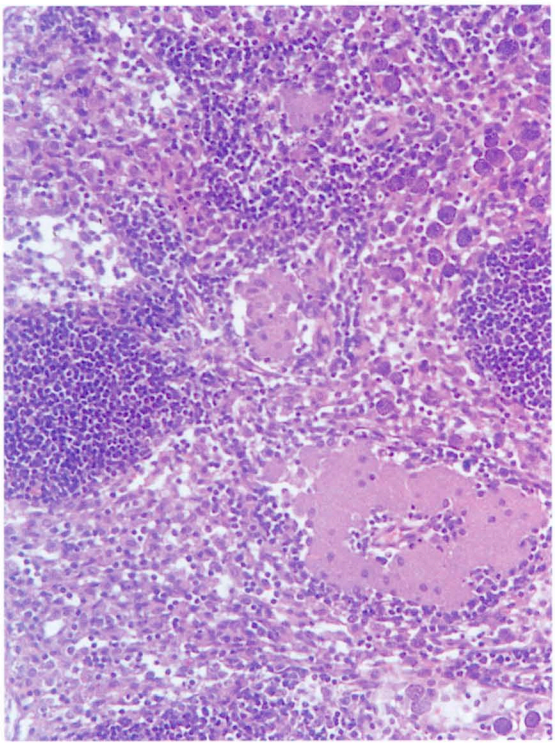


Fig.16 - *Granulomatous inflammation, lymph node.*

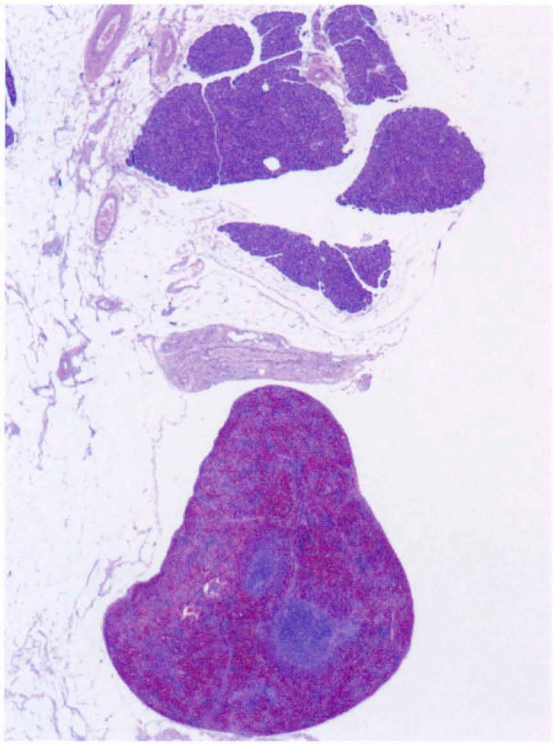


Fig. 17 - *Accessory spleen.*

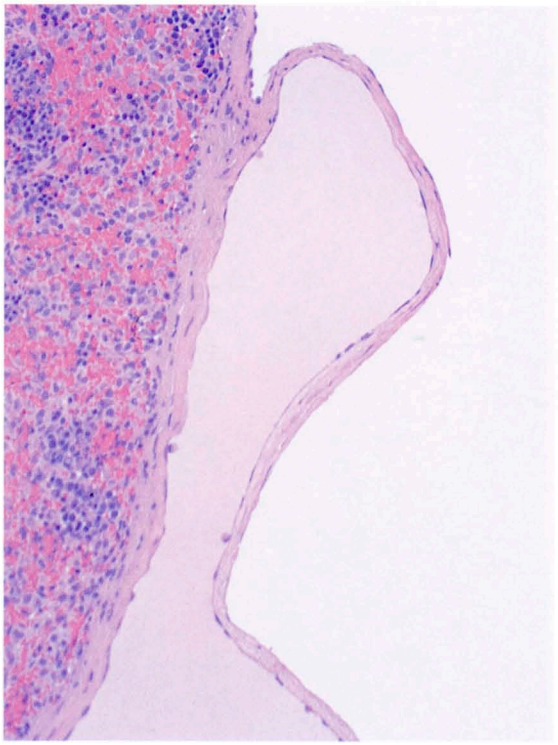


Fig.18 - *Splenic capsular cyst. Note endothelial cells and eosinophilic material.*

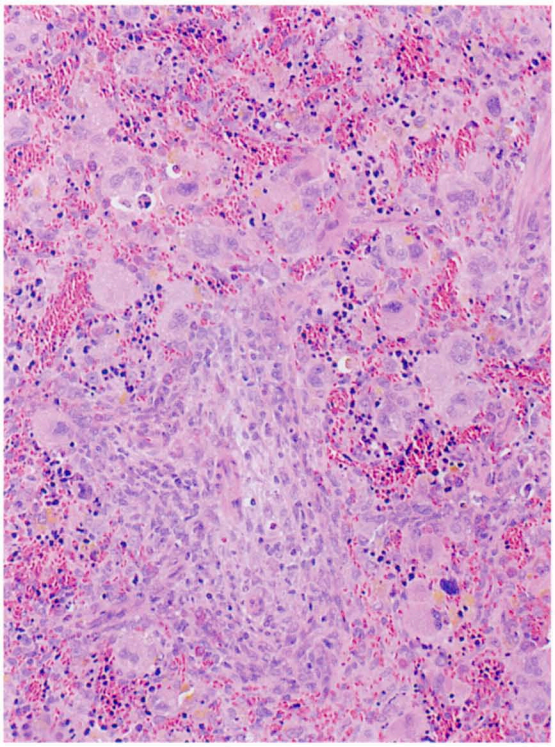


Fig. 19 - *Lymphoid hypoplasia, spleen from a nude rat. Note decreased white pulp.*

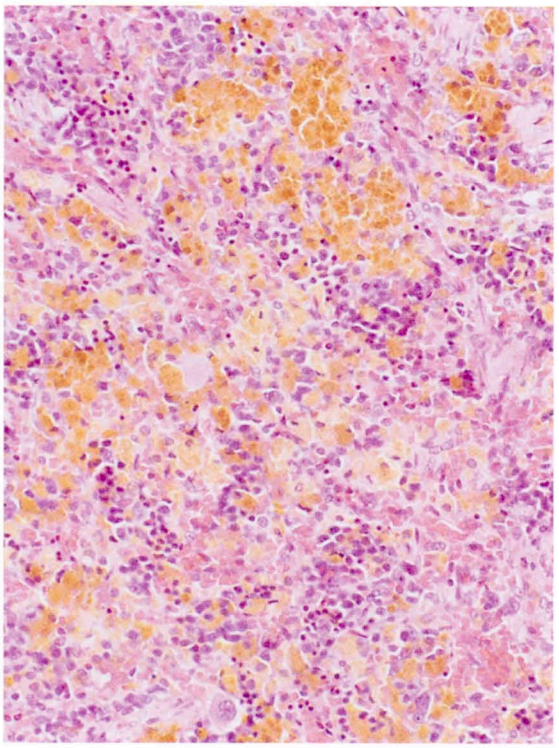


Fig. 20 - *Hemosiderin, spleen.*

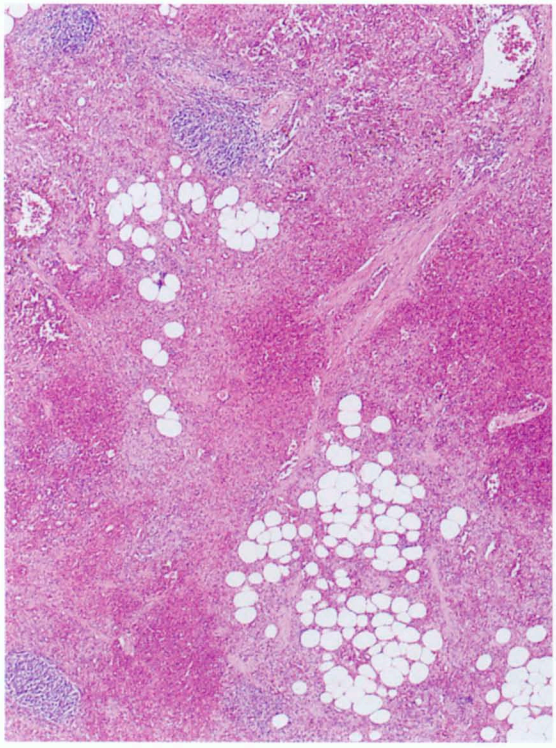


Fig. 21 - *Lipid accumulation, spleen.*

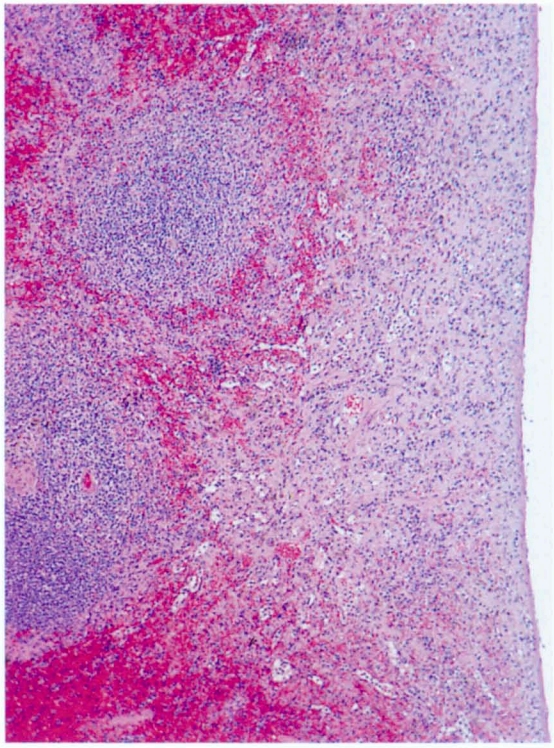


Fig. 22 - *Fibrosis, splenic capsule, extending into the parenchyma.*

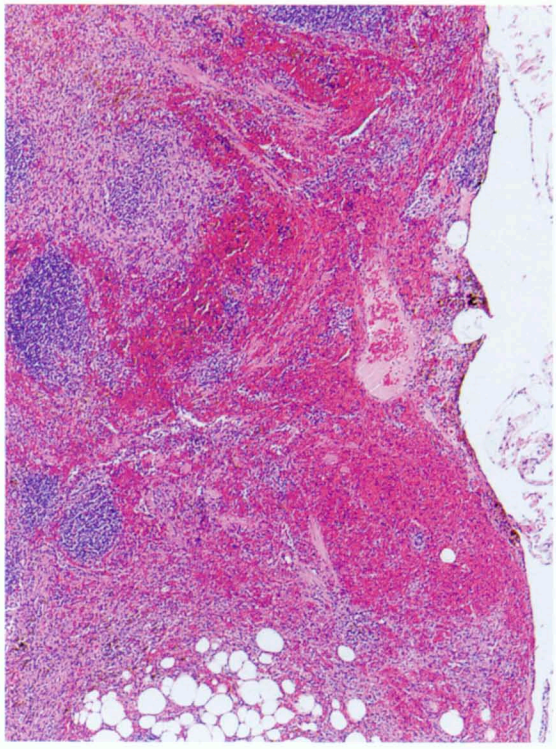


Fig. 23 - *Hemorrhage, spleen.*

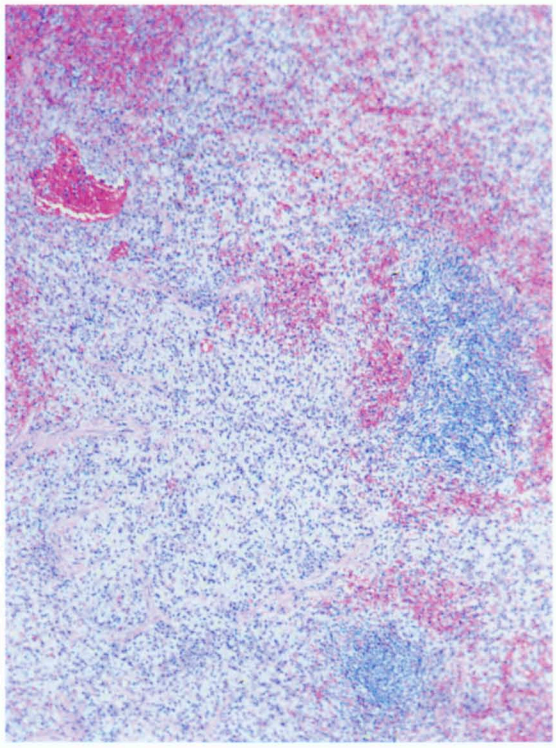


Fig. 24 - *Chronic inflammation, spleen.*

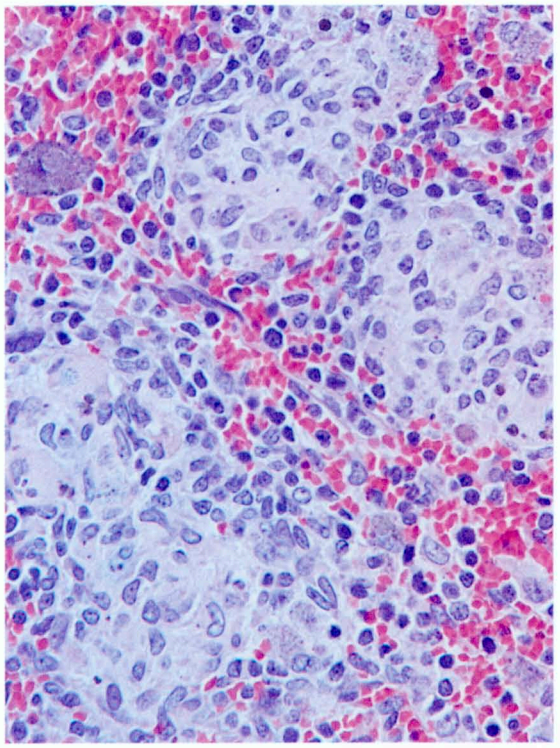


Fig. 25 - *Granulomatous inflammation, spleen.*

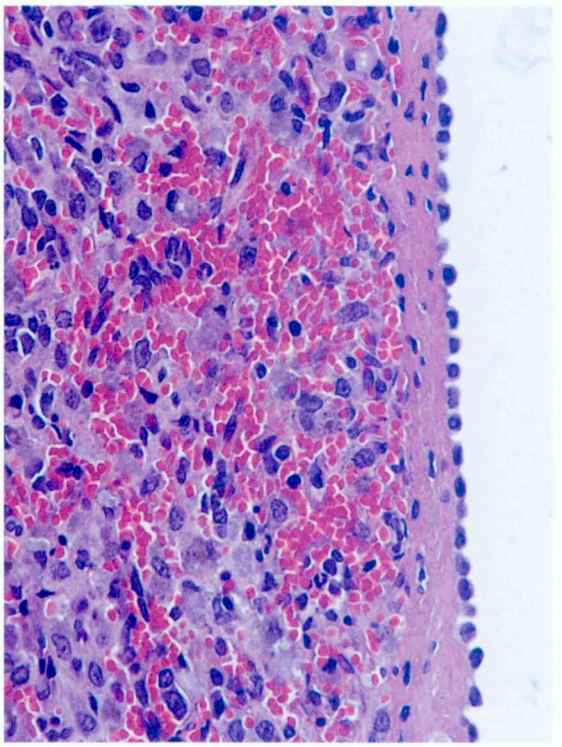


Fig. 26 - *Mesothelial hypertrophy, capsular surface of spleen.*

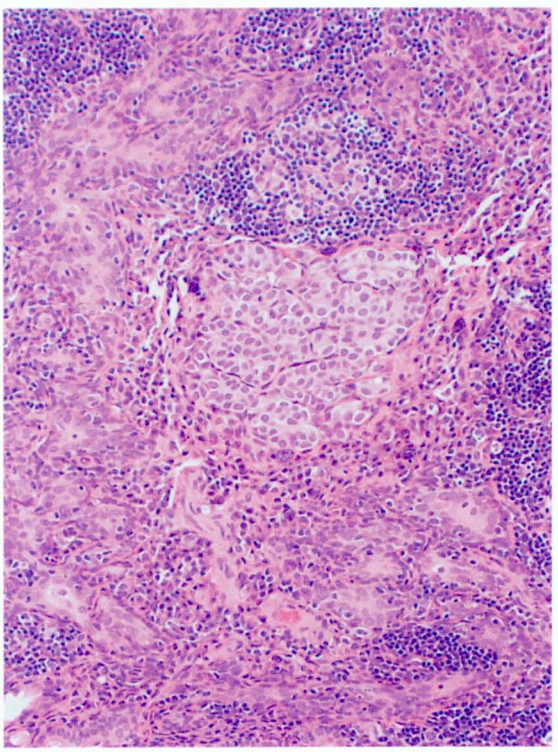


Fig. 27 - *Ectopic parathyroid, thymus.*

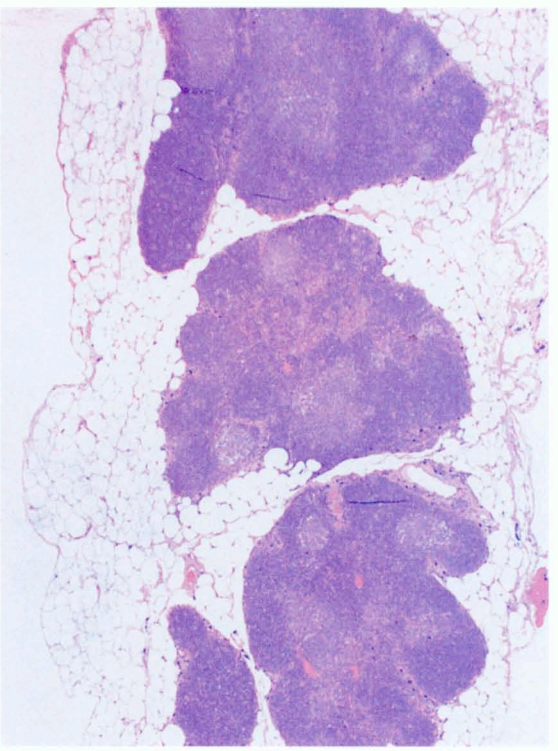


Fig. 28 - *Physiologic involution (atrophy), thymus of a sexually mature rat.*

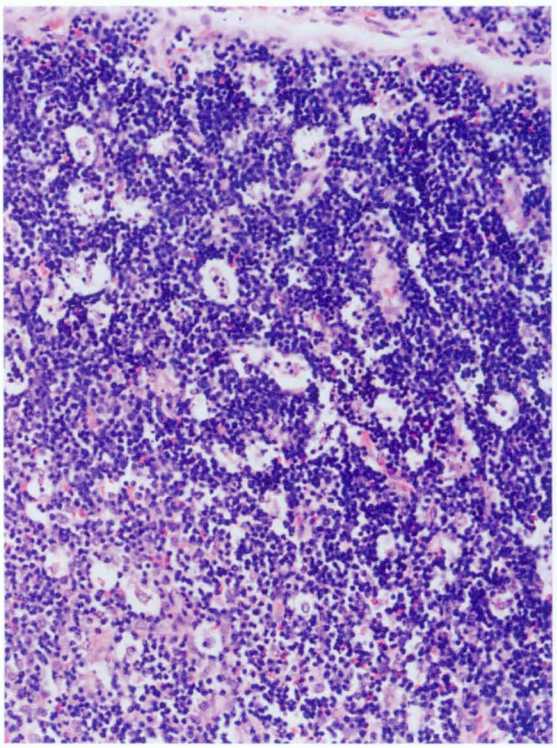


Fig. 29 - *Lymphoid necrosis, thymus.*

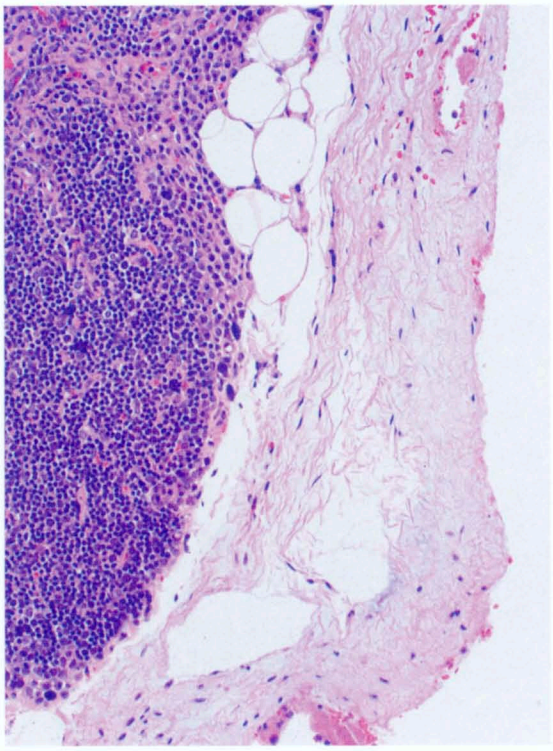


Fig. 30 - *Fibrosis of capsular surface, thymus.*

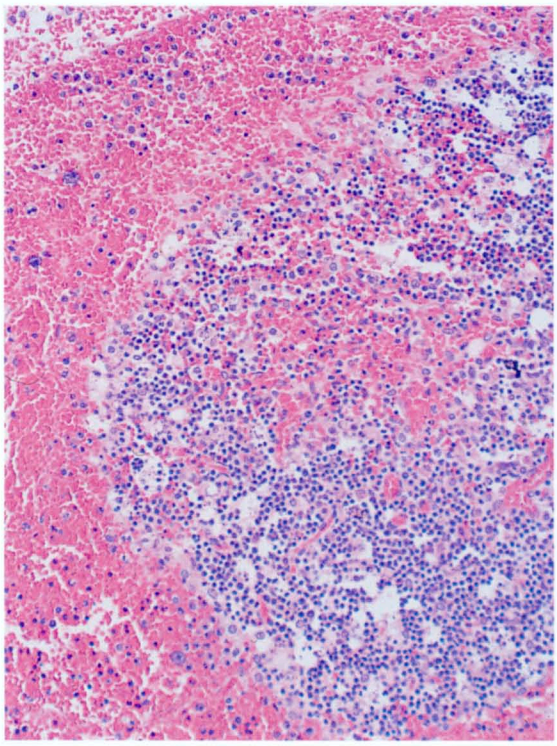


Fig. 31 - *Hemorrhage, thymus.*

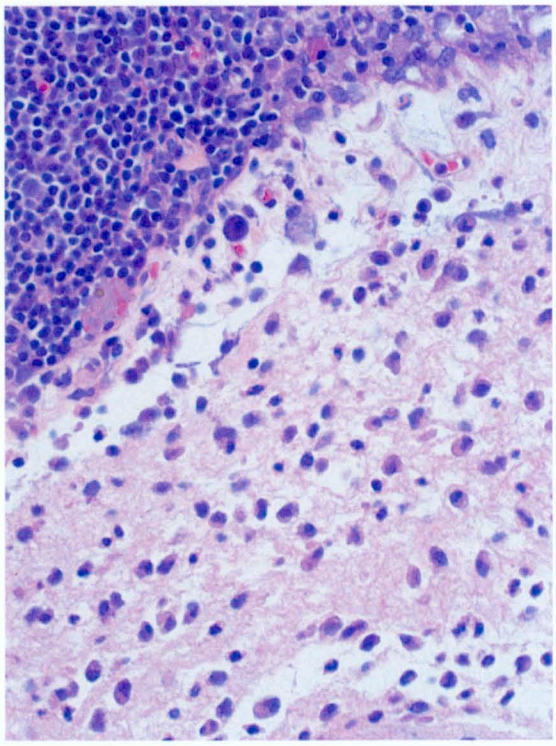


Fig. 32 - *Acute inflammation, thymus, from gavage injury.*

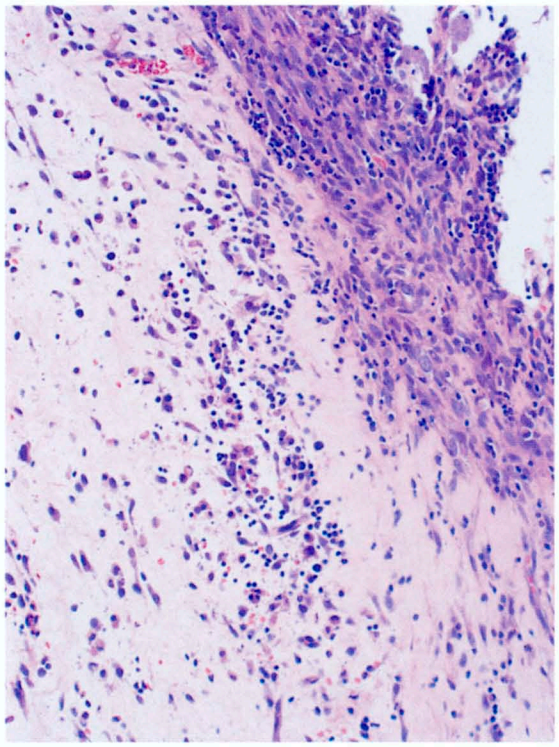


Fig. 33 - *Chronic inflammation, thymus. Note increase in collagen.*

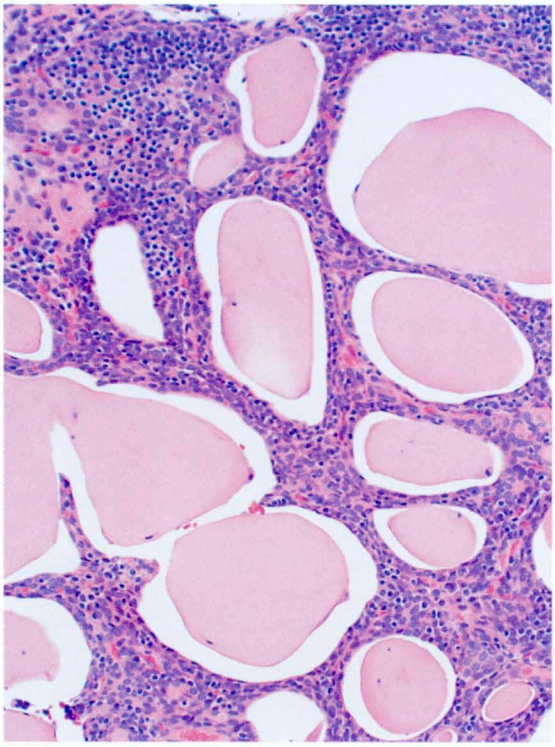


Fig. 34 - *Epithelial cysts, thymus.*

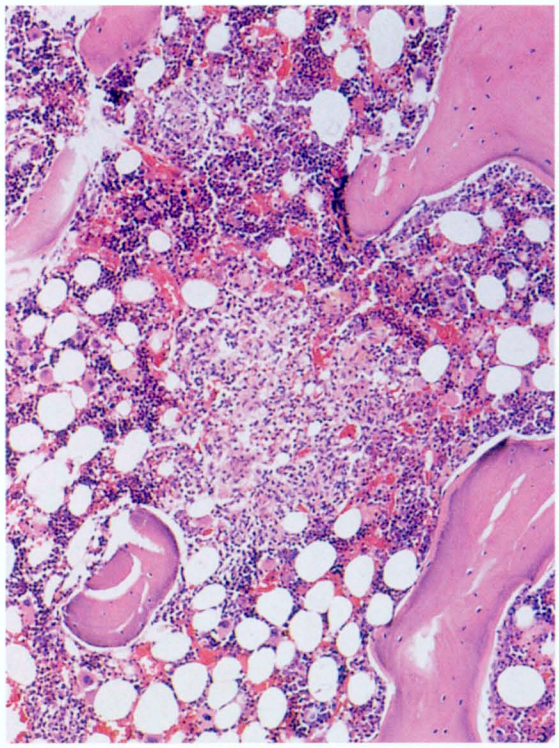


Fig. 35 - *Focal atrophy, bone marrow.*

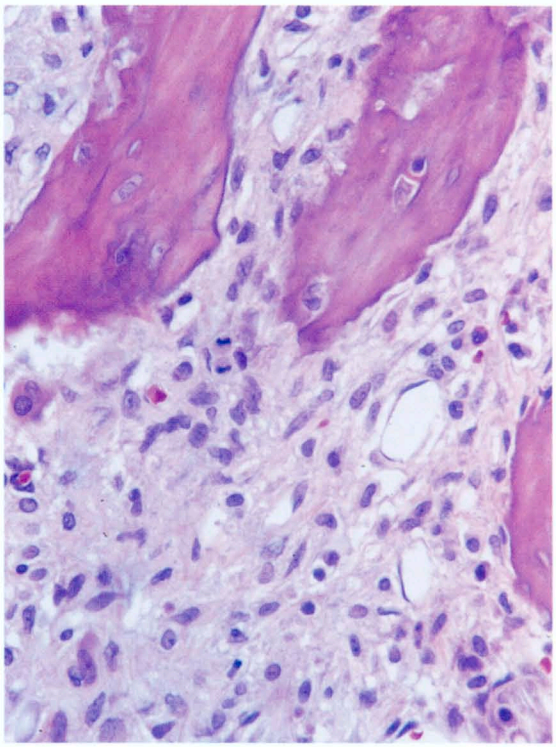


Fig. 36 - *Myelofibrosis, bone marrow.*

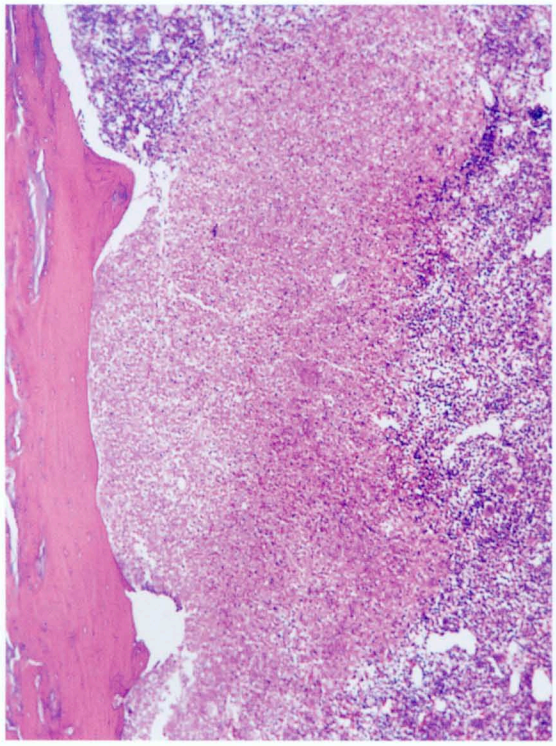


Fig. 37 *- Necrosis, bone marrow.*

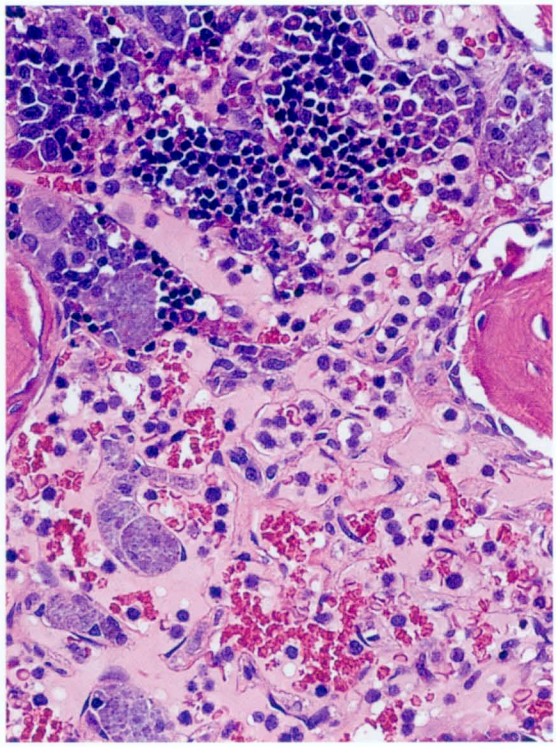


Fig. 38 - *Pigmentation, bone marrow.*

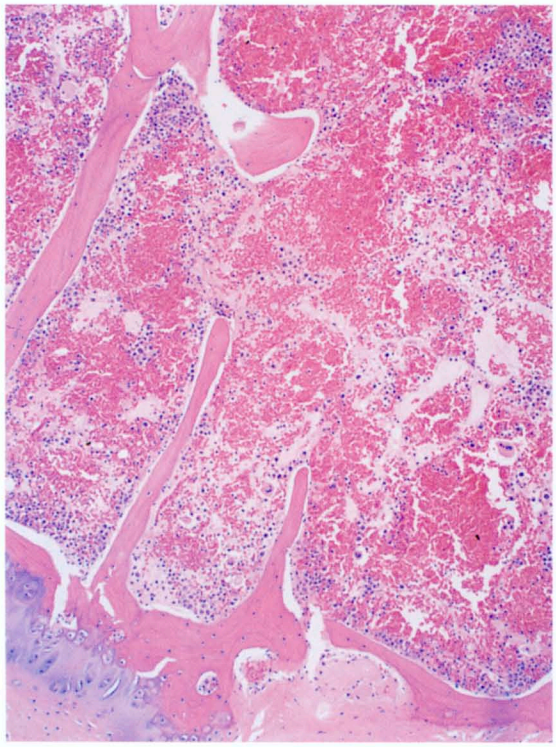


Fig. 39 - *Hemorrhage, bone marrow.*

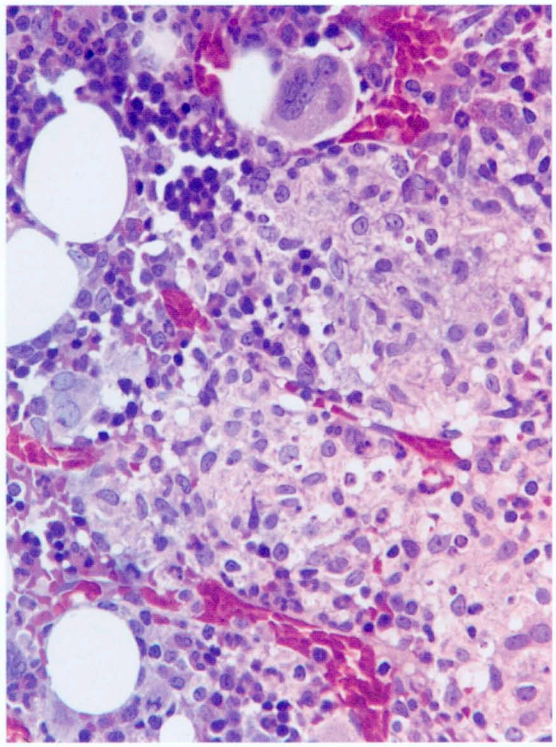


Fig. 40 - *Granulomatous inflammation, bone marrow.*